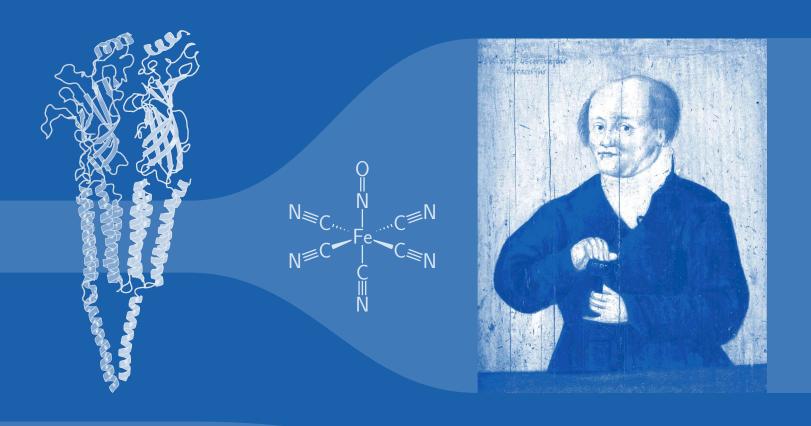
Biochemical Pharmacology

Lecture Notes



Michael Palmer, MD

with contributions by Alice Chan, Thorsten Dieckmann, and John Honek

Biochemical Pharmacology lecture notes

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This version is from Saturday 16th April, 2022. The latest electronic version of these notes and accompanying slides is available for free at

mpalmer.heresy.is/webnotes/Pharmacology/

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Preface

These notes, and the accompanying slides, are intended for teaching the subject to science students at the advanced undergraduate or graduate level. They may also be of use for self-study; a glossary that explains some of the medical terminology used in the text has been included to aid the independent reader.

The focus is on principles, not on comprehensive coverage, and not solely on clinical relevance either. Many drugs, and even some entire classes of drugs, that are important in clinical pharmacotherapy are not covered here. On the other hand, some drugs that are no longer clinically used have been included here for their interesting and instructive modes of action.

These notes use a simple layout: they present all slides in sequence, augmented with some explanatory text below each slide. This may not always produce a polished look, but it makes for easier on-screen reading than a traditional book layout, in which the figures are grouped and tend to migrate away from the corresponding text. It also helps me stay on topic and advance the plot with each successive slide. I have used this format previously with another of my teaching subjects, and it seems to work well for my students.

The content of these notes is based on the book "Biochemical Pharmacology" (Wiley, 2012) that I wrote together with my colleagues Alice Chan, Thorsten Dieckmann, and John Honek, whom I thank for their permission to include adapted versions of their book chapters here (14, 13, and 15, respectively). Most of the figures in this text have been adapted, with enhancements and sometimes with corrections, from that book. However, the text is entirely new and more condensed, which should make it easier to cover most or all of the content in a single term.

I thank several of my students, and in particular Stephanie Malatesta, Julia Plakhotnik, and Maxim Vasiliev, who helped to improve these notes by challenging and questioning me about the material and its presentation. Thanks also to Katharina Glatz of http://pathorama.ch, who kindly gave permission to reproduce the histological slides shown in these notes.

Update 2016: While these notes and slides were originally published by Wiley in 2013, Wiley agreed in 2016 to let the copyright revert to me. Therefore, I can now make them freely available.¹ If I may ask one thing in return from you, it would be that you bring to my attention any errors that you find in the text.² Even though (as stated above) my goal here is illustration by example rather than comprehensive, I would also regard as errors any omissions so substantial that they create a distorted impression of the state of the art in the field. Any other comments and suggestions you may have are also welcome.

Update 2022: My former university has fired me for my refusal to get "vaccinated." I will therefore just briefly state that I used to teach biochemistry for 20 years at a major Canadian university, with biochemical pharmacology being one of my main (and favorite) teaching subjects. I will keep these teaching materials in the public domain.

Having been originally a decade ago, this lecture notes certainly are showing their age and could do with a major update. I may or may not have time and occasion for that in the future.

¹For questions concerning permitted use and reproduction, please refer to the "Copyrights" section on page 303.

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Chapter 1

Introduction

1.1 What is biochemical pharmacology?

What is it?

- pharmacology, but with a focus on how drugs work, not on whether we should take them before or after dinner
- fascinating—you will love it, or double your money back

What is it not?

- just *molecular* pharmacology—physiological context is important, too
- a claim that we completely understand the biochemical action modes of all practically useful drugs—we don't

Let me repeat the last two points—the action modes of many drugs are incompletely understood, and physiology is important. I will try to cover the key aspects of physiology as far as they are needed to understand the course material, but you may find it useful to consult an actual physiology text on the side from time to time.

1.1.1 On drugs and poisons: Paracelsus' maxim

We will now consider the question: "What are drugs?" Our first witness will be the gentleman shown in this slide. In his day—the 16th century—Paracelsus acquired a reputation not just as a physician, but also for his fondness of a good drink; rumor has it that this latter inclination was useful to him in the discovery of his famous maxim.

As you know, many drugs turn poisonous if overdosed, and many poisonous natural compounds may become useful as drugs when properly diluted. That holds for both legal and illegal drugs; we will consider examples of both classes in this course. "Alle Ding' sind Gift und nichts ohn' Gift; allein die Dosis macht, dass ein Ding kein Gift ist."

"All things are poison and nothing is without poison; only the dosage makes it so that something is not a poison."

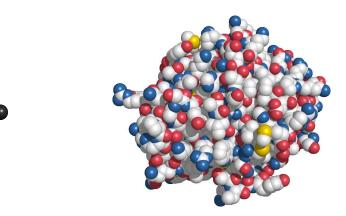
"Dosis sola facit velenum."



Picture from wikimedia

1.1.2 A very small drug particle and a very large one

Lithium is the smallest drug particle—it is not even a molecule but just a single atom. It is also an example of a drug whose mode of action is still unknown, despite many decades of research; various biochemical effects have been described, such as the inhibition of certain protein kinases, but no single effect has been conclusively linked to its antidepressant activity in humans.¹

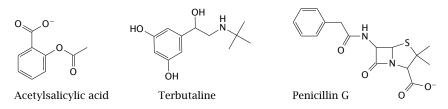


Urokinase is a protein and as such is much larger than most other drug molecules. It is used to dissolve intravascular blood clots in order to restore perfusion in acute myocardial infarction and stroke.

Note that neither lithium nor urokinase is a very typical drug; some more typical ones are shown in the next slide.

¹An interesting study on knockout mice [1] suggests that lithium signaling involves β -arrestin (see Section 5.7); however, the accompanying in vitro experiments intended to elucidate the intracellular signaling mechanism employed lithium concentrations much higher than those that can be expected in vivo.

1.1.3 Some drug molecules of more typical size



Most drugs are organic molecules with molecular weights of no more than 500 Da, such as the examples shown here. This rule of thumb holds for drugs of very different functional classes.² As you may know, acetylsalicylic acid inhibits cyclo-oxygenase, and penicillin inhibits bacterial cell wall synthesis; terbutaline activates β_2 -adrenergic receptors. We will discuss all these drug actions in more detail in later chapters.

Organic drug molecules may be natural compounds or obtained through organic synthesis. Some drugs are obtained *semisynthetically*, that is, through the synthetic modification of natural compounds; for example, penicillin G and other penicillin derivatives are obtained through semisynthesis.

1.2 Drugs and drug targets

Practically all drugs—with the exception of those that act osmotically; see below must bind to target molecules in order to exercise their effects. Most drug targets are proteins.

1.2.1 Functional classes of protein drug targets

- 1. Enzymes
- 2. Hormone and neurotransmitter receptors
- 3. Ion channels
- 4. Membrane transporters
- 5. Cytoskeletal proteins

As we work through the example drugs in this course, you will notice that most of the drug targets fall into one of the above categories.

1.2.2 Non-protein drug targets

- 1. DNA: alkylating anti-tumor drugs
- 2. RNA: anti-ribosomal antibiotics, antisense oligonucleotides

²You may have heard of Lipinski's "rule of five", which stipulates a molecular weight of \leq 500 Da, places limits on the number of polar atoms, and so forth, stating all of these constraints using numbers that are multiples of five. These additional aspects of drug molecular structure will be considered in Chapter 3.

- 3. Lipid membranes: antibiotics (amphotericin B, polymyxin); gaseous narcotics, alcohol?
- 4. Free space, or rather no target at all: osmolytes

Drug targets other than proteins are less common. DNA is important with anticancer drugs. The RNA of bacterial ribosomes is targeted by various classes of antibiotics. It is noteworthy, however, that many regulatory effects of RNA have only recently been discovered and appreciated. In experimental research, it has become quite common to inhibit the expression of specific genes at the level of mRNA, and in a few cases this strategy has already found clinical application. This is discussed in more detail in chapter 13.

Drugs that target lipid membranes are again most clearly exemplified by some antibiotics, which recognize specific lipids that occur in microbial cell membranes but not mammalian ones. The accumulation of alcohol and of gaseous narcotics in the cell membranes of neurons may contribute to the effects of these compounds; the long-standing question as to what extent these effects arise from direct interaction with membrane proteins as opposed to membrane lipids has not yet been fully resolved.

Osmotically active drugs are used in order to influence the distribution of fluids between compartments. An important application is the use of dextran or hydroxyethyl-starch as blood plasma expanders, that is, to replace lost blood volume. The osmotic activity of such macromolecules counteracts the hydrostatic pressure difference that exists across the capillary walls of the circulation. If lost blood were replaced with physiological saline solution instead, this fluid would rapidly leak out into the tissues due to the intracapillary pressure.³

While plasma expanders are clinically important, they are not very interesting from a biochemical point of view, and therefore we will not consider them any further.

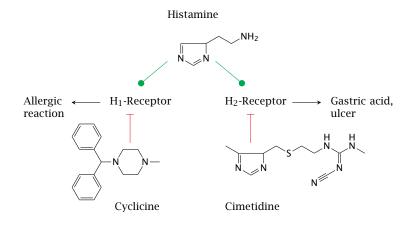
1.2.3 Histamine receptor antagonists

Among the hormone and neurotransmitter receptors, a particularly prominent class are the G protein-coupled receptors (GPCRs). These receptors are found in the cytoplasmic membranes of all kinds of cells and respond to a large number of different ligands. It is often said that approximately 50% of all clinically used drugs target G protein-coupled receptors. We will devote all of Chapter 5 to this important receptor class; here, we will briefly look at histamine receptors and angiotensin receptors as examples.

Histamine receptors occur in several subtypes. While all subtypes are activated by the same physiological ligand—that is, histamine—they may differ in their susceptibility to inhibition by receptor antagonists. For example, cyclizine inhibits H₁-receptors, whereas cimetidine selectively blocks H₂-receptors. Subtype-selective

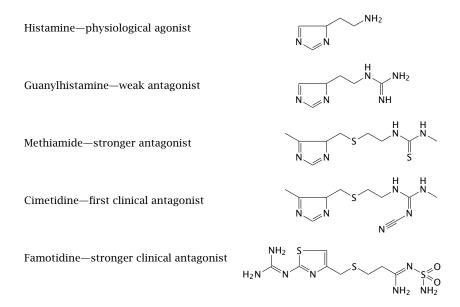
³Breakdown of the capillary permeability barrier leads to *capillary leak syndrome*, a life-threatening condition with extreme generalized edema.

drugs are often useful in pharmacotherapy; for example, H₁-selective inhibitors can be used to treat allergies without interfering with acid secretion, and H₂-selective drugs can be used to treat gastric hyperacidity without causing drowsiness, which is a common side effect of H₁-receptor blockade.



While cyclizine has only remote structural similarity to histamine, the resemblance is more obvious with cimetidine. This similarity is not coincidental.

1.2.4 The development of H₂-receptor blockers



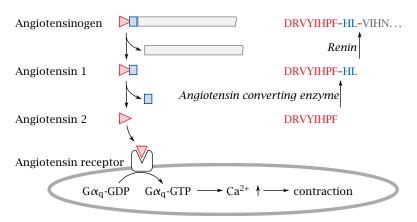
The development of cimetidine began in the 1960s. The histamine receptors had not been molecularly characterized at the time; all that was known about them was that they existed and were activated by histamine, and that some of them were involved in the secretion of gastric acid. Gastric acid was known to be a factor in the causation of gastric ulcers.⁴ The guiding idea was to modify the structure of

⁴Indeed, excess gastric acid was considered *the* cause of gastric ulcers, since the bacterial pathogen *Helicobacter pylori* had not yet been discovered. *H. pylori* damages the gastric mucous membrane and

histamine so as to turn it from an activator into an inhibitor, which could then be used to treat the ulcers.

This plan proved remarkably successful. The slide illustrates intermediate stages of the development process. Guanylhistamine was the first derivative that retained affinity for the receptor but inhibited it. Further modification yielded cimetidine, which improved upon the receptor affinity of guanylhistamine and became the first clinically used H₂-receptor antagonist, serving as the mainstay of ulcer therapy for more than a decade. Even more avidly binding antagonists, such as famotidine, can be administered at dosages of just a few milligrams per day, rather than the several hundreds of milligrams that were required with cimetidine.

1.2.5 Angiotensin: Proteolytic release from angiotensinogen, and mode of action



Vascular smooth muscle cell

Angiotensin 2 is a peptide mediator that plays an important role in the control of blood pressure. It binds to a GPCR at the surface of vascular smooth muscle cells. An intracellular signaling cascade then induces contraction of the cells, which causes constriction of the blood vessels and increased blood pressure. Inhibition of angiotensin 2 action is an important principle in the treatment of hypertension.

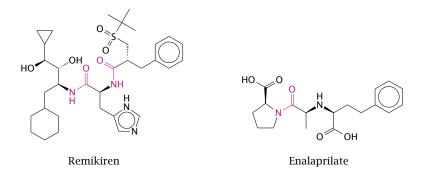
Angiotensin is released in two successive proteolytic steps from angiotensinogen, a plasma protein. Accordingly, angiotensin action can be inhibited by preventing either its proteolytic release or its binding to the receptor.

1.2.6 Two inhibitors of proteolytic angiotensin release

Remikiren inhibits the protease renin, which catalyzes the first proteolytic cleavage of angiotensin, whereas enalaprilate inhibits angiotensin-converting enzyme, which performs the second cleavage step. Both drug molecules contain peptide bonds, which are highlighted. Peptide bonds are subject to cleavage by proteases and peptidases in the digestive tract. In the case of remikiren, this prevents oral drug

initiates ulceration, and antibacterial therapy is now key in the treatment of ulcers. However, since ulceration is promoted by gastric acid, its reduction remains important as well.

application, since the drug is destroyed by these enzymes before it can be taken up. In contrast, the single peptide bond in enalaprilate has a greater degree of steric protection, and enalaprilate can therefore be used orally.



It is interesting to compare the structure of these protease inhibitors to the peptide sequences of the angiotensinogen substrate near the respective cleavage sites (see preceding slide).

1.2.7 Sequence of saralasin, a peptide inhibitor of the angiotensin 2 receptor

Angiotensin Asp-Arg-Val-Tyr-Ile-His-Pro-Phe Saralasin **Sar**-Arg-Val-Tyr-**Val**-His-Pro-**Ala**

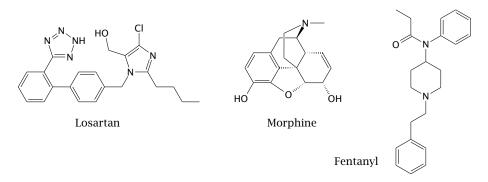
Saralasin is a synthetic peptide whose sequence is a variation of that of angiotensin. Sarcosine (Sar) is *N*-methyl-glycine.

Saralasin cannot be applied orally, because as a peptide it gets degraded by proteases and peptidases in the intestine. Even when applied intravenously, saralasin is fairly rapidly degraded by peptidases in the blood plasma, much like angiotensin itself. While drugs with such short lifetimes are impractical for routine application, they can occasionally be useful. For example, a hypertensive crisis is a dangerous condition that is characterized by spontaneous spikes in blood pressure that are very high yet of short duration. In this situation, drugs with short lifetimes make it possible to rapidly increase and decrease the antihypertensive effect so as to match these spontaneous changes in blood pressure. A longer-acting drug might overshoot when the blood pressure spike subsides spontaneously and drive the blood pressure below a safe target value.

1.2.8 Non-peptide ligands of peptide receptors

Like cimetidine, saralasin was developed through the stepwise modification of a physiological receptor agonist (that is, an activating ligand) so as to turn it into an antagonist. However, as we have seen, peptide molecules are inherently unstable *in vivo*. The question therefore arises whether peptide receptors can be controlled

with more stable non-peptide ligands. The drugs in this slide illustrate that this is indeed possible.



Morphine is a natural compound that binds and activates *opioid receptors*, a class of GPCRs activated by endogenous peptide agonists called *endorphins*. Morphine was the first non-peptide ligand of a peptide receptor to be identified. Fentanyl is a synthetic opioid receptor agonist that has a considerably simpler structure.

Losartan is a synthetic inhibitor of the angiotensin 2 receptor. In contrast to saralasin, it is not susceptible to degradation in the intestinal tract, and it therefore can be orally applied. Losartan itself and several similar drugs are widely used in the treatment of hypertension.⁵

1.3 Drug discovery and development

In the development of cimetidine and of saralasin, the physiological receptor agonists were used as lead compounds. Starting with a lead compound is an important approach to drug development, but not the only one. Some drugs have been found through *phenotypic screening*, without any previous knowledge or assumptions about suitable molecular targets. This approach is alive and well, although nowadays it is often possible to start with a known target molecule, and often even with the chosen target's complete molecular structure.

1.3.1 Arsphenamine, the first modern antibacterial drug

The antibacterial activity of arsphenamine was discovered in the early 20th century by Paul Ehrlich and Sachahiro Hata, who screened more than 600 compounds for activity against syphilis. Since *Treponema pallidum*, the bacterium that causes syphilis, cannot be grown *in vitro*, the screening had to be done in animal experiments.

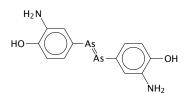
Arsphenamine can be considered an early example of drug development by brute-force phenotypic screening. It was used for several decades in the treatment of syphilis, until penicillin became available.

⁵If you are intrigued by the tetrazole group in the losartan structure, you can find out more about it in slide 14.2.2.

EM photo credit: CDC image library



Treponema pallidum



Arsphenamine

1.3.2 Paul Ehrlich, the discoverer of arsphenamine and originator of the receptor concept

A 200 Deutschmark bill (no longer valid; image from wikimedia) with Paul Ehrlich's portrait and the structure of arsphenamine. Ehrlich's research institute was located in the city of Frankfurt; the background of the image shows historical buildings from the city center.⁶



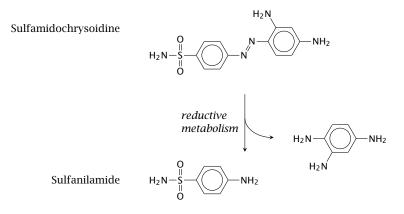
1.3.3 Drug discovery by brute force: sulfamidochrysoidine

Like arsphenamine, sulfamidochrysoidine emerged from an early large-scale drug screening program. While arsphenamine was active against syphilis only, sulfamidochrysoidine was the first drug with activity against a broad spectrum of bacterial pathogens.

Sulfamidochrysoidine itself is inactive *in vitro*; if you add it to a bacterial culture, nothing happens. The active component, sulfanilamide, is released from sulfami-

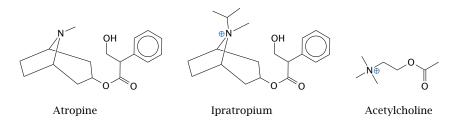
⁶The Deutschmark was not only fiscally but also artistically superior to the Euro. Maybe when the Euro finally collapses, we can get it back.

dochrysoidine through reductive drug metabolism *in vivo*.⁷ Therefore, as with arsphenamine, the antibacterial effect could only be observed in animal experiments.



Sulfanilamide and other sulfonamides are antimetabolites of folic acid synthesis. Most bacteria synthesize folate themselves and are unable to take it up from the environment. In contrast, folic acid is a vitamin in humans, which means that it is acquired from the diet, and therefore inhibition of folic acid synthesis doesn't affect us. Therefore, sulfonamides can be used to treat infections in humans.

1.3.4 Natural compounds and semisynthetic derivatives



Atropine is an alkaloid found in the plant *Atropa belladonna* (deadly nightshade). It acts as an antagonist of certain types of acetylcholine receptors. This has a range of effects on physiological regulation, such as acceleration of heartbeat and relaxation of bronchi and of pupils. At higher dosages, atropine also interferes with brain function. Atropine is a very good example of the drug-poison duality observed by Paracelsus.

Ipratropium is a semisynthetic derivative of atropine. Attachment of an isopropyl group to the nitrogen turns the latter into a quaternary amine. This gives the molecule a permanent charge, which prevents it from penetrating the brain as easily as atropine does, and therefore reduces side effects. We will consider the basis of this effect in Chapter 3.

⁷I suppose that something might happen in *anaerobic* cultures, with their reducing conditions. I have not looked into the literature on this question, however.

- 1.3.5 Protein structure-based drug discovery: HIV protease bound to its inhibitor saquinavir

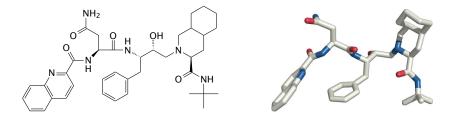
The HIV protease inhibitor saquinavir illustrates the modern paradigm of protein structure-based drug development.

Like several other viruses, HIV expresses its proteins in the form of a single polyprotein precursor, which is then proteolytically cleaved into the individual proteins. This cleavage is performed by the HIV protease, a viral protease which releases first itself and subsequently the other domains (see slide 11.10.7). If this cleavage is prevented, the proteins will remain trapped and not become functional; HIV protease therefore is essential for the maturation of virus particles. Accordingly, inhibition of the protease is an effective strategy for treating HIV infection.

The protease consists of two subunits that enclose the active site. Once the structure of the protease had been determined, inhibitors such as saquinavir could be designed that fill and block the active site. Structure rendered from 1fb7.pdb.

HIV infections always must be treated with a combination of different drugs in order to prevent the emergence of resistance. Protease inhibitors are a crucial part of such drug combinations, and their availability has greatly extended the life expectancy of HIV patients.

1.3.6 Structure of saquinavir, and its conformation in the active site of HIV protease



This slide shows the conformation of saquinavir when bound to HIV protease. It is taken from the same structure file as the slide above.

1.3.7 Drug discovery by accident (1): From a letter by Reverend Edmund Stone to the Royal Society, 1763

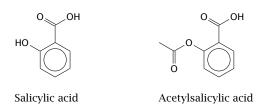
Among the many useful discoveries, which this age hath made, there are very few which, better deserve the attention of the public than what I am going to lay before your Lordship.

There is a **bark of an English tree**, which I have found by experience to be a powerful adstringent, and very efficacious in curing anguish and intermitting disorders.

About six years ago, **I accidentally tasted it**, and was surprised at its extraordinary bitterness ... As this tree delights in a moist or wet soil, where agues chiefly abound, **the general maxim**, **that many natural maladies carry their cures along with them**, or that their remedies lie not far from their causes, was so apposite to this particular case, that I could not help applying it; and that this might be the intention of Providence here, I must own had some little weight with me ...[2]

I continue to be puzzled by the kind of "accident" that might have caused the Reverend to involuntarily taste a piece of willow bark. After all, bicycles or roller blades were not in common use yet, so exactly how did he manage to hurl himself teeth-first into a willow tree? Nevertheless, for the rest of us, the Reverend's accident certainly proved most fortunate.

1.3.8 The active ingredient of willow bark, and its more widely known derivative



Both salicylic acid and acetylsalicylic acid inhibit cyclooxygenase, a key enzyme in the synthesis of pro-inflammatory prostaglandins and related mediators (see Chapter 9). While salicylic acid is a noncovalent inhibitor, acetylsalicylic acid inhibits the enzyme covalently, and thus irreversibly; this explains its longer lasting action.

The discovery of acetylsalicylic acid, too, was due to luck more than design. Felix Hoffmann, a chemist with Bayer AG, started playing with the structure of salicylic acid, trying to spare his father, who had been prescribed the drug, the intensely bitter taste that had already been noted by Reverend Stone. One of the modifications he tried was acetylation. The resulting drug turned out to have a significantly stronger and longer lasting effect than salicylic acid itself. We will examine the reason for this improved activity in Section 9.5.

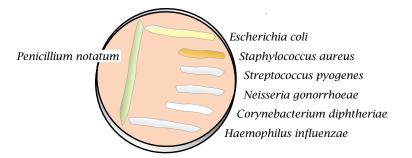
Penicillium notatum Staphylococcus aureus

1.3.9 Drug discovery by accident (2): The discovery of penicillin

This is a sketch of the original petri dish on which Alexander Fleming first observed the production of penicillin by the mold *Penicillium notatum*; it is drawn after a photograph in Fleming's paper [3]. The antibacterial effect of penicillin can be observed by the lysis of *Staphylococcus aureus* colonies near the mold colony.

The accident that gave rise to this discovery was the mold contamination normally, microbiologists try to protect their cultures from such contamination. It is to Fleming's credit that he immediately recognized the importance of this observation. However, his report was not the first of its kind; several others preceded it, and even the term "antibiosis" predates Fleming's discovery [4].⁸

1.3.10 Not all bacteria are susceptible to penicillin



Some bacterial species are resistant to penicillin and therefore can grow right up to the mold colony. This is observed with *Escherichia coli* and *Haemophilus influenzae*, both of which are Gram-negative bacteria. *Neisseria gonorrhoeae* is Gram-negative also but is nevertheless inhibited by penicillin.

Most Gram-negative bacteria are resistant to penicillin because the drug cannot penetrate the outer membrane of these bacterial cells (see slide 11.4.1). In contrast, Gram-positive bacteria such as *Staphylococcus aureus*, *Corynebacterium diphtheriae* and *Streptococcus pyogenes* don't have an outer membrane and are susceptible. Note, however, that these bacteria can still acquire resistance. *S. aureus* strains, in particular, have now mostly become resistant to penicillin. We will consider the

⁸Stigler's law of eponymy states that "no scientific discovery is named after its original discoverer." Stigler himself names Robert Merton as the original discoverer of Stigler's law.

underlying mechanisms of susceptibility and resistance to penicillin later (see slide 11.4.7ff.).

Chapter 15 covers the subject of drug discovery in a more detailed and systematic manner.

1.3.11 Drug development and approval

- preclinical, in-house: synthesis, in vitro and preliminary animal testing
- investigational drug application to Food and Drug Administration (FDA)—must be approved before clinical testing
- clinical trials in three phases:
 - (1) Healthy volunteers; focus on pharmacokinetics, toxicity
 - (2) Small number of patients with targeted disease
 - (3) Larger patient collective (several hundred to several thousand), comparison to established reference therapies
- new drug application—review by FDA
- post-introduction market surveillance

Several of the historic examples may convey the impression that drug discovery can be done with modest means. That may have been true at the time; however, nowadays the process has become strongly regulated, and these regulations have turned drug discovery into an enormously laborious and costly exercise. The total cost of development of a new drug, from scratch, is on the order of one billion dollars. Therefore, while an academic setting may suffice to support the initial stages of development, only major pharmaceutical companies have the means to see the entire process through to the end.

Chapter 2

Pharmacodynamics

2.1 General principles of drug action

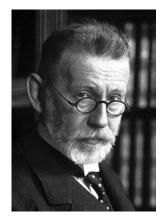
Drugs act on many different targets and in diverse ways. Nevertheless, there are some general principles of drug action that are applicable to many drugs; these are the subject of pharmacodynamics. In this chapter, we are going to cover the following concepts:

- Theory of drug-receptor interaction
- The two-state model of receptor activation
- Dose-effect relationships and their modulation by signaling cascades
- Potency, efficacy, and therapeutic index

2.1.1 The invention of the receptor concept

... I therefore assumed that the tetanus toxin must unite with certain chemical groupings in the protoplasm of cells ... As these receptors, which may be regarded as lateral chains of the protoplasm ... become occupied by the toxin, the relevant normal function of this group is eliminated ...

Paul Ehrlich, from his Nobel Lecture, 1908



In this excerpt from his Nobel lecture [5], Paul Ehrlich develops the idea of receptor blockade. Although this concept is viable and important, it does not account for the effect of tetanus toxin. Instead, tetanus toxin is a protease that cleaves synap-

tobrevin, an intracellular protein that is important for neurotransmitter exocytosis (see slide 6.9.1).

The receptor concept is fundamental to pharmacology. As stated above, most receptors are protein molecules; in this chapter, we will confine the discussion to this case.

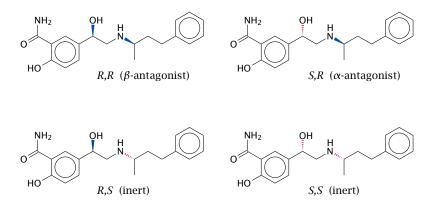
2.1.2 How do drugs affect their receptors?

- Mode of binding: reversible vs. irreversible
- Binding site: orthosteric vs. allosteric
- Functional effect: activation vs. inhibition

Most drugs bind their targets reversibly and non-covalently, but some important exceptions exist. Furthermore, binding is orthosteric in most cases, which means that the drug binds to the receptor within the same site as the receptor's physiological ligand. For example, all of the antagonists of histamine and angiotensin that were discussed in slides 1.2.3-1.2.8 bind orthosterically. This mode of binding implies that the receptor can not bind its physiological ligand and the drug at the same time; if both are present, they will compete for the same binding sites. Allosteric binding occurs with benzodiazepines, barbituric acid derivatives and several other ligands of the GABA_A receptor in the brain (see slide 6.10.8).

A drug that activates its receptor is referred to as an agonist, whereas an inhibitory drug is also called an antagonist. Drugs that target enzymes are virtually always antagonists, while with hormone and neurotransmitter receptors there usually are both agonistic and antagonistic drugs. Where this is the case, only one or the other may have therapeutic value; for example, with histamine receptors, only antagonists are clinically useful. On the other hand, with β -adrenergic receptors, both agonists and antagonists are used as drugs.

2.1.3 Labetalol as an example of stereoselective drug action



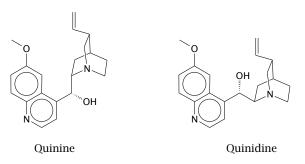
As with enzymes and substrates, the mutual selectivity of receptors and drugs is based to a great extent on the "lock and key" principle, that is, on steric complementarity. Like enzyme substrates, many drug molecules are chiral, which means that

they occur as R and S (or L and D) enantiomers. Since proteins consist of L-amino acids only, they are also chiral molecules. Therefore, we should expect protein receptors to interact with drugs in an enantioselective manner. This is indeed the case; a good example of this is the drug labetalol.

Labetalol, an α - and β -adrenergic receptor antagonist, is a racemic mixture of four stereoisomers. The *R*,*R* isomer carries most of the β -adrenergic blocking activity, whereas the *S*,*R* isomer carries most of the α -blocking activity. The *R*,*S* and *S*,*S* isomers are dead freight.

Many drugs occur as racemic mixtures, and as with labetalol the isomers will often differ in specificity and activity. Ideally, one would purify or selectively synthesize the isomer that has useful pharmacological activity, and remove others that are inactive and might only contribute to toxicity. However, in practice, achieving stereoisomeric purity may be difficult and costly, and it is usually done only where necessary to avoid significant drug toxicity.

2.1.4 Two natural stereoisomers with separate therapeutic uses



Both quinine and quinidine are obtained from the bark of the *Cinchona* tree.¹ Quinine is used against malaria; its mechanism of action is believed to resemble that of chloroquine (slide 11.9.1).

Quinidine also has some antimalarial activity, but is used in clinical medicine for its inhibitory effect on sodium channels in the heart, which is useful in certain types of cardiac arrhythmias.

2.2 Mass-action kinetics in drug-receptor interaction

Both in its assumptions and its conclusions, the theory of drug-receptor interaction is quite similar to enzyme kinetics, and it might be a good idea to dust off your biochemistry textbook to refresh your memory on that subject.

¹Pronunciation: 'Sin-cona'.

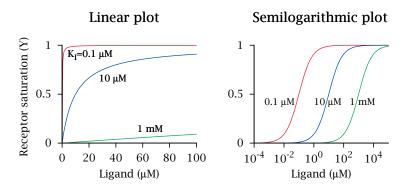
2.2.1 Mass action kinetics and receptor occupancy

$$K = \frac{[L][R_{\text{free}}]}{[LR]}$$

Receptor occupancy = $Y = \frac{[LR]}{[R_{\text{total}}]} = \frac{[L]}{[L]+K}$

The law of mass action represents the simplest possible case, but nevertheless an experimentally important one. The key parameter here is the *receptor occupancy*, that is, the fraction of receptor that is bound to the ligand and therefore subject to the ligand's functional effect. The second equation assumes that the ligand is present in excess over the receptor, which is almost always the case.

2.2.2 Linear and semi-logarithmic plots of receptor occupancy



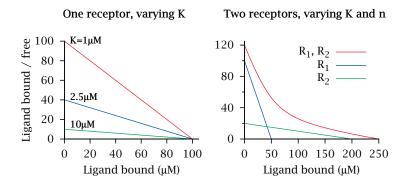
Receptor saturation, as a function of ligand concentration, can be plotted in various formats. The linear plot shows that, at ligand concentrations well below the dissociation constant, receptor saturation increases in an approximately linear fashion. At concentrations significantly greater than the dissociation constant, it approaches saturation and changes very little.

An advantage of the linear plot is freedom from distortion. However, it is not good at covering a wide range of ligand concentrations or affinities, which is often required for comparing different ligands in the same plot. For this purpose, a semilogarithmic plot—with a logarithmic scale for the ligand concentration—is more convenient. In such a plot, all curves that obey mass-action kinetics are transformed into the same sigmoidal shape. The inflection point of each curve is at Y = 0.5 and gives the dissociation constant; the horizontal offset between two curves represents the difference in affinity. We will mostly use this plot format in the following.

2.2.3 The Scatchard plot

The Scatchard plot is another way to depict the binding of ligands to receptors. In order to construct this plot, the concentrations of both receptor-bound ligand and

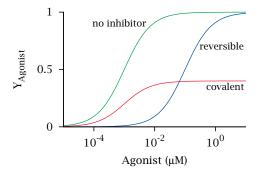
free ligand are measured, and the ratio of bound to free ligand is plotted against the concentration of bound ligand.



It is easy to show that, if all drug molecules bind to a single class of receptors with uniform affinity, all data points will fall on a straight line. Therefore, if the plotted line is not straight, this suggests that the binding sites are inhomogeneous and vary in affinity. Note, however, that this requires ligand binding to follow simple mass action kinetics, which is not always the case (see slide 2.5.1).

Also note that the Scatchard analysis does *not* assume that the concentration of the ligand always exceeds that of the receptor—in fact, some of the data points in the curve must be obtained at limiting ligand concentrations; otherwise, the y coordinate would be very low for all data points. This is usually accomplished by the use of radioactively labeled drug molecules, which can be measured accurately at such low concentrations.

2.3 Reversible and covalent receptor inhibition



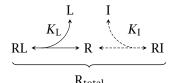
As pointed out above, most drugs bind their targets orthosterically, that is, they dislodge some physiological agonist—such as histamine or angiotensin—from its receptor. If such a drug does not itself activate the receptor, this will cause inhibition.

A drug may bind either noncovalently and reversibly, or covalently and irreversibly.² It turns out that the two cases affect the receptor quite differently: With

²While covalent and irreversible binding coincide with the great majority of drugs, there are exceptions; see for example slide 15.3.3.

a reversible inhibitor, the receptor saturation curve for the physiological agonist is shifted to the right, while with a covalent inhibitor it is vertically compressed.

2.3.1 Theory of competitive inhibition



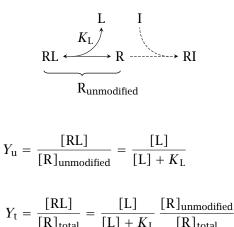
$$Y = \frac{[\mathrm{RL}]}{[\mathrm{R}_{\mathrm{total}}]} = \frac{[\mathrm{L}]}{[\mathrm{L}] + K_{\mathrm{L}} \left(1 + \frac{[\mathrm{I}]}{K_{\mathrm{L}}}\right)} = \frac{[\mathrm{L}]}{[\mathrm{L}] + K'}$$

Competitive inhibition occurs when a physiological agonist and an inhibitory drug bind reversibly to the same binding site on their receptor. The two binding equilibria are linked by the free receptor, which can only engage in one reaction at any time.

Let K_L and K_I be the dissociation constants that govern binding of the physiological agonist and the inhibitor, respectively. Then, if the concentration of the inhibitor [I] is fixed, we can comprise the entire term $K_L(1 + [I]/K_I)$ into a single number, K'. Since K' is necessarily greater than K_L , the resulting curve in the semilogarithmic plot is shifted to the right relative to the situation without inhibitor, as illustrated in slide 2.3.

Try to derive this equation on a rainy day—it's all basic high school math. You'll be glad you did!³

2.3.2 Theory of irreversible or covalent inhibition



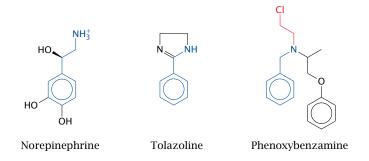
With a drug that inhibits its receptor through covalent reaction, there is no simple way to predict the exact extent to which the receptor population will be affected by

³And in case you aren't, just think of all the money you had no time to spend while deriving it.

the drug. However, after sufficient time, most of the drug will have either reacted with receptor molecules or been inactivated or eliminated in some other way, and the fraction of inhibitor-bound receptor [RI] will no longer change.⁴ The complementary fraction of unmodified receptor will continue to bind the agonist at equilibrium, governed by $K_{\rm L}$.

If you compare the second equation to the one given in slide 2.2.1, you will see that irreversible inhibition causes a vertical compression of the simple mass action receptor saturation curve, again as illustrated in slide 2.3.

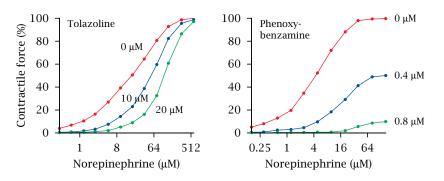
2.3.3 Two inhibitors of α -adrenergic receptors



As an experimental example of competitive and covalent inhibition, we will compare two inhibitors of α -adrenergic receptors. These receptors, which are activated by the catecholamine hormones epinephrine and norepinephrine, are found on different cell types. On smooth muscle cells, α receptor activation raises the cytoplasmic calcium concentration, which results in muscle cell contraction.

This slide shows the structures of the physiological agonist norepinephrine, and of the two antagonists tolazoline and phenoxybenzamine.

2.3.4 Inhibition of spleen strip contraction by tolazoline and phenoxybenzamine



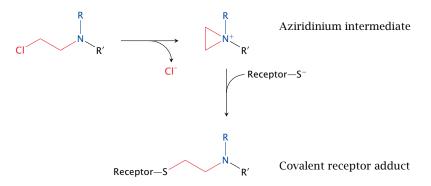
The effect of tolazoline and phenoxybenzamine on α -adrenergic receptors can be observed in spleen strips. The spleen resembles a large sponge that is soaked

⁴This statement neglects the turnover of the receptor itself by degradation and synthesis, which we here assume to be slow relative to the reaction and turnover of the drug.

with blood. Contraction of smooth muscle cells embedded in the spleen tissue will squeeze out the blood and inject it into the circulation. This contraction is triggered by epinephrine or norepinephrine.

The graphs shown here are replotted from original data in [6]. In the experiment, spleen strips were placed between two elastic hooks, bathed in norepinephrine with or without added inhibitor, and the resulting contractile force was measured. If one compares these experimental curves to the theoretical ones shown in slide 2.3, it is pretty clear which inhibitor binds to the receptor reversibly, and which one acts irreversibly.

2.3.5 Mechanism of covalent receptor blockade by phenoxybenzamine

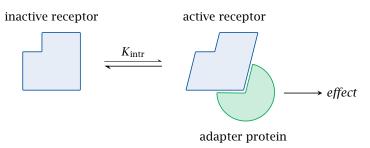


Phenoxybenzamine has a benzylamine moiety that structurally resembles the catecholamines and facilitates its initial, noncovalent binding to the receptor. This sets the stage for the subsequent covalent reaction, which is brought about by the chloroethyl group. This group undergoes spontaneous cyclization to the aziridine derivative, which then reacts covalently with a cysteine residue in the receptor.

Antagonists of α -adrenergic receptors cause smooth muscle relaxation not only in the spleen but in the vascular system in general, and they are useful in the treatment of arterial hypertension. Competitive antagonists such as tolazoline are more widely used for this purpose than covalent ones like phenoxybenzamine. However, phenoxybenzamine is useful in *pheochromocytoma*, which is a tumor of the adrenal gland that produces and intermittently releases excessive amounts of epinephrine and norepinephrine. These high amounts of hormones could overrule competitive inhibitors, but they will not overcome the irreversible inhibition imposed by phenoxybenzamine.

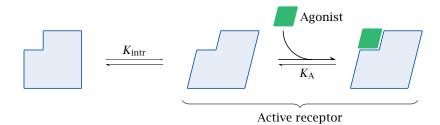
2.4 The two-state model of receptor activation

Mass-action theory is good enough for competitive inhibition; however, it cannot explain how agonistic drugs (or physiological ligands) might activate their receptors. The simplest theory to account for receptor activation assumes that a receptor occurs in exactly two conformational states. In one state, the receptor is active, for example because this conformation can bind some adapter protein that triggers the downstream effect; in the other conformation, it is inactive.



The two conformations are at equilibrium. With most receptors, this intrinsic equilibrium favors the inactive conformation; therefore, in the absence of ligands, receptors tend to be inactive. However, the equilibrium will be shifted by ligands that preferentially bind to one or the other conformation.

2.4.1 Agonist behavior in the two-state model

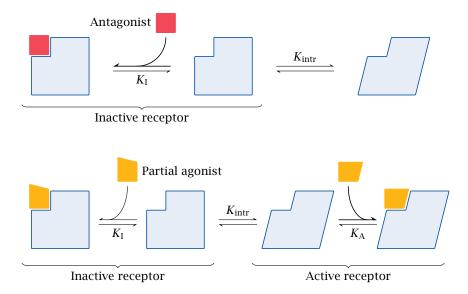


In the two-state model, an agonist is a ligand that binds selectively to the active receptor conformation, according to simple mass-action kinetics. The free energy released by binding will trap a fraction of the receptor molecules in the active state; only the residual fraction of unbound receptor will continue to equilibrate between the active and the inactive conformation.

Note that, if the affinity of the drug for the inactive conformation is indeed zero, the active fraction of the receptor will slightly exceed the ligand-bound fraction. Such a drug is referred to as a *full agonist*.

2.4.2 Antagonists and partial agonists

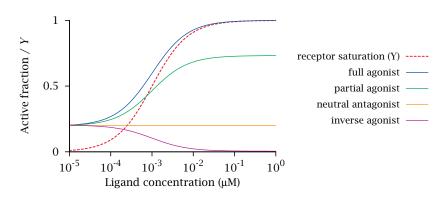
After the foregoing, it will not surprise you that the two-state model explains the effect of antagonists by assuming that they bind selectively to the inactive receptor conformation. In principle, the receptor equilibrium will then be shifted toward this conformation; however, a decreased receptor activity can only be observed if the receptor in question had measurable activity in the unbound state. Antagonists that measurably decrease spontaneous receptor activity are also referred to as *inverse agonists*.



An interesting case are the *partial agonists*, which bind to both the inactive and the active states. In order to achieve partial activation, that is, to shift the receptor equilibrium partway mpMihctoward the active state, the drug's affinity for the active state has to be greater than that for the inactive state.

A drug with exactly equal affinities for the active and the inactive state would not shift the conformational equilibrium at all, and therefore on its own would not change receptor activity. Nevertheless, such drugs are referred to as *neutral antagonists*; presumably, this is because an antagonistic effect will result when such a drug is applied together with an agonist. Neutral antagonism would seem to be mostly a theoretical case; it would surprise us to find a compound which binds the two different conformations with *exactly* the same affinity.

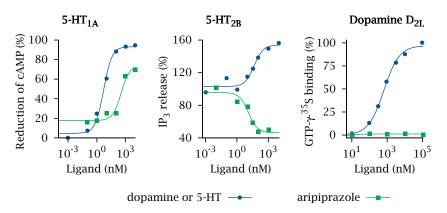
2.4.3 Dose-effect curves in the two-state model



This slide shows numerical examples for receptor active fraction as a function of ligand concentration, for various types of ligands. Each curve represents a different set of values for K_A and K_I . However, all combinations have been adjusted so as to correspond to the same receptor saturation curve (Y; dashed red line).

At the left end of the *x* axis, the receptor saturation is negligible, and the γ axis intercepts of the active fraction curves reflect the fraction of the receptor that is in the active conformation due to its intrinsic conformational equilibrium. This fraction was arbitrarily set to 0.2,⁵ which is higher than observed with most real-life receptors. This choice was made to more clearly distinguish the curves for the different ligand types from each other and from the receptor occupancy.

2.4.4 Application of the two-state model: Effects of aripiprazole on serotonin and dopamine receptors



Earlier, we noted that receptors for a given hormone or neurotransmitter may occur in multiple variants (see slide 1.2.3). A drug may bind to more than one receptor variant. Where this is the case, the two-state model allows that a drug may be an agonist on one receptor variant, yet an antagonist at another. Such behavior is indeed observed with the antipsychotic drug aripiprazole, which binds to various serotonin (5-hydroxytryptamine, 5-HT) receptors and also to several dopamine (D) receptors.

In this slide, which shows data replotted from [7], it can be seen that aripiprazole increases the activity of the 5-HT_{1A} receptor subtype, but to a lesser extent than serotonin does; it therefore is a partial agonist at this receptor. In contrast, at the 5-HT_{2B} receptor, aripiprazole is an inverse agonist. It also fails to activate the dopamine D_{2L} receptor. Since this receptor has no detectable basal activity, it cannot be discerned whether aripiprazole is a neutral antagonist or an inverse agonist at this receptor.⁶

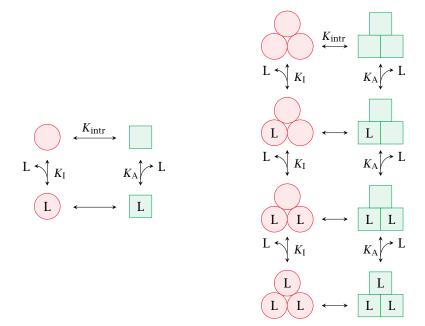
⁵If we define K_{intr} as the ratio of active fraction over inactive fraction, then an active fraction of 0.2 in the absence of ligand corresponds to $K_{intr} = 0.2/0.8 = 0.25$.

⁶You may have noticed that each of the three panels in this plot has a different parameter plotted along the γ axis. These will be explained in Chapter 5.

2.5 Beyond the two-state model

As we have just seen, the two-state model can account for a fair variety of experimental observations, and accordingly it is widely used in the scientific literature. However, some observations cannot be explained within this model's rules.

2.5.1 Cooperative behavior of oligomeric receptors

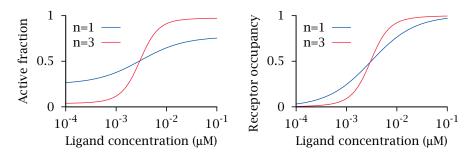


The two-state model assumes mass-action equilibrium for ligand binding to both the active and the inactive state. While this is indeed commonly observed with monomeric receptors, oligomeric ones often bind their ligands *cooperatively*. In such receptors, individual subunits may still bind the ligand according to mass-action kinetics. However, the subunits are not free to change their conformation individually; instead, the transitions of all subunits are tied together and occur in concert. This changes the shape of both receptor activity and ligand saturation curves, such that they become steeper and sharper than those of comparable single-subunit receptors.⁷

This slide illustrates a hypothetical receptor that contains three subunits and another one that has only a single subunit. The equilibrium constants— K_{intr} , K_a and K_i —are assumed to be the same for each receptor subunit, regardless of subunit stoichiometry. In the trimeric receptor, partial conformational changes are forbidden; all subunits change conformation simultaneously, regardless of saturation with ligand.

 $^{^{7}}$ This is analogous to the oxygen binding kinetics of hemoglobin *vs.* myoglobin, which is commonly treated at length in introductory biochemistry classes.

2.5.2 Effect of cooperativity on receptor activity



This slide illustrates hypothetical dose-response curves for the two receptors shown in the previous slide. The value of K_{intr} (1/3) was chosen to favor the inactive conformation only slightly over the active one. In addition, the values of K_a and K_i were chosen so as to make the ligand a partial agonist. With these parameters, the active fraction of the monomeric receptor (left, n=1) shows a rather shallow response to increasing ligand concentrations.

In the trimeric receptor, all the equilibrium constants have been retained; the only change is that the conformational transitions of all three subunits must now occur in unison. This single change suffices to make the transition from the inactive to the active state much steeper and more decisive. A steeper transition is also evident in the receptor saturation curve (right).

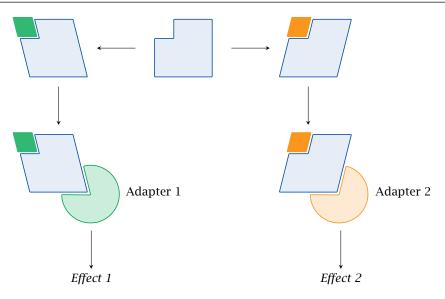
What this amounts to is that cooperative receptors have a better signal-to-noise ratio than monomeric receptors. This is an important advantage in cellular signal processing.

You will note that our hypothetical oligomeric and cooperative receptor assumes only two different functional states; therefore, in an intuitive sense, it still conforms to the two-state model; it deviates only with respect to the mass-action kinetics, which we have somewhat arbitrarily stipulated as an element of the two-state model.

2.5.3 Agonist-specific coupling

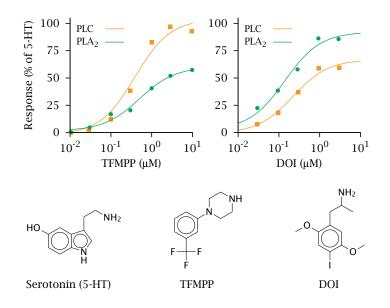
Some receptors couple to more than one adapter protein; the latter can initiate separate signaling cascades inside the cell. With such receptors, it is sometimes observed that a drug activates one of these downstream cascades more strongly than the other. This effect is referred to as *agonist-specific coupling* or *stimulus trafficking*.

To understand agonist-specific coupling, one has to assume the existence of more than one active receptor conformation. Agonist-specific coupling therefore cannot be accounted for within the confines of the two-state model.



2.5.4 Experimental example: Agonist-specific coupling of 5-HT₂ receptors

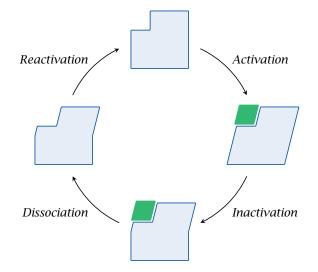
The 5-HT₂ serotonin receptor subtype binds two different G proteins. The first one, $G\alpha_q$, activates phospholipase C (PLC), which releases inositol-triphosphate; the second one, $G\alpha_{12}$, indirectly activates phospholipase A₂ (PLA₂), which releases arachidonic acid.



Two agonists were compared to serotonin with respect to the activation of PLC and of PLA₂. TFMPP (3-Trifluoromethylphenylpiperazine) resembles serotonin with respect to PLC activation, but it activates PLA₂ to a lower degree. Conversely, DOI (1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane) shows the opposite behavior. Figure prepared from original data in [8].

While the selectivity of these two drugs for either downstream signalling pathway is not very strong, more compelling examples exist (see for example slide 7.4.5).

2.5.5 Some receptors have refractory states



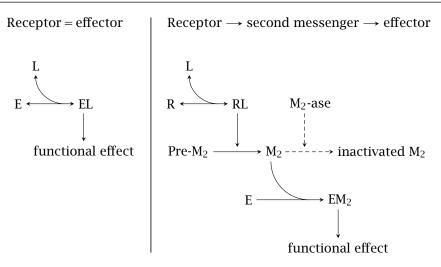
So far, all receptors—whether or not they comply with the two-state model—were assumed to freely go back and forth between active and inactive conformations. However, in some receptors, another functional state exists in which the receptor is functionally inactive and at the same time not amenable to activation.

One receptor that can assume such a *refractory state* is the nicotinic acetylcholine receptor. This receptor, a ligand-gated ion channel, is activated (opened) by binding of acetylcholine. While still bound to the ligand, the receptor transitions from the active (open) state to its refractory state. As in the inactive state, the channel is now closed; however, as long as acetylcholine remains bound, it cannot be activated again directly. Only once acetylcholine dissociates is the receptor free to revert to the proper inactive state, in which it is once more susceptible to activation (see slide 6.10.6).

Obviously, the existence of such a third functional receptor state is incompatible with the two-state model.

2.6 Dose-effect relationships in biochemical cascades

In the nicotinic acetylcholine receptor, it is possible to observe both the binding of a ligand and its functional effect—namely, the conductivity of the closed or open channel—in the same molecule. However, very often, the functional response is observed not in the receptor itself but a good way downstream of it. An example is the contraction of smooth muscle cells induced by norepinephrine (see slide 2.3.4) or by angiotensin (slide 1.2.5). Here, receptor activation triggers a lengthy biochemical cascade that involves phospholipase C activation, calcium release, calmodulin activation, and protein phosphorylation.



If we observe the effect of a drug on its receptor downstream of such a biochemical cascade, the latter can significantly affect the shape of the measured dose-response curves. To better understand this effect, we will consider a simple model cascade, which is shown on the right hand side of this slide. It consists of a receptor R, a second messenger M_2 , and an effector E. The following quantitative assumptions were made:⁸

- 1. Binding of receptor ligand (L) to the receptor, as well as of M₂ to the effector, conforms to mass action kinetics. Additionally, the receptor conforms to the two-state model.
- 2. The strength of the functional effect—that is, the response parameter that is plotted along the γ axis in a dose-response curve—is proportional to the saturation of E with M₂.
- 3. M_2 is inactivated by M_2 -ase, at a rate that is proportional to the concentration of M_2 itself.

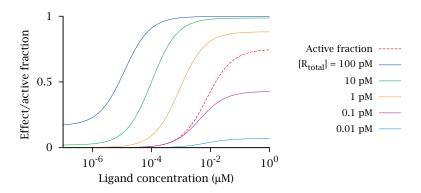
The next slide illustrates the consequences of these assumptions, using some arbitrarily chosen numerical values for the various parameters of the model.

2.6.1 The response of a biochemical cascade depends on receptor density

The curves in this graph characterize the behavior of the hypothetical cascade described in the preceding slide. The only thing that varies between the solid curves is the abundance of receptor; imagine, for example, that the receptor is expressed downstream of an inducible promoter that we can activate with some inducer molecule to different degrees.

The dashed curve shows the active fraction (f_A) of the receptor (R); notice that this *relative* value stays the same regardless of receptor density, even though the *absolute* number of activated receptor molecules does not. The solid curves measure the activity of the effector (E), for different densities of receptor (R_{total}) as indicated. We can make the following observations:

⁸These assumptions follow [9], with the addition of explicit two-state behavior for the receptor.



- 1. The ligand is a partial agonist, since f_A maxes out significantly below 1. As already noted, f_A itself does not change with receptor abundance; therefore, the same f_A curve applies to all of the dose-effect curves.
- 2. The maximal receptor response increases with increasing receptor abundance, ultimately approaching 1. Therefore, the ligand, which remains a partial agonist with respect to the receptor, behaves as a full agonist with respect to the downstream response when the receptor density is sufficiently high.
- 3. At higher receptor densities, the dose-response curve also shifts significantly to the left—the effector is already going strong before the receptor even gets out of bed!
- 4. At the highest receptor abundance, the functional response observed without any ligand also becomes noticeable. This is due to our assumption of receptor two-state behavior; with a large enough number of receptor molecules, the small fraction of spontaneously active ones begins to make itself felt.

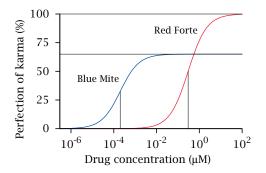
Cascade effects such as the one modeled here are readily observed *in vivo*. For example, the contractile force of the heart can be stimulated through β -adrenergic receptors. A half-maximal response can be obtained with just 2% of receptor saturation, and the response will be virtually maximal long before the receptor becomes saturated [10].

Greater than physiological receptor density is often attained when receptors are expressed in cell culture for *in vitro* studies. We can expect the dose-response curves in such systems to be higher and left-shifted from those that would be observed *in vivo*.

The opposite effect—namely, right-shifted and vertically depressed curves—can be expected when the receptor density is artificially diminished. This is exactly what we noted earlier with the gradual depletion of α -adrenergic receptors with phenoxybenzamine (see slide 2.3.4).

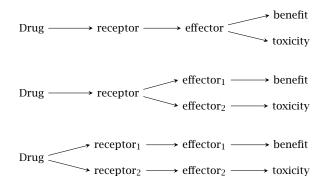
2.7 Potency and efficacy

This slide introduces two basic concepts that are frequently used in the characterization of drug activity. The *efficacy* of a drug is the maximal strength of its effect; therefore, the efficacy of Red forteTM exceeds that of Blue mite[©].



The potency is defined as the inverse of a drug's EC_{50} , which in turn is the concentration at which the drug exhibits 50% of its maximal effect. In our example, the potency of Blue mite[©] exceeds that of Red forte^{\mathbf{M}}.

2.8 Therapeutic and toxic drug effects



Most drugs exercise not only beneficial but also toxic effects. The two effects may be related in different ways. Toxicity may arise as an extension of the beneficial effect and be unseparable from it (top). For example, barbituric acid derivatives exercise a useful sedative effect but become narcotic and finally lethal with increasing dosage; all of these effects arise from activation of the GABA_A receptor in the brain. Such drugs tend to have a low *therapeutic index* or therapeutic range, which means a low ratio of toxic over therapeutic concentration.

At the other end of the scale (bottom), toxicity and benefit arise downstream of entirely separate receptors, both of which are activated by the same drug. In this case, it is desirable to develop a drug with specificity for the receptor that mediates the beneficial effect; we have already discussed the histamine H₂ receptor antagonists as an example.

Finally, it is also possible that benefit and toxicity arise downstream of the same receptor, but that their respective signaling chains diverge at some point upstream of the ultimate effector molecules (middle). In such cases, it may be possible to avoid toxicity by developing drugs that act downstream of the branching point.

An example of this latter case is provided by antagonists of the nicotinic acetylcholine receptor. These drugs reduce blood pressure and have been used in the past to treat hypertension. However, since they inhibit both the sympathetic and the parasympathetic nervous system (see slide 6.13.2), they are prone to side effects. Drugs that act specifically on downstream receptors, such as the adrenergic receptors of the sympathetic nervous system, have better selectivity and fewer side effects.

This figure was adapted from one found in [11].

Chapter 3

Pharmacokinetics

3.1 The scope of pharmacokinetics

Pharmacokinetics deals with the following questions:

- Will the drug reach its intended site of action? If not, can we improve the drug's uptake and distribution to help it reach its target?
- After uptake, how long will the drug stay in the system? How is it eliminated from the system?

As we will see, getting a drug to its target can be a considerable challenge. Many experimental drugs that are viable in principle and which can be shown to work *in vitro* are not useful *in vivo* because of this difficulty.

And no, the latest and greatest nanoparticles aren't going to be the answer to this problem. You may have noticed that most people who flog their revolutionary nanoparticle contraptions for "drug delivery" don't even talk about any specific drugs that they are going to deliver, or to what location exactly. This should tell us something.

3.2 Stages of drug transport

- Absorption: Uptake of the drug from the compartment of application into the blood plasma
- Distribution: Equilibration of the drug between the blood plasma and the rest of the organism
- Elimination: Excretion or metabolic inactivation of the drug

These stages do not occur strictly successively but overlap in time: As soon as some drug molecules have been taken up, they will start to distribute and undergo elimination, even while most others are still waiting for uptake. Moreover, strictly speaking, equilibrium of distribution can be reached only when a drug is administered continuously, *e.g.* by intravenous infusion, which is not usually the case. With these caveats out of the way, we will now consider these stages separately and in turn.

Route	Advantage	Disadvantage	
Oral	Convenience—route of choice where possible	Multiple barriers between in- testine and circulation	
Intravenous	No barriers to absorp- tion	Involved; risk of infection; al- lergic reactions more severe	
Pulmonary	Fast, quantitative uptake	Limited to gases (mostly nar- cotics)	

3.2.1 Absorption depends on the route of application

Oral application of drugs is the most common case, because it is the most convenient for the patients. However, the obstacles to uptake from the gut into the circulation are formidable; we have already seen examples of drugs—remikiren and saralasin, see slide 1.2.6f—that fail entirely to be absorbed after oral application.

The second-most common route of application is intravenous injection or infusion. In this case, the absorption stage is bypassed altogether. This route is used with most protein drugs, as well as with small molecules that fail to be taken up after oral ingestion.

In clinical emergencies, intravenous application is usually preferred even with drugs that are suitable for oral application in principle, in order to ensure their rapid and quantitative uptake.

3.2.2 The need for distribution varies with the location of the drug target

Location of target	Example		
Inside circulation, outside cells	Proteases in blood coagulation and fibrinolysis		
Inside circulation, inside cells	Chemotherapy of malaria parasites		
Outside circulation, on cell sur- faces	Histamine receptors		
Outside circulation, inside cells	Cyclooxygenase		

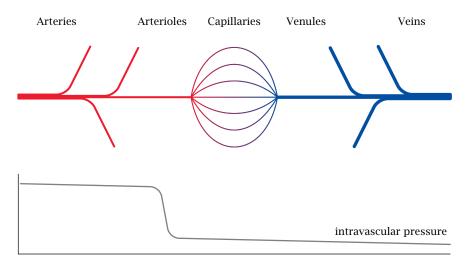
After reaching the circulation, a drug may have to cross fewer or more barriers along the way to its target. In the most favorable case, the targets are located right within the circulation, and not hidden within any cells. This applies to the proteases involved in blood clot formation and dissolution (fibrinolysis), as well as to receptors that are located on the surfaces of thrombocytes or leukocytes.

Targets are less accessible when they are placed outside the circulation, yet less so when placed within cells, and least when placed inside the central nervous system. We will now consider the anatomical reasons for this order of precedence.

3.3 Drug transport across anatomical barriers

Anatomical barriers concern all stages of drug transport. They differ in their active and passive transport properties. Because of its central place in drug transport, the blood circulation and its anatomical properties are particularly pertinent.

3.3.1 Sections of the blood circulation

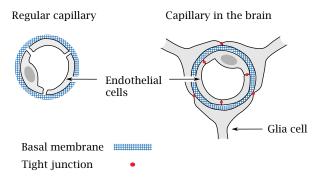


In the general circulation, oxygen-rich blood flows in the arteries. The final branches of the arteries are the *arterioles*. These vessels have the greatest flow resistance, and therefore the intravascular blood pressure, which is high in the arteries, here drops steeply to the much lower level that prevails in the capillaries and veins.

Both the arteries and veins have walls with multiple continuous layers of tissue that have low permeability for gases and metabolites. The exchange of oxygen, carbon dioxide, and other solutes between blood and tissues is mostly confined to the capillaries.

3.3.2 Capillaries as barriers to drug transport

Capillaries are the ubiquitous, tiny blood vessels—just wide enough to permit passage of blood cells in a single file—that mediate the exchange of oxygen, CO_2 and metabolites between the blood within and the tissue without. Unlike the multilayered arteries and veins, a capillary has just a single cell layer, the *endothelium*, which is surrounded by an acellular meshwork composed of glycans and proteins, the *basal membrane*.

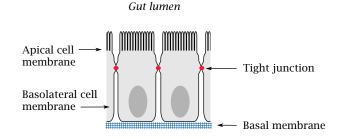


In almost all tissues outside the brain, the capillary walls have a loose, leaky structure, such that there are gaps between, and fenestrations (windows) across the endothelial cells. The only continuous structure that spans these gaps and fenestrations is the basal membrane, which therefore forms the only barrier to substance exchange by diffusion. It acts like a dialysis membrane: molecules smaller than 10 kDa can pass it freely, whereas blood plasma proteins and other macromolecules are retained.

In the brain and the spinal cord, the endothelia of the capillaries don't have fenestrations, and adjacent cells are connected by tight junctions. On the outer side, the basal membrane of each capillary is surrounded by another tightly sealed cellular layer, which is formed by glia cells. Therefore, instead of a single, porous basal membrane, the lumen of the capillary is separated from the surrounding tissue by two continuous cell layers. This structure, which is much less permissive toward substance exchange than an ordinary capillary wall, forms the *blood brain barrier*.

The stacked cell membranes of the blood brain barrier prevent many small molecules from entering the central nervous system. In addition to passively impeding diffusion, the endothelial cells also contain ABC transporters that actively eject many drug molecules from the cytosol right back into the circulation. These transporters further reduce the penetration of drugs into the brain (see slide 3.5.3).

3.3.3 The intestinal epithelium as a barrier to drug absorption



The small intestine is the place in which most orally ingested drugs are taken up. The epithelial cells sit atop a basal membrane and are joined to each other by tight

junctions; therefore, drug molecules (and nutrients) have to be taken up across the cell membranes.

Like the endothelial cells in the blood brain barrier, the intestinal epithelia express ABC transporters, which extrude some solutes from the cells back into the gut lumen. Moreover, these cells also express cytochrome P450 enzymes, which metabolize and inactivate many drug molecules. The role of cytochrome P450 enzymes in drug metabolism is covered in detail in Chapter 4.

3.4 Drug transport across cell membranes

As we have seen, cell membranes restrict drug transport across the intestine and into the brain. In addition, they will also restrict the cellular uptake of drugs that act on intracellular targets.

3.4.1 Mechanisms of solute transport across cell membranes

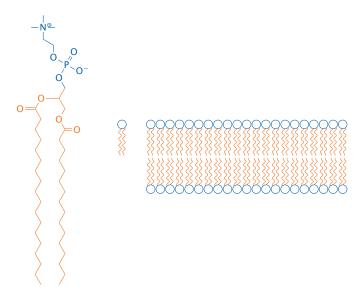
- 1. Active transport
 - (a) Primary: ATP-coupled
 - (b) Secondary: driven by ion gradients
- 2. Passive transport
 - (a) Facilitated diffusion: protein-mediated transport, not coupled to ATP or ion gradients
 - (b) Non-facilitated diffusion of lipophilic compounds; non-ionic diffusion

Transport of types 1a, 1b, and 2a is mediated by transport proteins and is substrate-specific. Some drugs can take advantage of active transport; an example is cytosine arabinoside, which is taken up from the gut by the same transporter that also mediates uptake of cytidine and deoxycytidine (see slide 12.6.7). However, most drugs don't fit the substrate specificities of any transport protein that would aid in uptake and distribution. Therefore, they have to cross cell membranes by non-facilitated diffusion, which occurs directly across the lipid bilayer and is independent of membrane proteins. The efficiency of this mode of transport depends mostly on the drug's physicochemical properties.

3.4.2 Structure of a phosphatidylcholine bilayer

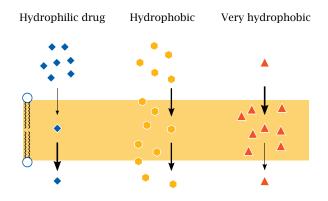
Obviously, cell membranes are more complex than a simple phosphatidylcholine (PC) bilayer; about 50% of a cell membrane consists of various membrane proteins, and the remainder is composed of a fairly complex mixture of lipid molecules. Nevertheless, a simple PC membrane is a useful model for drug transport across cell membranes by passive diffusion.

The solvent interfaces of a PC bilayer consist of the hydrophilic glycerophosphocholine headgroups, whereas the core consists of the aggregated fatty acyl side chains.



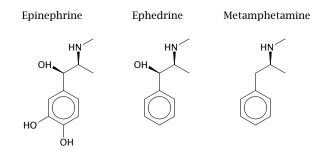
3.4.3 The polarity of drug molecules affects their rate of diffusion across lipid bilayers

The ability of drug molecules to cross a lipid bilayer by passive diffusion correlates with their ability to partition into and out of the hydrophobic membrane interior. A fairly but not excessively hydrophobic character is most conducive to transport; very polar molecules will fail to enter the membrane, whereas extremely hydrophobic molecules will enter readily but may have a hard time leaving it on the other side.



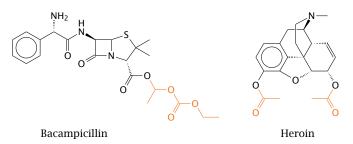
3.4.4 The membrane permeability of drugs can be improved by removing polar functional groups

Removal of charged and polar functional groups can improve the ability of a drug molecule to cross lipid bilayers. For example, ephedrine and metamphetamine are analogues of epinephrine that have shed some hydroxyl groups. Accordingly, they penetrate cell membranes more readily than the parent compound, which promotes their uptake from the intestine and their distribution to the brain.



However, the structural changes also cause a shift in the molecular mode of action. Like epinephrine, both ephedrine and metamphetamine stimulate adrenergic synapses; however, while epinephrine acts directly on adrenergic receptors at the cell surface, ephedrine and metamphetamine act primarily on intracellular targets (see section 6.14.3). This example illustrates that the scope of structural alterations for the sake of improving pharmacokinetic properties is limited.

3.4.5 Resorption esters can improve the diffusion of drugs across membranes



An alternate approach to improving the membrane penetration of drug molecules is to turn them into prodrugs, in which polar or ionizable groups are masked with hydrophobic residues. After uptake, these hydrophobic groups must be able to undergo enzymatic cleavage in order to release the original drug molecules. Most commonly, such functional groups are organic esters.

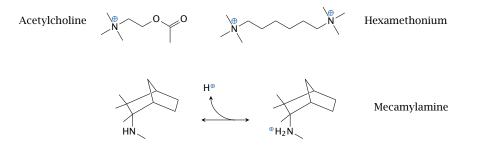
Bacampicillin is such a *resorption ester*; it contains ampicillin, which after uptake into the intestinal epithelium is released by esterases. Heroin is morphine with two acetyl groups added so as to mask two polar hydroxyl groups and facilitate distribution of the drug to the central nervous system. Once there, heroin undergoes cleavage by esterases, which releases the morphine again. In both prodrug molecules, the cleavable ester groups are highlighted.

3.4.6 History of heroin



Heroin is a semisynthetic drug, which is obtained from morphine through acetylation.¹ It was originally intended not as a narcotic drug but rather as an analgesic milder than morphine that would be suitable for everyday use; and as these pictures illustrate, it was indeed marketed for a while in this vein. However, because of its improved penetration into the brain, it is actually stronger and more addictive than morphine.

3.4.7 Ionizable drug molecules may cross bilayers by non-ionic diffusion



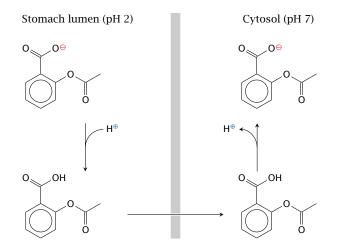
The physiological mediator acetylcholine possesses a quaternary amino group whose positive charge is essential for the interaction with the nicotinic acetylcholine receptor (NAR; see slide 6.10.1).

Hexamethonium is an inhibitor of the NAR. Since it contains two quaternary amino groups, it is transported across membranes very poorly and cannot be orally applied. In contrast, the inhibitor mecamylamine contains a tertiary amine. Therefore, it can cross bilayers in its unprotonated form and then bind a proton to acquire the charge necessary for receptor binding. This drug is amenable to uptake after oral

¹The acetylation reaction is straightforward and is carried out with acetyl chloride or acetic anhydride. Police routinely track shipments of these compounds in order to ferret out illicit heroin-producing labs.

ingestion. The transport across membranes of an ionizable drug in its non-ionized form is called *non-ionic diffusion*.

3.4.8 Gastric acid promotes accumulation of acetylsalicylic acid in the cells of the mucous membrane



Non-ionic diffusion will be affected by pH gradients. The steepest such gradient exists at the surface of the gastric mucous membrane. The high proton concentration inside the stomach lumen can cause accumulation of acetylsalicylic acid inside the gastric epithelial cells and promote gastritis and ulcer formation.

To prevent the accumulation of acetylsalicylic acid in the gastric mucosal cells, the drug can be given an *enteric coating*, that is, be encapsulated in some sort of polymer that resists dissolution at the low pH in the stomach but readily dissolves in the slightly alkaline milieu of the small intestine (see slide 14.4.1).

3.5 Protein-mediated drug transport

Non-specific, passive diffusion is the most common mode of drug transport across cell membranes; nevertheless, many drugs, and in particular drug metabolites, are also subject to protein-mediated active or passive transport. An important class of membrane proteins involved in drug transport are the so-called ABC transporters. The "ABC" in this name stands for "ATP-binding cassette," which denotes a structural motif that is conserved among these proteins. ABC transporters are found in many places, but prominently in the small intestine, the liver, the kidneys, and the blood brain barrier. Typically, they oppose the intracellular accumulation of drug molecules by extruding them back out of the cell, for example into the gut lumen or into the blood stream.

ABC transporters tend to have rather broad substrate specificities, and they can therefore transport/extrude a rather large number of drugs. Overexpression of ABC transporters in tumor cells is an important mechanism of resistance to anticancer drugs. Similarly, ABC transporters in bacteria can cause resistance to antibiotics. Organic cation transporters and organic anion transporters are other major types of membrane proteins involved in drug transport, particularly in the kidneys and the liver. They are not driven by ATP, but some are powered by ion co-transport or antiport; that is, they perform secondary active transport. Like ABC transporters, organic anion and cation transporters tend to have broad substrate specificities.

3.5.1 Inward- and outward-facing conformations of ABC transporters

Bacterial and mammalian ABC transporters share a high degree of structural homology. The left panel of this slide shows the structure of P-glycoprotein in its inward-facing conformation [12]). P-glycoprotein—also called MDR-1, for multi-drug resistance—is a human ABC transporter notorious for its role in the resistance of tumor cells to anticancer drugs.

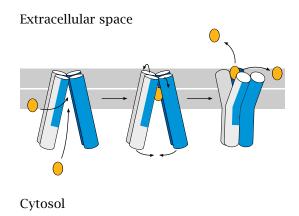
The right panel shows the outward-facing conformation of the bacterial ABC transporter Sav1866 [13]. In each panel, one of the two homologous functional domains (MDR-1) or separate monomers (Sav1866) is rendered in cartoon mode and in blue, while the other is shown in white and in surface representation.

The Sav1866 structure also contains two molecules of a non-hydrolyzable ATP analogue—shown in red—within the ATP binding sites, which are located at the interface of the two subunits.

3.5.2 The functional cycle of ABC transporters

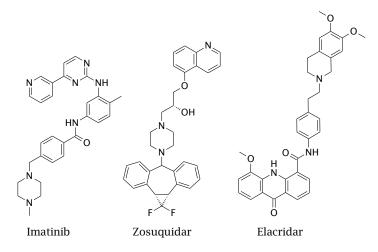
The ABC transporters alternate between the inward- and the outward-facing conformation; the cyclical transition between them is powered by ATP hydrolysis.

ABC transporters are involved in the transport of both drugs or other solutes and of membrane lipids. Accordingly, substrates may be accepted from the cytosol or from the inner leaflet of the cell membrane. The ATP-powered conformational change first sequesters the cargo molecule inside the transporter and then expels it into the outer membrane leaflet or the extracellular space.



3.5.3 ABC transporters and the blood brain barrier (1)

Imatinib is an anticancer drug. Its penetration into the brain across the blood brain barrier is inhibited by ABC transporters, which capture the drug inside the endothelial cells of the capillaries and extrude it back into the circulation.

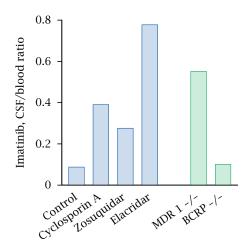


Zosuquidar and elacridar are ABC transporter inhibitors. They have been developed primarily to overcome ABC transporter-mediated drug resistance in cancers. However, they also inhibit ABC transporters in normal cells.

3.5.4 ABC transporters and the blood brain barrier (2)

This experiment illustrates the effect of ABC transporters on the penetration of imatinib through the blood brain barrier. The concentration of imatinib was measured both in the cerebrospinal fluid (CSF) and the blood. A low ratio of CSF to blood concentration indicates a low degree of brain penetration.

When imatinib is applied alone, its concentration in the CSF is only 10% of that in the blood. Penetration of imatinib increases when it is applied together with cyclosporin A—an immunosuppressive drug, which inhibits ABC transporters as a side effect—or with zosuquidar, both of which are known inhibitors of MDR-1. An even stronger effect is observed with elacridar, which inhibits both MDR-1 as well as BCRP, another ABC transporter involved in cancer cell resistance (BCRP stands for "breast cancer resistance protein").



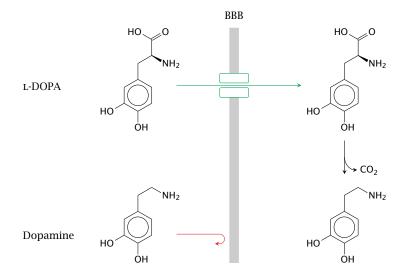
In order to identify the transporters whose inhibition caused the increased brain penetration of imatinib, the drug effects were compared with genetic knock-out of the transport proteins. The effect of knocking out MDR-1 (MDR-1 –/–) is strong enough to account for the action of zosuquidar and cyclosporin A, but it falls short of the inhibition observed with elacridar. Surprisingly, however, a genetic knock-out of BCRP (BCRP –/–) has hardly any effect on imatinib penetration. This suggests that transporters other than MDR-1 and BCRP participate in imatinib extrusion and are inhibited by elacridar. Figure prepared from original data in [14].

Inhibition of ABC transporters also occurs as a side effect of other, more commonly prescribed drugs. In a clinical case report [15], the inhibition of ABC transporters by verapamil, a calcium channel blocker which had been prescribed for a heart ailment, occasioned CNS penetration and toxicity by colchicine, which had been prescribed simultaneously for gout.

3.5.5 L-DOPA reaches the brain by specific transport

Occasionally, active transport can work in favor of drug distribution. An important example is L-DOPA, which is the biosynthetic precursor of the neurotransmitter dopamine. In Parkinson's disease, a specific group of dopamine-producing cells in the brain stem—named the *substantia nigra*—degenerates, and the ensuing lack of dopamine causes the symptoms of the disease.

Parkinson's disease can be treated (not cured) by the replacement of dopamine. However, dopamine itself is not able to cross the blood brain barrier; we therefore need a prodrug. Fortunately, the immediate precursor of dopamine, L-DOPA, structurally resembles the aromatic amino acid tyrosine, and it is transported across the blood brain barrier by the same transporter as is tyrosine.



Once inside the brain, L-DOPA can undergo enzymatic decarboxylation to dopamine. Application of L-DOPA is an important element in the treatment of Parkinson's disease.²

3.6 Anatomy and physiology of drug uptake and distribution

In addition to the general structures of endothelial and epithelial cell layers, some aspects of organ anatomy and physiology are important in controlling the uptake and distribution of drugs.

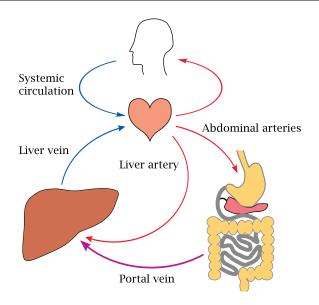
3.6.1 The portal circulation

In most organs, the circulation consists of arteries, which supply oxygen-rich blood from the left heart, the capillaries, which enable gas and substrate exchange, and the veins, which drain the oxygen-depleted blood back to the right heart.

The intestinal organs—that is, the stomach, small and large intestine—as well as the pancreas and spleen also receive arterial blood from the left heart. However, their venous blood is not transported back to the heart directly; instead, it is drained into the portal vein, which then feeds it into the liver. This allows the liver to act as a checkpoint; any solutes that have been taken up in the intestines can be extracted and modified here. Following this liver passage, the blood flows back into the general circulation via the liver vein.

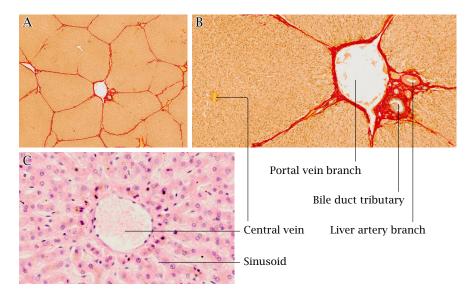
In addition to the already oxygen-depleted blood from the portal vein, the liver also receives a direct supply of oxygen-rich blood via the liver artery.

²The transport protein that takes L-DOPA across the blood brain barrier also transports tryptophan and phenylalanine and is referred to as the L-aromatic amino acid transporter. This same transporter is also involved in the pathogenesis of phenylketonuria (see slide 10.2.2).



3.6.2 Tissue structure of the liver

The liver tissue is organized into lobules that are a few millimeters in diameter (A).³ Each liver lobule receives blood from the terminal branches of both the portal vein and the liver artery (B). The blood leaves these branches and seeps through the hollows (sinusoids) of the liver lobule; it is collected by the central vein (C).



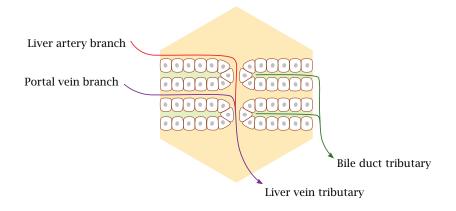
Next to the branches of the portal vein and liver artery, we also find tributaries of the bile duct, which drains the bile produced in the liver lobule toward the small intestine. The very thin bile conduits that originate within the lobule itself are not

³The lobules are separated by thin septa of connective tissue, which are stained bright-red in this tissue sample.

visible in this picture; are so thin that they can only be visualized using special histological stains or by electron microscopy.

Histological slices in A-C reproduced with permission from pathorama.ch.

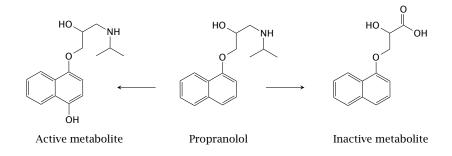
3.6.3 Blood flow and bile flow in the liver lobule



The epithelial cells in each liver lobule are arranged in parallel layers. One side of each cell faces the blood-filled sinusoid, the other faces a bile duct tributary.

The liver cells extract solutes from the blood, modify them, and export them either back into the bloodstream or directly into the bile. This process can be very efficient; with some solutes, extraction and modification are almost complete during a single pass through the liver.

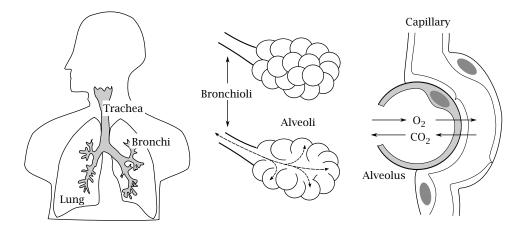
3.6.4 Propranolol and the first-pass effect



Propranolol is a β -adrenergic receptor antagonist propranolol that is used to treat some patients with cardiovascular disease. During its initial passage through the intestine and the liver, it undergoes metabolic transformation to an extent of >50%. The two metabolites shown here are formed by cytochrome P450 enzymes. The metabolite on the left (4-hydroxypropranolol) has a pharmacological activity similar to propranolol; it is an active metabolite. In contrast, naphthyloxylactate (right) is inactive.

While a first pass effect of >50% may seem high, it is not uncommon, and propranolol is indeed nevertheless given orally.

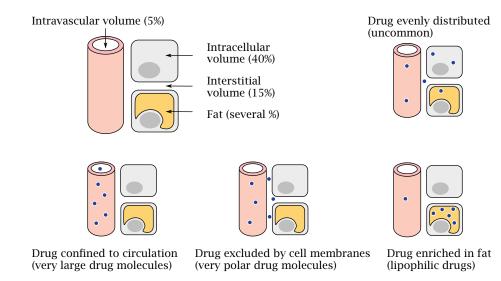
3.6.5 Outline of lung anatomy



For gaseous drugs, most commonly inhalation anesthetics, inhalation is the most straightforward route of application and results in very rapid uptake. The efficiency of uptake is due to the organ structure of the lung.

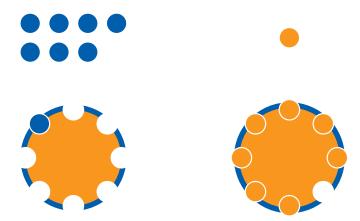
The trachea branches out into bronchi and then bronchioli that open out into alveoli. The alveoli collectively have a very large active surface (~80 m²), and the distance between the air-filled space and the blood in the surrounding capillaries is only a few micrometers. This combination of large area and short distance of diffusion enables a very rapid gas exchange.

3.6.6 Major compartments of drug distribution



This picture summarizes the different compartments between which drugs may distribute. These spaces are not divided according to organs but rather according to tissue architecture. Drugs need to traverse different barriers on their way from one compartment to the other. Very large drug molecules—such as proteins and polysaccharides used as plasma expanders—cannot cross the basal membranes of the capillaries to reach the interstitial space and will remain confined to the circulation; they will therefore occur in the blood plasma at high concentrations. On the other hand, drugs that are small enough to leave the capillaries and apolar enough to easily cross cell membranes will have low plasma concentrations, particularly when they are very lipophilic and accumulate inside fat cells.

One useful, easily measured parameter that characterizes the distribution of a drug is the *volume of distribution* (V_d). It is defined as the ratio of the drug's number of molecules in the body (which is known from the total dosage) divided by the plasma concentration; therefore, it varies inversely with the fraction of the drug that is present in the blood plasma. The name of this parameter only reflects the fact that it has the *physical dimension* of a volume; it is not an actual volume. Indeed, some very lipophilic drugs have V_d values that are many times greater than the actual body volume. More often than not, the volume of distribution is normalized to the body weight and therefore stated in units of l/kg; even though this form no longer has the dimension of a volume, it is still called by the same name.



3.6.7 Hydrophobic drugs tend to bind to proteins

The surfaces of protein molecules are mostly hydrophilic, but they often have hydrophopic patches and crevices that may bind hydrophobic drug molecules. Protein binding can also be mediated by other molecular interactions such as hydrogen bonds and Coulomb forces.

Protein binding is particularly common in blood plasma, which has a very high protein content (~60–80 grams per liter). Two thirds of the total plasma protein is made up by a single protein, namely albumin. Each albumin molecule has eight hydrophobic binding pockets, which in regular physiology serve to transport free fatty acids. These binding pockets also accommodate hydrophobic drug molecules.

Protein binding delays the distribution from the plasma into the tissues. I also slows down filtration in the kidneys (see below), and it thus tends to delay the elimination of drug molecules.

Drug	Structure	V _d (l/kg)	Protein-bound	Oral availability
Ibuprofen	OH OH	0.15	99%	80%
Olanzapine		16.4	93%	60%
Amoxicillin	$HO \xrightarrow{NH_2} H \xrightarrow{N} S \xrightarrow{NH_2} H \mathsf$	0.21	18%	93%

3.6.8 Example drugs and their uptake and distribution parameters

Ibuprofen and olanzapine are fairly hydrophobic, whereas amoxicillin is more hydrophilic. Nevertheless, all three drugs are readily taken up from the intestine; amoxicillin even has the best oral availability.⁴ Note that the oral availability is not only affected by the traversal of the intestinal mucous membrane but also by the hepatic first-pass effect.

Olanzapine has a very high V_d value, suggesting that it accumulates in some sort of sink outside of the circulation, most likely inside fat cells. On the other hand, both ibuprofen and amoxicillin have low V_d values, suggesting that they are largely confined to the extracellular compartment. In the case of ibuprofen, a fairly high blood plasma level is likely caused by strong binding to albumin.⁵

The ineffective penetration of the intracelluar compartment observed with amoxicillin is also observed with many other penicillins and with cephalosporins, and it limits the usefulness of these drugs with bacterial pathogens such as *Listeria monocytogenes* that persist intracellularly.

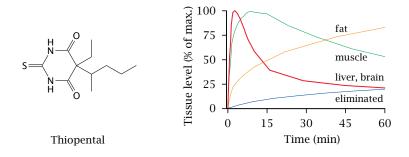
3.6.9 Kinetics of thiopental distribution

In our above treatment of drug distribution, we implicitly assumed that drugs equilibrate readily between the different compartments, and we neglected distinctions between different organs. One of these neglected distinctions between organs concerns their different relative rates of blood perfusion.

With the drug thiopental, the unequal rates of perfusion create a characteristic time course of distribution that is essential for this drug's clinical use, namely the induction of a short-lived state of narcosis.

⁴Amoxicillin is ampicillin with an aromatic hydroxyl group attached. Surprisingly, it has better oral availability than ampicillin. There may be a rational explanation for this, but I have not found it.

⁵As noted above, albumin transports fatty acids. Ibuprofen looks a bit like a fatty acid, and its mode of action involves competing with a fatty acid (arachidonic acid) for binding to the active site of cyclooxygenase (see Chapter 9).



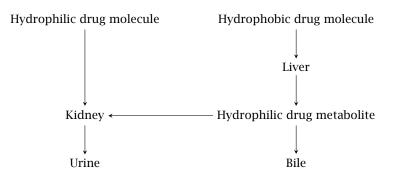
The plot on the right shows the time course of thiopental levels in various tissues after intravenous application. After a very short initial phase of accumulation in the lung (not shown), thiopental redistributes to the next most strongly perfused organs such as the brain and the liver. It is during this phase that the patient, for a few minutes, experiences (or rather, doesn't experience) narcosis.

From the brain, the drug then redistributes to skeletal muscle—which, in a resting patient, is *not* very strongly perfused—and finally to fat tissue, which has the lowest rate of perfusion but accumulates the drug because of its lipophilicity. Ultimate elimination from the body is very slow, but this does not affect the time course of the narcotic effect. Figure prepared from original data in [16].

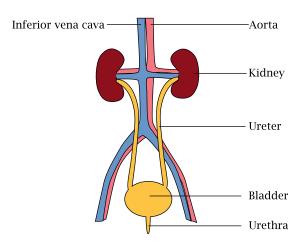
3.7 Kidney function and drug elimination

The kidneys and the liver share in the work of getting rid of drugs and their metabolites. Drug elimination by the kidneys will be covered in this chapter; the role of the liver is considered in the next chapter.

3.7.1 Overview of drug elimination



Very broadly speaking, hydrophilic drug molecules tend to be eliminated via the kidneys directly, whereas hydrophobic drugs require metabolic transformation in the liver before undergoing renal or biliary elimination. There are, however, numerous exceptions to this rule.



3.7.2 Location and perfusion of the kidneys

The kidneys are situated close to two major blood vessels, the aorta and the inferior vena cava. In terms of blood flow per tissue mass, the kidneys are the most strongly perfused organs in the human body. This high rate of blood flow is an important aspect of kidney function.

3.7.3 Overview of kidney function

Urine is "distilled" from blood plasma in several stages:

- 1. Ultrafiltration: 10-20% of the blood plasma volume flow is squeezed out; macromolecules are retained
- 2. Solute reuptake: glucose, salts, amino acids etc. are recovered from the primary filtrate by active transport
- 3. Water reuptake: driven by osmotic gradient
- 4. Solute secretion: some substrates are actively secreted into the nascent urine

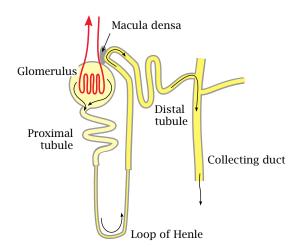
Blood flows through the kidneys at a rate of $\sim 1.2 \, \text{l/min}$, or $\sim 1700 \, \text{l/day}$. A bit more than half of that volume is blood plasma, and if we assume a filtration rate of 15%, we obtain a filtrate volume of approximately $150 \, \text{l/day}$. Obviously, therefore, most of the water and of the solutes contained in the filtrate are reclaimed, and only the leftovers appear in the final urine. On the other hand, some solutes are secreted into the urine only after filtration.

Kidney physiology is fascinating, and I encourage you to read about it in a good physiology textbook. Here, I will not try to give a complete picture, but focus on the aspects that are most relevant to drug elimination.

3.7.4 The nephron

Just as the liver consists of many lobules that each comprise the whole organ function in a small microcosm, the kidney consists of many instances of a repeating

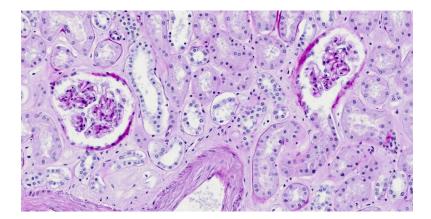
functional unit, the *nephron*, that comprises all stages of urine production. Each of the two kidneys contains about 1.3 million nephrons, all working in parallel.



A nephron consists of a glomerulus and a tubular part that can be divided into the proximal tubule, the loop of Henle, and the distal tubule; the latter joins a collecting duct that drains several nephrons.

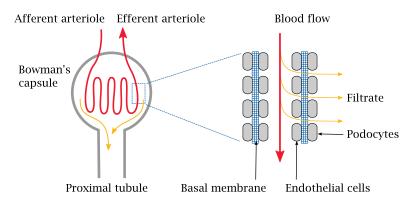
The primary filtrate is formed in the glomerulus. It flows into the proximal tubule, where glucose, amino acids, and most of the salt ions are reclaimed by specific active transporters; water follows by osmosis. More water is reclaimed in Henle's loop. Along this stretch, a very high salt concentration prevails in the interstitial space of the surrounding tissue, which allows the nascent urine to become significantly more concentrated than blood plasma. Fine tuning of concentration, pH, and salt content occurs in the distal tubule.

3.7.5 Cross sections of glomeruli and tubules in kidney



The tissue slice (from pathorama, with permission) shows two glomeruli, surrounded by cross sections of various types of tubules. The sheath containing each glomerulus is known as Bowman's capsule. In the glomerulus on the right, the capsule has been cut away where it opens into the proximal tubule.

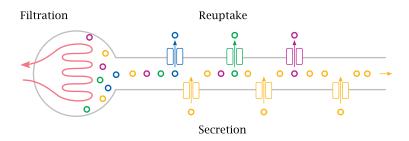
3.7.6 Plasma ultrafiltration in the glomerulus



The glomerulus contains a coiled arteriole, that is, a small artery. Unlike the arterioles in the remainder of the body, the endothelium of the glomerular arterioles has gaps; these line up with gaps in a second cellular layer, formed by the so-called podocytes, that surrounds the outer side of the basal membrane of the arteriole. As with the capillaries in the general circulation, it is the basal membrane that forms the actual molecular sieve in the filtration. The molecular weight cutoff for filtration is similar, too; molecules with less than 10 kDa get across, larger ones are retained. For small molecules that are not retained in the plasma by protein binding, the concentration in the filtrate will be the same as in the plasma.

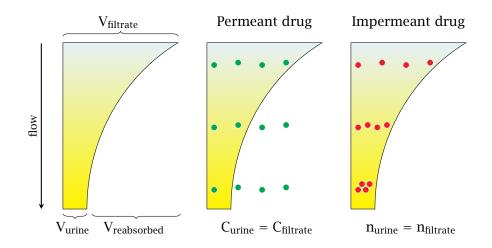
Glomerular filtration is driven by the hydrostatic pressure gradient across the wall of the arteriole. In capillaries, the hydrostatic pressure is fairly low, and since it is almost entirely balanced by the osmotic activity of the plasma proteins, very little net filtration occurs. The pressure within the glomerular arterioles is much higher, and therefore filtration proceeds apace.

3.7.7 Filtration, reuptake, and active secretion



The glomerular filtrate is extensively post-processed as it passes down the tubule. Many solutes are recovered from the filtrate, whereas some other solutes are secreted into the nascent urine by specific active transport.

Drugs can be substrates at all three stages; that is, they can be subject to filtration, reuptake, and secretion.



3.7.8 A drug's rate of urinary excretion depends on its membrane permeability

As we had seen before, most of the water is recovered from the primary filtrate as it passes down the tubules. As the volume of the filtrate diminishes, the concentration of a drug contained in it increases, creating a gradient between the tubule and the surrounding interstitial space. Whether or not (or the extent to which) the drug will travel downhill this concentration gradient, across the tubular epithelium, depends on its membrane permeability.

The slide depicts the two limiting cases. A fully membrane-permeant drug may freely equilibrate between the tubule and the surrounding interstitial fluid. Its concentration in the urine will therefore be the same as in the interstitial fluid, and the rate of its urinary excretion will be determined simply by the product of this concentration and the urine volume flow.⁶

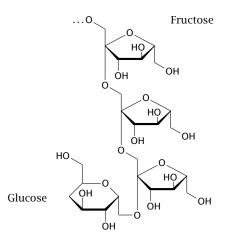
On the other hand, a completely impermeant drug will be quantitatively retained in the nascent urine. Therefore, the absolute number of drug molecules remains the same between the primary filtrate and the final urine, and the urine concentration exceeds the filtrate concentration in inverse proportion to the volume fraction of the filtrate that makes it to the final urine. Furthermore, the rate of elimination will depend only on the rate of glomerular filtration, but not on the urine volume flow rate.

We had previously encountered the principle of non-ionic solute diffusion across lipid bilayers (see slide 3.4.7). We can use this principle to advantage in order to accelerate the renal elimination of certain drugs. Apart from xenobiotics and metabolic end products, the kidneys also eliminate excess acid or base. Through the application of (buffered) acid or base, we can therefore induce the kidneys to lower or raise the urine pH. This can for example be used in phenobarbital poisoning. This drug (see structure on slide 4.1.4) is a weak acid. To accelerate its elimination, we can raise the urine pH using sodium bicarbonate infusions. This will cause

⁶A somewhat old-fashioned method to accelerate the excretion of such compounds is *osmotic diuresis*, that is, the application of an osmolyte such as mannitol that is quantitatively filtrated and retained in the urine, and thus increases the urine volume due to its osmotic effect.

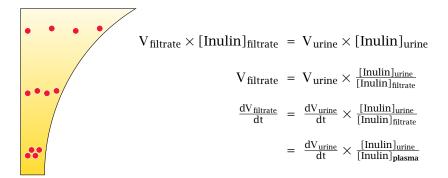
phenobarbital in the urine to be deprotonated, which will inhibit its escape by nonionic diffusion and thereby promote its excretion [17].⁷

3.7.9 Inulin, a model compound that is quantitatively filtrated and retained in the urine



Inulin is a polysaccharide that is small enough to undergo quantitative filtration in the glomeruli. It is also polar enough to avoid protein binding and reuptake by diffusion across bilayers; therefore, it represents the second limiting case presented in slide 3.7.8). Based on these properties, inulin can be used to non-invasively measure the volume flow rate of glomerular filtration.

3.7.10 The volume flow of glomerular filtration can be measured from the renal clearance of inulin



This slide shows what parameters we require in order to measure the glomerular filtration rate (GFR; $dV_{filtrate}/dt$). The flow rate of the urine can be obtained simply by collecting the urine for 24 hours. The concentrations of inulin in the urine and in the filtrate and the blood plasma are obtained with chemical methods. This

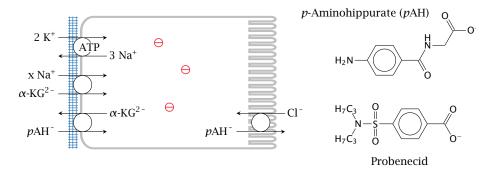
⁷An even faster way to eliminate phenobarbital is the oral application of copious amounts of charcoal, also described in [17], which works by "reverse absorption" into the intestines.

analysis assumes steady state for the inulin plasma concentration, which can be established by continuous infusion. Since inulin is quantitatively filtrated, we can equate the experimentally determined plasma concentration with the concentration in the filtrate.

The last term in the last equation—urine volume flow times urine concentration over plasma concentration—is referred to as the *renal clearance* of a compound. The inulin clearance equals the GFR because inulin is quantitatively filtrated but not subject to tubular secretion or reuptake. Most other compounds don't meet all of these criteria, and thus their renal clearances do *not* equal the GFR. Nevertheless, the GFR is a major determinant in the elimination of many drugs, and it therefore is of considerable interest in pharmacokinetics and pharmacotherapy.

In clinical practice, the GFR is measured not with inulin but with the endogenous metabolite creatinine. This metabolite is formed at a fairly constant rate in skeletal muscle, obviating the need to administer an infusion. While the behavior of creatinine is not as "ideal" as that of inulin, the creatinine clearance provides an estimate for the GFR that is good enough for the practical need of adjusting dosage regimens for drugs that undergo renal elimination to the kidney function of individual patients.

3.7.11 Tubular secretion of *p*-aminohippurate



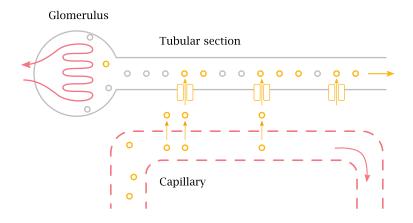
A model compound for studying the tubular secretion of drugs is *p*-aminohippurate (*p*-AH). Import of *p*-aminohippurate from the interstitial space, across the basolateral membrane of the cells of the tubular epithelium, is driven by exchange for α -ketoglutarate (α -KG). The required α -ketoglutarate gradient is maintained by Na⁺-symport, and the Na⁺ gradient in turn is maintained by Na⁺/K⁺-ATPase. The excretion of *p*-aminohippurate across the apical membrane into the tubular lumen is driven by antiport with Cl⁻ or HCO₃⁻. This step can be inhibited by the drug probenecid.⁸

The free energy of solute transport across the basolateral membrane is affected not only by concentration gradients but also by the negative-inside membrane po-

⁸Probenecid was developed to slow down the elimination of penicillin, which is eliminated in the same way as p-aminohippurate, though not quite at the same rate.

tential, which makes the exchange of the singly negative *p*-aminohippurate for the doubly negative α -ketoglutarate more exergonic.

3.7.12 Tubular secretion of *p*-aminohippurate is almost quantitative



In each nephron, blood vessels run alongside the tubular segments to facilitate the efficient transfer of solutes from the blood to the nascent urine. The solutes permeate out of the capillaries and are then picked up by transport proteins that eject them into the tubules.

In the case of *p*-aminohippurate, the transport rate is so high that $\ge 90\%$ of the compound is extracted from the blood and transferred to the urine in a single passage of the blood through the kidney.

3.7.13 The *p*-aminohippurate clearance measures the renal plasma flow

$$\frac{\mathrm{dn}_{p\text{-AH, plasma}}}{\mathrm{dt}} \approx \frac{\mathrm{dn}_{p\text{-AH, urine}}}{\mathrm{dt}}$$

$$\mathbf{n}_{p\text{-AH}} = [p\text{-AH}] \times \mathbf{V}$$

$$\frac{\mathrm{dV}_{\text{plasma}}}{\mathrm{dt}} \times [p\text{-AH}]_{\text{plasma}} \approx \frac{\mathrm{dV}_{\text{urine}}}{\mathrm{dt}} \times [p\text{-AH}]_{\text{urine}}$$

$$\frac{\mathrm{dV}_{\text{plasma}}}{\mathrm{dt}} \approx \frac{\mathrm{dV}_{\text{urine}}}{\mathrm{dt}} \times \frac{[p\text{-AH}]_{\text{urine}}}{[p\text{-AH}]_{\text{plasma}}}$$

Since *p*-aminohippurate is almost quantitatively extracted from the plasma, we can use its clearance to measure the renal plasma flow. The first equation simply states that, over time and at steady state, the number of *p*-aminohippurate molecules appearing in the urine is approximately equal to the number of molecules carried to the kidneys with the plasma flow. The second equation expands the number of solute molecules into the product of concentration and volume, and the third equation substitutes both terms in the first equation according to the second. The

fourth equation is obtained by rearranging the third; it tells us that the renal plasma flow is approximated by the *p*-aminohippurate clearance.

The practical side of determining the plasma flow from the *p*-aminohippurate clearance is analogous to the use of inulin clearance to measure the filtrate flow: Steady state is established using a continuous infusion of *p*-aminohippurate, urine is collected, and plasma and urine concentrations are measured.

3.8 Non-equilibrium kinetics of drug elimination

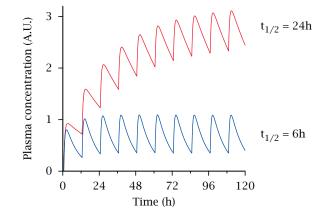
 $n = [D]_{\text{plasma}} \times V_{\text{d}} \times \text{body weight}$ $\frac{\text{dn}}{\text{dt}} = -k \times [D]_{\text{plasma}}$ $\frac{[D]_{\text{plasma,t}}}{[D]_{\text{plasma,0}}} = e^{-\frac{k}{V_{\text{d}} \times \text{body weight}}t}$ $0.5 = e^{-\frac{k}{V_{\text{d}} \times \text{body weight}}t_{1/2}}$ $t_{1/2} = \ln 2 \times \frac{V_{\text{d}} \times \text{body weight}}{k}$

The elimination rates of inulin and *p*-aminohippurate were examined at steady state; however, a practically more important case is the elimination of a drug that is applied only once or intermittently. In the simplest case, elimination then follows first-order kinetics. This case is also very common in practice.

The first equation simply states the number of drug molecules in the body; V_d is the volume of distribution in units of liters per kilogram of body weight. The second equation postulates first order kinetics, that is, it states that the rate of elimination is proportional to the plasma concentration. The kinetic constant k is the big unknown here: factors like protein binding, which prevents filtration, tubular secretion, and membrane permeability, which promotes nonspecific reuptake, will all be comprised in this rate constant.

The third equation is obtained by integration of the second and substitution using the first, and the fourth one by taking the logarithm; $t_{1/2}$ is the half-life of elimination. While this does not get rid of k, the big unknown, it is interesting to note that the half-life of elimination increases with the volume of distribution. This makes sense: filtration or tubular secretion can only operate on the fraction of the drug that currently resides in the circulation. A high V_d implies that this circulating fraction is small, which slows down elimination.

Note that our equations make no assumptions regarding the mechanism of the elimination—it might be filtration, secretion, even metabolic transformation in the liver, or some combination of the above. Indeed, the concepts of clearance and half-life are useful with any drug, regardless of its route of excretion.



3.8.1 Repeated drug application can result in accumulation

In practical pharmacotherapy, we typically want to keep the concentration of a drug within its therapeutic range throughout, which means that we have to repeat its application before the last dosage has been completely eliminated. Such repeated application will cause accumulation, the extent of which will depend on the dosage interval and the drug's half-life of elimination. In the slide, this is illustrated for two hypothetical drugs, which have half-lives of 6 hours and of 24 hours, respectively. With both drugs, application was assumed to occur in intervals of 12 hours, and absorption with a half life of 0.5 hours. It is clear that, all else being equal, a longer half-life of elimination gives rise to a greater extent of accumulation.

Accumulation through repeated application needs to be taken into account with drugs that have a small therapeutic range. One way to deal with this is to use a larger initial loading dose that brings the concentration to the therapeutic level straight away, followed by smaller repeating doses that are calculated to maintain the level established by the initial one. While this approach is logical in principle, I have rarely seen it used in practice.

Chapter 4

Drug metabolism

4.1 Overview

In the last chapter, we considered the renal elimination of drugs. While some drugs are eliminated via the kidneys unchanged, most of them undergo metabolic transformation at least in part before being excreted.

4.1.1 Types of reactions in drug metabolism

- 1. Oxidation
- 2. Conjugation
- 3. Reduction
- 4. Hydrolysis

These categories of metabolic transformations are very broad; for each category, there is a multitude of enzymes and specific reactions. We have already seen examples of drug metabolism by hydrolysis, which is exploited in the design of resorption esters (see slide 3.4.5). As an example of reductive metabolism, we have seen the activation of sulfamidochrysoidine via azoreduction (see slide 1.3.3). As we will see in this chapter, oxidative metabolism and conjugation reactions are even more common.

4.1.2 Functional outcomes of drug metabolism

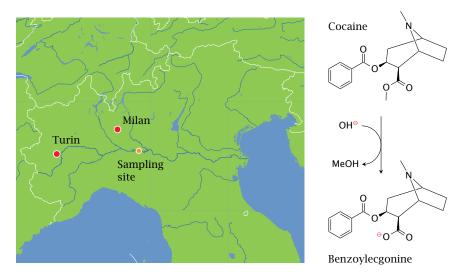
- 1. Inactivation and accelerated elimination of drugs
- 2. Activation of prodrugs
- 3. Formation of active metabolites with similar or novel activity
- 4. Detoxification of toxic xenobiotics
- 5. *Toxification* of non-toxic xenobiotics

Drugs are a subset of *xenobiotics*, that is, exogenous substances. Examples of xenobiotics other than drugs are plant alkaloids or fungal toxins. The metabolic pathways and enzymes that have evolved to dispose of these natural xenobiotics have broad substrate specificity, and they are therefore active on many synthetic drugs also.

In most cases, drug metabolism results in inactivation and accelerated elimination of drugs from the body. However, as this list indicates, other outcomes of metabolism are possible; we will see examples below.

In the list above, outcomes 2. and 3. both yield active products. The difference between them is that, in 3., both the original drug and the metabolite have pharmacological activity. In contrast, a prodrug has no activity before undergoing metabolic conversion.

4.1.3 A hydrolytic metabolite of cocaine can be detected in wastewater



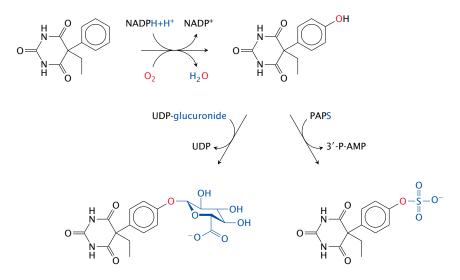
Cocaine is inactivated by hydrolysis. The metabolite, benzoylecgonine, is excreted with the urine. It is fairly stable and thus can be detected in wastewater.

A study from Italy [18] analyzed water samples from the river Po, which (together with the flow rate of the river) were used to estimate the consumption of cocaine upstream of the sampling site. The samples were taken at a point downstream of Turin and, in part, of Milan, two of Italy's four largest cities. Cocaine consumption in this area was found to be 4–5 times higher than had previously been estimated. Similar findings have since been reported from other European countries.

4.1.4 Metabolism of phenobarbital

Phenobarbital is a barbituric acid derivative. It is an agonist at the GABA_A receptor (see slide 6.10.8) that is sleep-inducing and antiepileptic. The drug molecule itself is quite hydrophobic; this enables it to penetrate the blood brain barrier, and it also

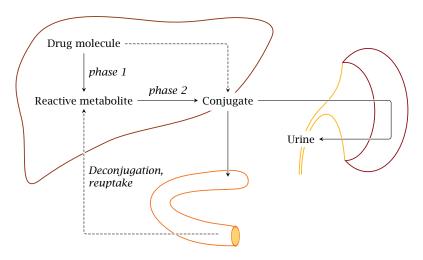
slows down renal elimination. Therefore, 75% of the compound is excreted in the form of conjugated metabolites.



Phenobarbital as such, however, is not a good substrate for conjugation reactions. To make it amenable to conjugation, a suitable functional group—an aromatic hydroxyl group—is first introduced by a cytochrome P450 enzyme. Aromatic or aliphatic hydroxylations by such enzymes are very common *phase I* reactions.

In *phase II* of phenobarbital metabolism, the hydroxyl group just introduced is conjugated either with glucuronic acid by a UDP-glucuronosyltransferase, or with sulfate by a sulfotransferase. Both of these metabolites are fairly polar and are effectively excreted through the kidneys.

4.1.5 With many drugs, metabolic transformation facilitates excretion

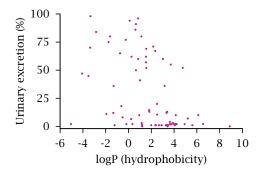


The metabolism of phenobarbital illustrates a pattern that applies to many other drugs as well. With many drugs, hydroxylation by cytochrome P450 or some other phase I reaction precedes conjugation with glucuronic acid, glutathione, or another

functional group. These conjugations are collectively referred to as phase II reactions. Excretion of conjugated drugs is sometimes referred to as phase III of drug elimination.

The most important organ in drug biotransformation is the liver. The conjugated drugs may be exported into the blood stream and be eliminated in the kidneys, or they may be excreted into the bile and then reach the intestine. In the latter case, they may undergo deconjugation by bacterial enzymes in the large intestine and subsequent reuptake. Reabsorbed drugs will be transported to the liver via the portal vein and may undergo another round of conjugation and biliary excretion; this is called entero-hepatic cycling.

4.1.6 Hydrophobicity does not strongly predict the extent of metabolism



This scatter plot of a randomly selected sample of 75 drugs (data from [19]) correlates the percentage of a drug that appears in the urine in unchanged form to the estimated logP value. The logP values is the logarithm of the octanol/water partitioning coefficient; therefore, drugs with high logP values are hydrophobic.

There may be a very slight trend for hydrophobic drugs to undergo renal elimination to lesser extent; however, regardless of logP, only very few drugs are quantitatively eliminated through the kidneys. In most cases, the remainder will undergo metabolic transformation, although a few drugs may be excreted into the bile unchanged.

So, the key point of this slide is not in the correlation of the two parameters, but rather in the fact that most drugs undergo metabolic transformation at least in part; and contrary to lore, this applies to both hydrophilic and hydrophobic drugs.

4.1.7 Major types of drug-metabolizing enzymes

Phase I

- Cytochrome P450 enzymes
- Diaphorase (NADH:quinone oxidoreductase)

Phase II

• UDP-glucuronosyltransferases

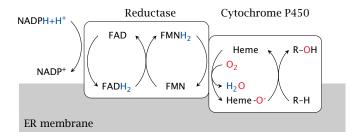
- Sulfotransferases
- Glutathione-S-transferases
- N- and O-acetyltransferases

Cytochrome P450 enzymes are the most important class of enzymes in phase I metabolism. Other oxidative enzymes in drug metabolism include flavin monooxygenase and monoamine oxidase. Reductive metabolism is carried out by various enzymes; diaphorase, also called quinone reductase, is a major one.

In addition to glucuronosyl- and sulfotransferases, glutathione-*S*-transferases and acetyltransferases also contribute significantly to phase II metabolism. Methyl conjugation and amino acid conjugation are less common.

While the number of enzymes involved in the degradation of xenobiotics is large, it is necessarily far smaller than the virtually limitless number of potential substrates. Therefore, many of these enzymes must be able to modify a large number of structurally diverse drugs and xenobiotics. The same also applies to transporters involved in phase III, that is, biliary and urinary excretion.

4.2 Cytochrome P450 enzymes



Cytochrome P450 isoforms are associated with the membrane of the endoplasmic reticulum (mostly) or the mitochondria (some). Membrane association facilitates interfacial processing of lipophilic substrates that often reside in the apolar phase of the membrane.

Cytochrome P450 enzymes cooperate with cytochrome P450 reductase, which acquires two electrons from NADPH and, via two flavin coenzymes,¹ passes them on one by one to the heme group in the cytochrome. Thereafter, they are used to reduce one of the oxygen atoms of O_2 to water. The second oxygen atom is retained in a highly reactive form, and subsequently carries out the actual attack on the substrate.

¹You may recall that nicotinamide cosubstrates (NAD and NADP) can only accept or donate electron pairwise, whereas flavin coenzymes (FAD and FMN) can accept or donate them both singly and in pairs. Flavins therefore can mediate between NAD or NADP and iron-containing hemes or iron-sulfur clusters that can only accept single electrons. In addition to cytochrome P450 reductase, we find this arrangement also in nitric oxide synthase and in the mitochondrial respiratory chain.

R

4.2.1 Reactions catalyzed by cytochrome P450

Halfway through the reaction, the active site of cytochrome P450 contains a single oxygen atom in a loosely bound, highly reactive state. This oxygen atom can react with many different functional groups, and in various ways, as summarized on this slide. The particular reaction that occurs with a given substrate depends more on which of its functional groups happens to be the nearest to the oxygen, rather than on which one might be the most reactive itself.

The next slide presents some examples of drugs that undergo one or the other of the cytochrome P450-mediated oxidative reactions shown here. In those examples, the reaction products also happen to be active metabolites; this, however, is not always the case.

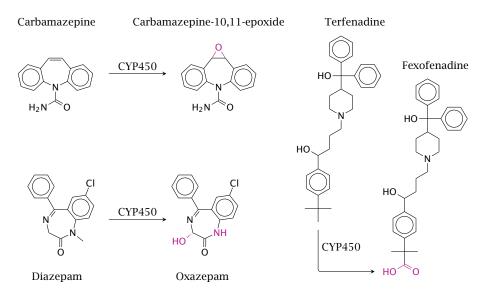
4.2.2 Formation of active metabolites by CYP450 enzymes

Diazepam is an agonist of the GABA_A receptor in the brain (see slide 6.10.8) that is used as a tranquilizer and in some forms of epilepsy. Both the hydroxylation and the demethylation of diazepam are performed by cytochrome P450 enzymes. The resulting metabolite, oxazepam, is also used as a drug in its own right. Since it no longer has to undergo phase I metabolism, it is conjugated and eliminated more rapidly than is diazepam, which is advantageous for example when used as a sleeping aid.

Carbamazepine blocks sodium channels (slide 6.5.4); this is useful in epilepsy and several other neurological conditions. The epoxide metabolite of carbamazepine significantly contributes to the pharmacological activity, but also to the toxicity, of carbamazepine. Like other epoxides, carbamazepine-10,11-epoxide may wreak havoc by reacting covalently with intracellular nucleophiles.

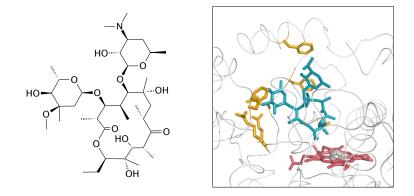
Fexofenadine and terfenadine are histamine H₁ receptor blockers. Fexofenadine was originally observed as an active metabolite of terfenadine. The metabolite has since supplanted the original drug, because it avoids some toxic side effects on

cardiac excitation that may occur when the parent compound accumulates due to inhibition of cytochrome P450-mediated metabolism. Such inhibition can arise from drugs such as ketoconazole (see below) but also from regular foods such as grapefruit [20].



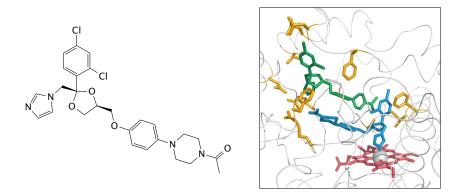
The metabolism of terfenadine also illustrates that it is possible for one substrate molecule to undergo several rounds of oxidation—in this case, all the way from a methyl to a carboxyl group—that will most likely occur in one sitting.

4.2.3 Erythromycin bound to the active site of cytochrome P450



This slide and the following ones illustrate the promiscuous substrate binding by cytochrome P450, subtype 3A4. The enzyme was crystallized in the presence of erythromycin or of ketoconazole, respectively. Erythromycin is shown in this figure, ketoconazole in the next one. Erythromycin is shown in blue, heme in red, and amino acid side chains in close contact with the drug are shown in yellow. Figures rendered from 2j0d.pdb and 2v0m.pdb [21].

4.2.4 Ketoconazole bound to the active site of cytochrome P450



In this structure, two molecules of the antifungal drug ketoconazole (shown in blue and green) are bound in the active site; the orientation of the upper one is similar to that of the structural formula.

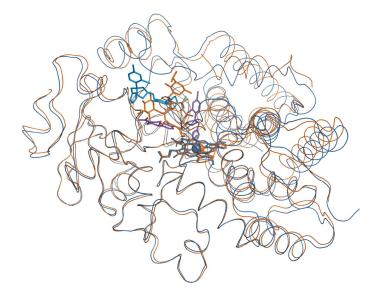
The binding of two drug molecules to one enzyme molecule is likely caused by the high concentrations used in the crystallography experiment; *in vivo*, only one molecule would likely be bound. Nevertheless, this structure does illustrate the enzyme's ability to accommodate substrates with rather diverse structures.

Note the participation of different side chains in binding erythromycin and ketoconazole, and also the direct association of the ketoconazole molecule shown in blue with the catalytic heme. Ketoconazole is not only a substrate but also an inhibitor of cytochrome P450; this inhibitory action can cause side effects in humans but is also responsible for the antifungal activity of the drug (see Section 11.8.2).

4.2.5 Superposition of the erythromycin- and the ketoconazole-bound CYP3A4 structures

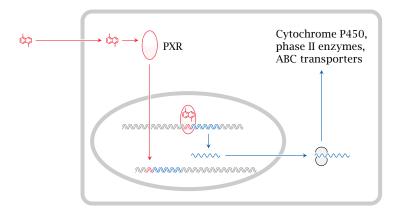
The erythromycin-bound structure is shown in orange, the ketoconazole-associated one in blue. The polypeptide backbones track each other for the most part but diverge noticeably in several places, particularly atop the active site. These local deviations illustrate the conformational flexibility of the enzyme that allows it to accommodate many different substrates.

Cytochrome P450 3A4 (CYP3A4), the subtype shown in these two structures, is involved in the metabolism of as many as 50% of all clinically applied drugs. While this observation also suggests an astonishingly broad substrate specificity, another reason for the prominent role of CYP3A4 in drug metabolism is its upregulation in the presence of drugs, which is mediated by transcriptional induction.



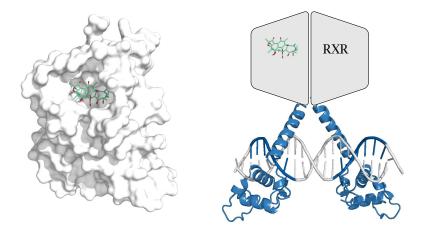
4.3 Transcriptional induction of drug metabolism

The transcriptional induction of CYP3A4 and several other drug-metabolizing enzymes and drug-excreting transporters is mediated by the pregnane X receptor (PXR). This receptor belongs to the family of nuclear hormone receptors, which also comprises the receptors for thyroid and steroid hormones (see chapter 7). The PXR binds to a wide range of drugs. Upon drug binding, the receptor translocates from the cytosol to the nucleus and binds to its cognate regulatory DNA sequences, named xenobiotic response elements (XRE), and thereby causes increased transcription of genes in the vicinity.



Induction of drug-metabolizing enzymes often leads to accelerated metabolism of multiple drugs, not just the inducing drug itself. For example, rifampicin, an antibiotic used in tuberculosis, or phenytoin and phenobarbital, which are used as anti-epileptic agents, all induce accelerated inactivation of each other and of contraceptive agents. Therefore, oral contraceptives may be rendered ineffective in patients being treated with such transcriptional inducers. This cartoon is criminally simplified, neglecting a host of other interacting and regulatory proteins, but then this is no class (and I'm no expert) on transcriptional regulation.

4.3.1 Transcriptional induction of cytochrome P450: Rifampicin bound to the pregnane X receptor



Complete structures of nuclear hormone receptors are apparently not yet available. The structure on the left [22] illustrates rifampicin bound to the drug-binding domain of the pregnane X receptor.²

The ligand-bound PXR forms a heterodimer with another nuclear hormone receptor, the retinoid X receptor (RXR) before binding to the DNA. The partial structure on the right shows the DNA-binding domains of a similar receptor dimer, along with outlines of the two ligand-binding domains. The interaction of a nuclear hormone receptor with DNA is illustrated in slide 7.3.1.

4.4 Conjugation reactions

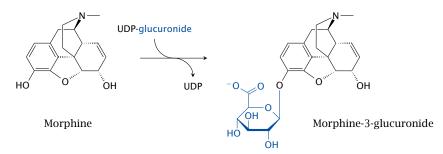
Phase II metabolism consists of conjugation reactions. Some of these reactions have already been illustrated; others will be shown below.

²The ligand does not seem to be rifampicin itself but a similar compound, although it is referred to as rifampicin in the pdb file.

Functional group	Enzyme	Cosubstrate
Glucuronic acid	UDP-glucuronosyl- transferases	UDP-glucuronide
Sulfate	Sulfotransferases	3'-Phosphoadenosine-5'- phosphosulfate (PAPS)
Glutathione	Glutathione- <i>S-</i> transferases / spon- taneous	Free glutathione
Acetate	N-acetyltransferases	Acetyl-CoA
Methyl	<i>N</i> -, <i>S</i> -, and <i>O</i> -methyl- transferases	<i>S</i> -adenosylmethionine (SAM)
Amino acids	Amino acid trans- ferases	Free amino acids/ATP

4.4.1 Morphine skips phase I and is conjugated directly

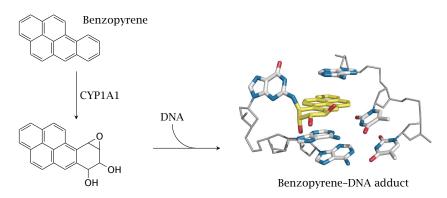
Phase I reactions are not necessary if a drug molecule already contains functional groups suitable for conjugation. An example is morphine, which has two hydroxyl groups. The conjugation of either, or both, with glucuronic acid is sufficient for excretion. The single glucuronide shown in this slide is the major metabolite found in the urine. Interestingly, the other single glucuronide retains pharmacological activity; therefore, even drug conjugates may be active metabolites, although this is less common than with the often more limited modifications introduced in phase I (cf. slide 4.2.2).



In addition to conjugation, morphine may also undergo *N*-demethylation by cytochrome P450; however, this makes little difference for urinary excretion.

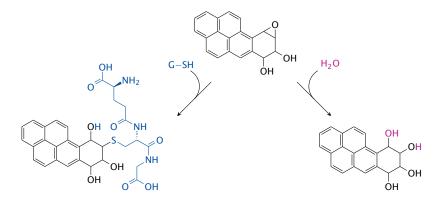
4.4.2 Epoxides of aromatic hydrocarbons can intercalate and covalently react with DNA

Benzopyrene and related aromatic compounds are contained in tobacco smoke and in industrial emissions. Their activation by cytochrome P450 enzymes, notably subtype 1A1, is an essential step in their carcinogenic action. The study that reported the structure of the DNA adduct shown here, which involves the epoxide on the benzopyrene metabolite and the 2'-amino group of a guanine base, also discusses how this adduct interacts with DNA polymerase to induce mutations [23].



Like CYP3A4, CYP1A1 is also subject to transcriptional induction, but this occurs downstream of a different nuclear receptor (the aromatic hydrocarbon receptor, AHR).

4.4.3 Enzymatic detoxification of benzopyrene epoxy-derivatives



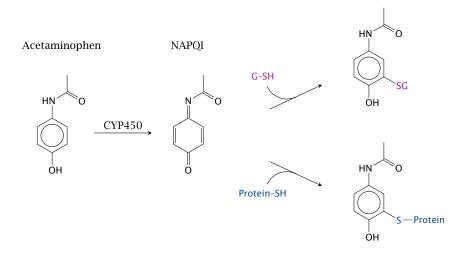
While reactive benzopyrene metabolites are harmful, most such molecules will actually be captured and inactivated before reacting with DNA—otherwise, famous smokers like Winston Churchill and Deng Xiaoping might not have lived long enough to become famous ...

The major detoxification pathways are hydrolysis, which is catalyzed by epoxide hydrolase, and glutathione conjugation, which is catalyzed by glutathione-*S*transferase (GST).

4.4.4 Hepatic metabolism of acetaminophen

A cytochrome P450-catalyzed oxidative reaction that we haven't seen so far is the conversion of aromatic rings to quinones. This is exemplified by acetaminophen, which is converted to *N*-acetyl-*p*-benzoquinone imine (NAPQI). This metabolite is

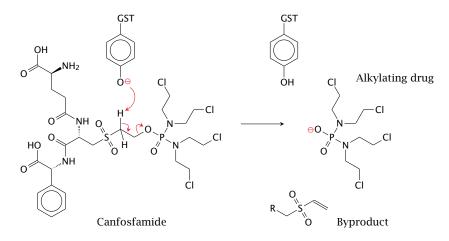
readily conjugated with glutathione and excreted. If acetaminophen is overdosed, this metabolic pathway can deplete glutathione in the liver cells and thereby cause severe, even fatal, liver toxicity.³



Acetaminophen is used for its inhibitory effect on cyclooxygenase. NAPQI is also formed in the reaction of acetaminophen with cyclooxygenase, and this forms the basis of its inhibitory activity (see slide 9.3.4).

As an alternative to the pathway shown here, acetaminophen can also undergo direct glucuronidation or sulfation at its hydroxyl group [24]. These two competing pathways will mitigate the cytochrome P450-mediated toxicity. The glucuronide is actually the most abundant metabolite found in the urine.

4.4.5 Activation of canfosfamide by glutathione-S-transferase



³Interestingly, the major cytochrome P450 isoform responsible for acetaminophen oxidation is 2E1, which is induced by ethanol and acetone and also degrades both these inducers. Also note that, as an alternate route to the pathway shown here, acetaminophen can be conjugated directly with glucuronic acid or sulfate.

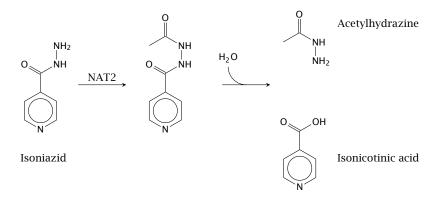
Glutathione reacts readily not only with epoxides but with many other cytotoxic compounds that are reactive toward cellular nucleophiles. Many such compounds are used as anticancer drugs; some examples are shown in slide 12.6.16. During treatment, cancer cells may become resistant to these drugs through mutations that increase the expression of glutathione-*S*-transferase (GST).

The anticancer drug canfosfamide was designed to turn this resistance mechanism on its head. The molecule, which contains a moiety that resembles glutathione, is cleaved rather than alkylated by GST. It is activated by this cleavage, and it should therefore be more active in tumor cells that overexpress the enzyme.

Canfosfamide is cytotoxic due to its reactive chloroethylamine groups; slide 2.3.5 shows how these groups will react with cellular nucleophiles. These groups are already present in canfosfamide itself, and they are not involved in GST-mediated cleavage; therefore, just how does cleavage by GST increase the drug's anticancer activity?

While I have seen no experimental evidence for or against it, the following hypothetical explanation seems plausible. The difference in activity between the prodrug and the cleaved product may be due to the extrusion of the prodrug. Glutathione conjugates make good substrates for ABC transporters, which will eject them from the cell. Canfosfamide resembles a glutathione conjugate and thus may also be subject to extrusion. Cleavage by GST releases the reactive moiety of the drug, allowing it to escape extrusion, stay in the cell and exert its cytotoxic effect.

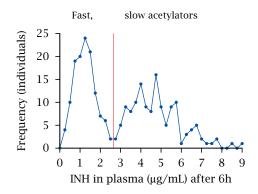
4.4.6 Acetylation of INH by N-acetyltransferase 2 (NAT 2)



Isoniazid (isonicotinic acid hydrazide, INH) is the classical example of drug metabolism through acetylation. The acetylated product may undergo hydrolysis to release acetylhydrazide, which is reactive and may cause cytotoxicity. Nevertheless, overall, *N*-acetylation *reduces* INH toxicity, since metabolites with even greater toxicity would otherwise arise through *N*-hydroxylation by cytochrome P450 (see below).

In spite of its potential metabolic toxicity, INH is tolerated better than most other tuberculostatic drugs, and it is also the most commonly prescribed one. Its antibacterial mode of action is discussed in slide 11.4.2.

4.4.7 Bimodal distribution of INH acetylation speed



In the depicted experiment, the speed of acetylation was measured in a group of patients. A fixed test dosage (presumably adjusted for body weight) was applied at t=0, and the amount of drug still remaining in the blood plasma after six hours was measured. There clearly are two separate peaks; the two groups represented by these peaks are referred to as fast and slow acetylators, respectively. Figure prepared from original data in [25].

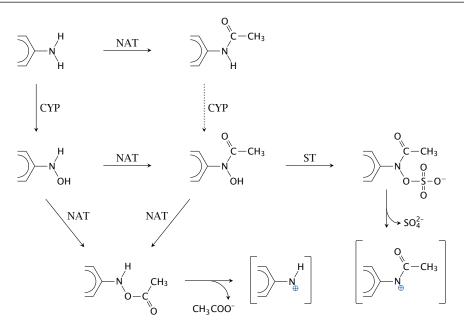
Among Caucasians, about 50% express an inactive NAT2 allele, which causes the slow-acetylator phenotype. The percentage of slow acetylators is lower among Asians (but was higher in a small study done on Kenyans [26]; I don't know how representative that study is).

Apart from isoniazid, the NAT2 enzyme and its polymorphism also affect the inactivation rates of some other drugs, such as procainamide and hydralazine, which are more likely to cause toxicity in slow acetylators. NAT2 has also been implicated in the susceptibility to bladder cancer caused by aromatic amines, which in Europeans was found to correlate with slow acetylator status. Surprisingly, the same correlation was not observed in Chinese [27]. I have not seen an explanation for this discrepancy.

4.4.8 Metabolic activation of arylamine carcinogens

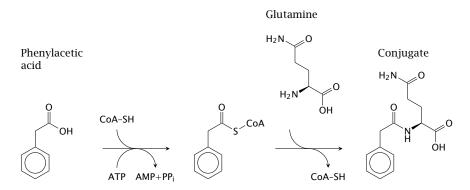
N-Acetyltransferases (NAT), cytochrome P450 (CYP) and sulfotransferases (ST) cooperate in the metabolic activation of arylamine carcinogens such as benzidine or 2-naphthylamine. The acetoxy and sulfohydroxamate products decay spontaneously to reactive electrophiles, which can then react covalently with cellular macromolecules, including DNA.

The activation is most efficient if CYP acts before NAT; acetylation of arylamines therefore affords partial protection from carcinogenic activation. This likely accounts for the lower susceptibility of fast acetylators to arylamine-induced tumors. Figure drawn after a scheme shown in [28].



4.4.9 Glutamine conjugation of phenylacetate

Glutamine, glycine and taurine can be conjugated to various xenobiotics that are organic acids; the figure shows glutamine conjugation of phenylacetic acid as an example.



Glycine and glutamine conjugation are exploited in an unusually ingenious manner for *alternate pathway therapy* in enzyme defects of the urea cycle (see slide 10.2.6).

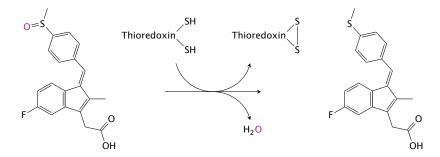
4.5 Reductive drug metabolism

- important functional groups in substrates: nitro, azo, sulfoxide, quinones
- diverse enzymology
- "incidental"—most enzymes that cause reductive drug metabolism primarily serve other roles in metabolism
- some reductive reactions can occur without enzyme catalysis

Reductive drug metabolism belongs to phase I. It is less regular than the oxidative and conjugative pathways covered so far, and also somewhat less common than the most prominent oxidative and conjugation pathways.

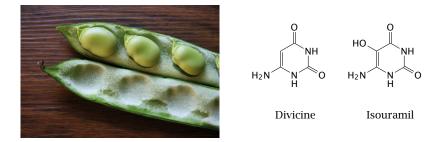
Among the various enzymes involved in reductive metabolism, there are a few surprises, such as cytochrome P450 enzymes and xanthine oxidase, which on occasion may transfer electrons to acceptors other than oxygen. We will look at some examples but make no attempt at exhaustive coverage.

4.5.1 Reductive activation of sulindac by thioredoxin



Sulindac is a prodrug that is activated by reductive metabolism, which is carried out by the reducing enzyme thioredoxin; the resulting thioether is a cyclooxygenase inhibitor. After reduction, the drug is excreted into the bile and then reabsorbed from the small intestine. This entero-hepatic cycling gives the drug a fairly long half life (~16 hours).

4.5.2 Redox-active ingredients of Vicia faba

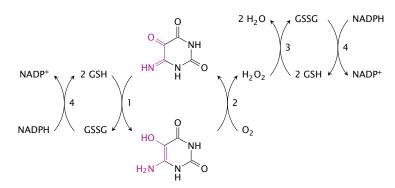


The broad bean (*Vicia faba*) contains isouramil and several other compounds isouramil and divicine that undergo repeated oxidation and reduction in the body, and in the process burn up reduction equivalents.

4.5.3 Redox cycling of isouramil

This scheme outlines the redox cycle that is induced by isouramil. Reactions 1 and 2 occur spontaneously, without the requirement for any enzymatic catalysis. The H_2O_2 formed in reaction 2 is then reduced by glutathione peroxidase. The glu-

tathione disulfide formed in reactions 1 and 3 is reduced at the expense of NADPH in reaction 4 by glutathione reductase. The NADPH, in turn, must be regenerated by other pathways.



4.5.4 Glucose-6-phosphate dehydrogenase deficiency leads to favism

- Most patients are healthy most of the time—hemolytic crises triggered by drugs or food ingredients that cause redox cycling
- Manifest in red blood cells because these cells lack protein synthesis—no replacement of deficient enzyme molecules during the lifetime of the cell
- Affords partial protection against malaria—similar to sickle cell anemia and other hemoglobinopathias

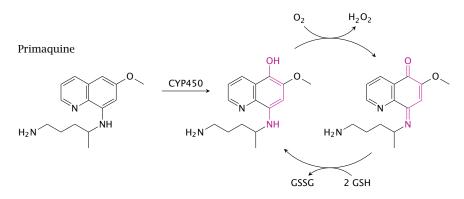
In red blood cells, the only pathway that provides NADPH is the hexose monophosphate shunt. The first enzyme in this pathway is glucose-6-phosphate dehydrogenase. Mutations that lead to a reduced activity of this enzyme limit the supply of NADPH and, therefore, the capacity of the cell to cope with redox cycles such as the one created by isouramil.

The toxicity of reactive oxygen species is mediated by the peroxidation of membrane lipids, which destroys the cell membrane—the red blood cells disintegrate. The occurrence of such episodes of *hemolytic anemia* upon ingestion of broad beans is called *favism*.

In contrast to red blood cells, nucleated cells can replace inactive enzyme molecules. Furthermore, they also contain mitochondria and can replenish cytosolic NADPH using mitochondrial NADH. Therefore, the enzyme defect is not manifest in nucleated cells.

4.5.5 Redox cycling of 5-hydroxyprimaquine

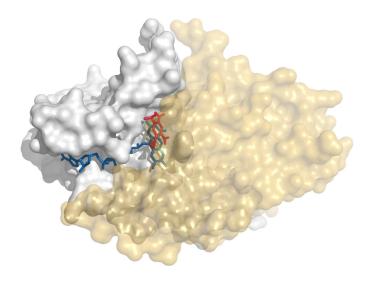
Like sickle-cell anemia and several other hemoglobinopathias, glucose-6-phosphate dehydrogenase deficiency is found more frequently among people indigenous to countries in which malaria is common. This is something to keep in mind when treating malaria patients with primaquine, which after hydroxylation to 5-hydroxyprimaquine by cytochrome P450 sets up a redox cycle analogous to the



one induced isouramil (only shown in part in this slide). Primaquine therefore may induce hemolytic anemia in these patients. Several other drugs can do the same.

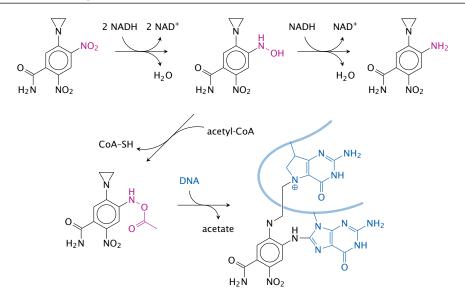
4.5.6 The anticancer prodrug CB1954 bound to quinone reductase 2

CB1954 is an experimental anticancer prodrug that is activated by reductive metabolism. In this structure [29], CB1954 (red) is shown bound to diaphorase, or quinone reductase 2. The cosubstrate NAD and the coenzyme FAD are shown in red. One of the enzyme's subunits is rendered transparent to make the substrate visible.



4.5.7 Two-step activation of the anticancer prodrug CB1954

CB1954 is not a quinone, from which we can infer that the name 'quinone reductase' for the enzyme in question is too narrow. Indeed, the same enzyme also reduces methemoglobin and cytochrome b5; considering such a broad range of endogenous substrates, it is not surprising that it accepts drugs in a fairly non-specific manner also.



The compound undergoes nitro reduction, followed by acetylation. The resulting acetoxy group is reactive toward DNA, as is the aziridine group already contained in the parent molecule. Such bivalent reagents—or multivalent ones such as canfos-famide, see above—can introduce crosslinks into DNA molecules, which are difficult for the cell to repair and therefore inflict much more effective cytotoxic damage than univalent reagents. Many anticancer drugs are, or give rise to, multivalent reagents (see section 12.6).

For reasons that are not well understood, the cytoplasm of tumor cells tends to be more reducing than that of healthy cells; so, drug activation by reduction is a way to preferentially target tumor cells. Note though that reductive activation also applies to some established anticancer drugs, such as mitomycin C or bleomycin. These two drugs are nevertheless quite toxic, so we should probably not expect particularly low toxicity from reductively activated synthetic drugs such as CB1954.

Chapter 5

G protein-coupled receptors

5.1 Overview

As already stated earlier (slide 1.2.3), G protein-coupled receptors (GPCRs) form the largest class of drug targets in the human body. It is therefore appropriate to examine and understand them in some detail.

The human genome contains genes for several hundred GPCRs. For a sizable number of these receptors, the ligands and physiological functions are not yet known. Once these "orphan" receptors will have become "deorphanized",¹ at least some of them will probably become drug targets also.

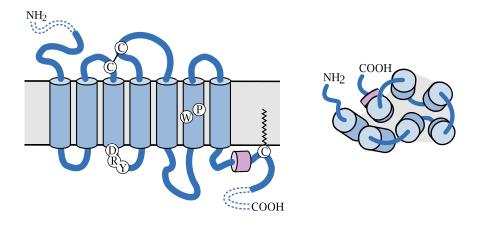
Drug	Major receptor	Drug action	Clinical use
salbutamol	β_2 -adrenergic	partial agonist	bronchodilation
fexofenadine	histamine H_1	inhibitor	antiallergic
atropine	muscarinic	inhibitor	pupil dilation
haloperidol	dopamine	inhibitor	antipsychotic
morphine	opioid	agonist	pain killer
losartan	angiotensin	inhibitor	antihypertensive
clopidogrel	adenosine	inhibitor	anticoagulation

5.1.1 Drugs that act on G protein-coupled receptors: Some examples

These selected examples suffice to illustrate the wide-ranging and diverse roles of GPCRs in physiology and pharmacology.

¹One might think the act of nominating parents for orphans could be described as "adoption," but apparently "deorphanization" is more scientific.

5.1.2 GPCR structure



All GPCRs (with the exception of rhodopsin) are located in the cytoplasmic membrane. The snake diagram on the left shows the arrangement of the seven transmembrane helices: the N-terminus faces outward, and the C-terminus is located inside the cell. The diagram also indicates the location of several amino acid residues that are conserved within the largest subfamily of GPCRs, and which play key roles in receptor activation. These include the DRY motif at the cytoplasmic end of helix 3, the proline and tryptophan residues in helix 6, and the short, horizontally positioned C-terminal helix 8.

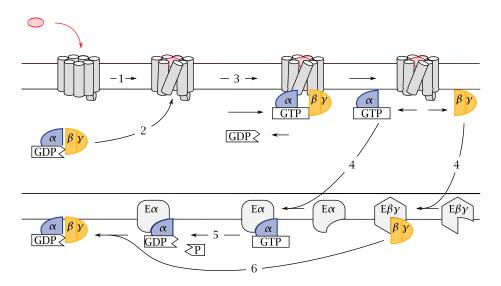
The schematic on the right shows the view from the extracellular side. The helices are arranged in an approximately circular fashion within the membrane plane. With many receptors, the crevice in the middle of the helices contains the ligand binding site, which is accessible from the extracellular side.

While the extracellular N-terminal domain is short in most GPCR molecules, some receptors have larger N-terminal domains that may contain ligand binding sites or other functional features. We will see some examples later.

5.1.3 The G protein cycle

GPCRs activate GTP-binding proteins, or G proteins for short, which in turn activate various effector proteins. G proteins are heterotrimers; their subunits are referred to by the Greek letters α , β and γ .

G proteins undergo repeated cycles of activation and inactivation. The cycle starts when an agonist binds to the extracellular face of a GPCR (1), which changes the conformation of the entire GPCR molecule, including its intracellular surface. In this activated conformation, the GPCR binds a G protein (2). The α -subunit of this G protein then releases a molecule of GDP, which was left behind by a previous turn of the cycle. Next, it binds GTP and then dissociates from the $\beta\gamma$ -dimer. The two dissociated G protein fragments are now free to seek out and bind to their respective effector proteins (4), which have various biochemical activities (see later).

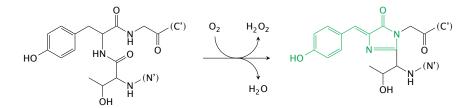


After some time has elapsed, the bound GTP molecule is cleaved by the built-in GTP'ase activity of the G α -subunit (5). This causes the α -subunit to leave its effector and to again associate with a G $\beta\gamma$ dimer (6). The inactive trimer then awaits another round of activation.

5.2 G protein binding and dissociation can be observed with GFP fusions

The experiments described in the following illustrate how the activities of GPCRs and of G proteins can be observed within cells using the green-fluorescent protein from *Aequorea* jellyfish and its recombinant derivatives. These methods are straightforward and commonly used today; they were, however, not yet available in the early studies that first discovered how these proteins work.

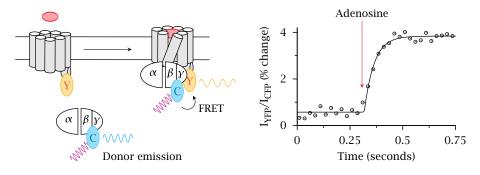
5.2.1 The fluorophore in green-fluorescent protein forms autocatalytically



The great power of the green-fluorescent protein (GFP) is due to its entirely spontaneous formation of a fluorophore—it needs no help from any other protein, and it therefore can easily be used in almost any type of cell or cellular compartment. GFP is typically used through translational fusion with a protein of interest.

While the wild-type GFP is excited with ultraviolet light, several mutants have been created that can be excited at different wavelengths within the visible spectrum, and which can be combined with one another for fluorescence resonance energy transfer (FRET) experiments. In the mutant cyan-fluorescent protein (CFP), the tyrosine residue that becomes part of the fluorophore is replaced by tryptophan. The yellow-fluorescent protein (YFP) retains the unmodified fluorophore of GFP; it contains a mutant tyrosine residue that is not part of the fluorophore, but engages in π -stacking interactions with the latter and thereby shifts its absorption and emission spectra.

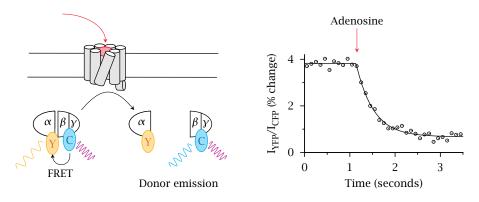
5.2.2 FRET detection of G protein binding to adenosine receptors



In this experiment, the yellow-fluorescent protein (Y) was translationally fused to the A_{2A} adenosine receptor, and the cyan-fluorescent protein (C) to the γ -subunit of the cognate G protein (G_s). Addition of adenosine activates the receptor and triggers binding of G_s to the receptor. The ensuing FRET from CFP to YFP is detected through an increase in the ratio of YFP emission to CFP emission.

The observed change in the extent of FRET is fairly small, suggesting that only a small fraction of the available G protein molecules associates with the activated receptor. Figure prepared from original data in [30].

5.2.3 FRET detection of G protein dissociation



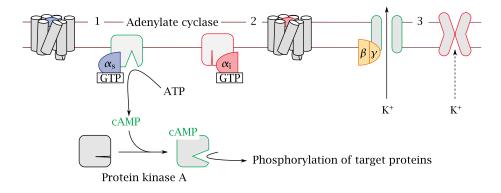
In this experiment, FRET between CFP and YFP was used to detect the dissociation of the heterotrimeric G protein. In this case, the extent of FRET decreases upon receptor activation. Again, the change in FRET is small.

Note the difference in time scales between this slide and the preceding one: Dissociation of the G protein is noticeably slower than its binding to the activated receptor. Figure prepared from original data in [30].

5.3 G protein effector mechanisms

For each of the three subunits of the heterotrimeric G proteins— α , β and γ —there are several subtypes, which may combine into heterotrimers in various permutations. However, overall, G proteins are less diverse than GPCRs; therefore, multiple types of GPCRs must converge upon the same G proteins and trigger the same intracellular responses.² We will now look at the major intracellular signaling cascades triggered by different G proteins.

5.3.1 Adenylate cyclase



One important effector protein is adenylate cyclase. This membrane-associated enzyme converts ATP to cyclic AMP (cAMP), which is an allosteric activator of protein kinase A.

Adenylate cyclase is controlled by two different $G\alpha$ subunits, which are activated by different GPCRs. The stimulatory α -subunit ($G\alpha_s$, 1) activates the enzyme, whereas the inhibitory α -subunit ($G\alpha_i$, 2) inhibits it.

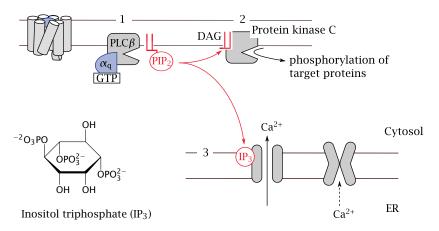
An important effector molecule for $G\beta\gamma$ dimers are K⁺ channels of the K_{ir} type (3). Opening of these channels will cause hyperpolarization of the cell membrane. In excitable cells, this will tend to reduce the level of activity.

5.3.2 The phospholipase C cascade

Another effector pathway that is activated downstream of many pharmacologically important GPCRs is the phospholipase C cascade. For example, the contraction of vascular smooth muscle cells downstream of α -adrenergic receptors (slide 2.3.4) or angiotensin receptors (slide 1.2.5) is mediated by this pathway.

In this cascade, the α -subunit of G_q stimulates phospholipase $C\beta$ (1). The activated enzyme then cleaves a specific membrane lipid, phosphatidylinositol-bisphosphate (PIP₂), into two secondary messengers, namely, diacylglycerol and inositoltriphosphate.

²On the other hand, individual GPCRs may also couple to more than one G protein; compare slide 2.5.4.



Diacylglycerol (DAG) is a very hydrophobic molecule that remains associated with the cytoplasmic membrane, where it activates protein kinase C (2). Phosphorylation of target proteins by this kinase causes various downstream effects.

The other messenger, inositoltriphosphate (IP₃), is water-soluble. It travels through the cytosol to the ER membrane, where it activates the IP₃ receptor, which is a calcium channel (3). Ca⁺⁺ ions released from the ER into the cytosol activate calmodulin, which in turn will affect numerous calmodulin-dependent proteins. In smooth muscle, a calmodulin-dependent protein kinase phosphorylates myosin, which triggers contraction.

Class	Effectors and Effects	Some activating GPCRs
Gαs	stimulation of adenylate cyclase (vari- ous types)	β-adrenergic, 5-HT ₄ , 5-HT ₆ , 5-HT ₇ , D ₁ , D ₅ ; ACTH
Gα _{i/o}	inhibition of adenylate cyclase; activa- tion of extracellular signal-regulated kinase (ERK)	α_2 -adrenergic, 5-HT ₁ , D ₂ , D ₃ , D ₄
$G\alpha_{q/11}$	stimulation of Phospholipase C β (various subtypes)	α -adrenergic, 5-HT ₂ , H ₁ , GABA _B
$G\alpha_{12/13}$	indirect activation of RhoA GTPase and of phospholipase ${\rm A_2}$	5-HT ₄ , AT ₁ , protease- activated receptors

5.3.3 Summary of G protein effector mechanisms

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); D, dopamine; AT, angiotensin; ACTH, adrenocorticotropic hormone; GABA, γ -aminobutyric acid.

The activities of the first three G proteins listed here were illustrated in the preceding two slides. The enumeration of activating receptors for each of these cascades is neither exhaustive nor recommended for memorization, but only intended to illustrate the convergence of different receptors on the same G proteins.

One effect that occurs downstream of the fourth listed G protein, $G\alpha_{12/13}$, is the activation of phospholipase A_2 , which in turn promotes the formation of eicosanoids (see chapter 9). The protein also activates RhoA GTPase, which influences transcriptional regulation.

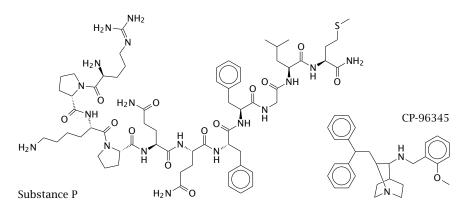
A single GPCR may interact with multiple G proteins, which may set off different downstream cascades. Occasionally, different receptor agonists may activate various downstream cascades to different extents, which is referred to as agonist-specific coupling (see slide 2.5.4).

5.4 Structure and function of GPCRs

Much experimental effort has been devoted to studying the conformational changes that are at the heart of GPCR function. Many of these studies have been performed on rhodopsin. This molecule differs from other GPCRs in being activated not by ligand binding and dissociation, but instead by photoisomerization of its covalently bound retinal chromophore. However, it is the easiest GPCR molecule to obtain in abundance, and it was the first one to be crystallized, which made it an attractive model. Since it shares extensive homology with many GPCRs that are drug targets, it is also a credible and useful model.

Crystal structures of other GPCRs have now been experimentally determined, and they will indubitably enhance our understanding and the precision of drug design. However, crystal structures are static, and other experimental methods remain relevant for understanding the molecular movements and interactions. We will consider a few selected examples below. The first example involves the receptor for substance P, a peptide neurotransmitter.

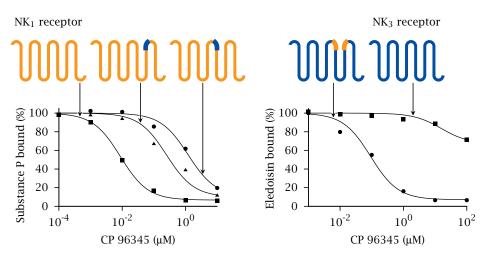
5.4.1 Substance P and its competitive antagonist CP-96345



Substance P, also known as neurokinin 1 (NK₁), is a peptide neurotransmitter that is involved in the perception of pain and in triggering emesis. NK₁ receptor antagonists such as CP-96345 are being developed for controlling chemotherapy-induced emesis in cancer patients.

Substance P and CP-96345, as well as eledoisin, another peptide that is related to substance P, were used in the following experiment that was designed to determine the ligand binding sites of the NK₁ and NK₃ receptors.

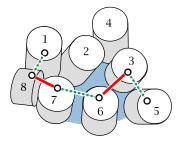
5.4.2 Using receptor chimeras to locate the ligand binding sites of NK receptors



CP-96345 displaces substance P from the wild-type NK_1 receptor (left) but does not effectively compete with eledoisin at the NK_3 receptor (right). When either of two short stretches located at the extracellular ends of transmembrane helices 5 or 6, respectively, in NK_1 is replaced by the homologous sequences from NK_3 , the inhibitory potency of CP-96345 is greatly reduced. This indicates that binding of CP-96345 to the chimeric receptors is disrupted.

The reciprocal exchange markedly increases the sensitivity of the NK₃ receptor to inhibition by CP-96345. Therefore, the short sequence stretches that were exchanged between the receptors carry determinants that are crucial for binding CP-96345. Note that the chimeric receptors retain affinity for substance P and eledoisin, respectively. Figure prepared from original data in [31].

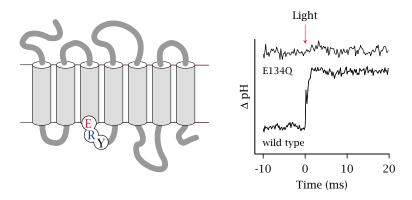
5.4.3 Engineered disulfide bonds pinpoint helix movements involved in GPCR function



This slide is supposed to show a view onto the cytoplasmic surface of rhodopsin. Lines between helices indicate disulfide bonds that were engineered into the molecule one by one. This is done by substituting pairs of amino acid residues in strategic locations with pairs of cysteines. If two cysteines are sufficiently close in space, they will form a disulfide bond spontaneously.

Bonds drawn with solid red lines prevent receptor activation, whereas those shown as dashed green lines do not. These findings indicate that helices 7 and 8, as well as helices 3 and 6, must be able to move relative to one another in order to allow receptor activation. Figure based on reference [32].

5.4.4 Protonation of residue E134 of rhodopsin in response to light stimulation



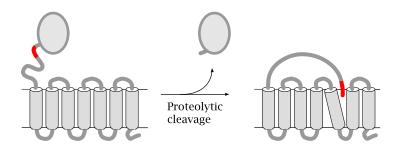
The cytosolic end of helix 3 of rhodopsin contains a functionally crucial tripeptide sequence, consisting of glutamate in position 134, followed by arginine and tyrosine. This sequence motif—with aspartate often taking the place of glutamate, see slide 5.1.2—is conserved in the entire group of rhodopsin-like receptors, which are the largest subclass of GPCRs. From the disulfide tethering experiment just discussed, we already know that this location is important in receptor activation. The experiment shown here looks at the role of the conserved ERY motif.

Purified and detergent-solubilized rhodopsin was suspended in unbuffered water, so as to maximize the pH change in response to a small change in the number of free protons, such as can be expected to occur through protein protonation or deprotonation. When wild type rhodopsin is exposed to a flash of light, the pH value jumps up, indicating uptake of protons by the protein. This protonation occurs at residue glutamate 134, as is shown by its absence in a mutant (E134Q) that contains glutamine instead of glutamate in this position.

A plausible explanation is that protonation of E134 breaks a salt bridge with the adjacent arginine and thereby removes an energetic constraint that ties rhodopsin down in its inactive conformation. Figure prepared from original data in [33].

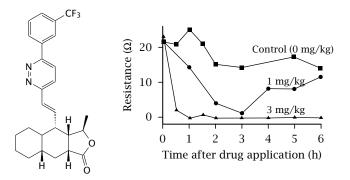
5.5 Protease-activated GPCRs

Protease-activated receptors contain an inhibitory domain that extends the extracellularly exposed N-terminus. A receptor is activated when a protease cleaves this domain and thereby exposes a built-in receptor ligand, which then associates noncovalently with a ligand binding site located in the transmembrane domain of the same receptor molecule.



The location of this binding site, and the events set in motion by the binding of the intramolecular ligand, are similar to those found in other, conventional GPCRs. However, there is one important difference: While activation of other GPCRs is reversed when the ligand is diluted, activation of protease-activated receptors is irreversible, since the ligand is part of the receptor and not amenable to dilution. Protease-activated receptors therefore can only be inactivated by endocytosis and degradation (see slide 5.7.1).

5.5.1 Pharmacological inhibition of protease-activated receptors



The protease-activated receptor PAR1 is found on thrombocytes and is activated by thrombin; it contributes to the activation of thrombocytes (see slide 10.6.7). Generally speaking, inhibition of thrombocyte activity is an important strategy in the prevention of myocardial infarction and stroke in patients with advanced atherosclerosis. Protease-activated receptors are interesting as a potential target in this application also.

The molecule shown on the left is a synthetic PAR1 inhibitor. Its activity as an inhibitor was assessed in the animal experiment summarized in the plot. The inhibitor was applied at time 0, and plasma samples were obtained at various times after application. The samples were then spiked with a peptide agonist of PAR1 in order to activate the receptor.³

³One might wonder why a protease was not used to activate the receptor. The reason may be the inherent difficulty in selectively cleaving the receptor without also cleaving and activating plasma coagulation factors. The use of a peptide receptor agonist avoids this problem.

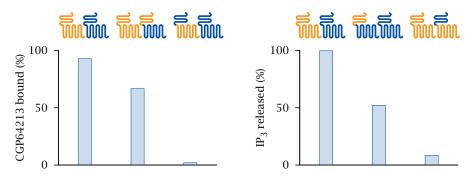
Thrombocyte aggregation was measured through the electrical resistance between two electrodes immersed in the sample; as thrombocytes aggregate atop the electrode surfaces, the ohmic resistance between the electrodes increases. A decrease in resistance therefore indicates inhibited thrombocyte aggregation. Figure prepared from original data in [34].

5.6 GPCR oligomerization

- · Oligomers can comprise identical or different subunits
- Potential for cooperativity
- Potential for novel ligand specificity
- When receptors for antagonistic mediators form heterodimers, these mediators can "duke it out" already at the cell surface, reducing noise inside the cell

While GPCRs were initially believed to function as monomeric molecules, there is now solid evidence that many receptors form oligomers. While in some cases the functional effect of oligomer formation may amount to no more than a modicum of cooperativity in the response to the ligand, in others oligomerization has a more profound effect on receptor function; we will look at a few examples presently.⁴

5.6.1 Functional specialization in GABA_B receptor heterodimers



GABA (γ -aminobutyric acid) is a major inhibitory neurotransmitter in the brain. There are two major types of GABA receptors; the GABA_A and GABA_C receptors are ligand-gated channels, whereas the GABA_B receptors belong to the GPCR family.

In contrast to most other GPCRs, GABA_B receptors contain an extended Nterminal domain that is extracellularly located and also contains the ligand binding site. The receptors occur in various subtypes, which can form homo- and hetero-oligomers.

The study summarized here used chimeric receptors to examine the functional roles of the different subunits in GABA_B-1/GABA_B-2 receptor hetero-oligomers. Cells

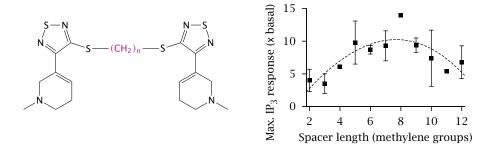
⁴In much of the literature on GPCR oligomers, these are assumed to be dimers, but most studies do not contain experiments that would stringently distinguish between dimers and larger oligomers. When talking about dimers below, I simply follow the authors of the cited studies, without examining whether or not they have proven a strictly dimeric subunit stoichiometry.

were transfected with both GABA_B-1 receptor (orange) and GABA_B-2 receptor (blue), or with combinations of either wild-type receptor with chimeric receptors as indicated.

In the experiment shown on the left, wild-type receptors were combined with chimeras so as to change all N-termini to either the $GABA_B-1$ type (center) or the $GABA_B-2$ type (right). When only $GABA_B-1$ N-termini are present, ligand binding is somewhat diminished, but still considerable. In contrast, with only $GABA_B-2$ N-termini present, ligand binding is abolished. Thus, in the heterodimer, the $GABA_B-1$ subunit dominates ligand binding.

In the experiment on the right, all transmembrane domains were converted to either the GABA_B-2 or the GABA_B-1 type, and IP₃ production downstream of receptor activation was measured. In this case, it is the GABA_B-2 domain that on its own retains significant activity, while the GABA_B-1 TM domain alone is inactive. Therefore, GABA_B-2 dominates G protein activation. Figure prepared from original data in [35].

5.6.2 Bivalent agonists of muscarinic acetylcholine receptors

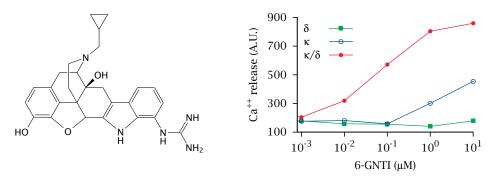


Like GABA, acetylcholine has receptors both among the ligand-gated channels and in the GPCR family. The latter are also referred to as muscarinic receptors (see Section 6.15). Muscarinic receptors form tetramers [36]. In the experiment depicted here, two molecules of the receptor ligand 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5thiadiazole (phew!) were tied together with flexible spacers varying in length, and the extent of receptor activation was measured as IP_3 release downstream of phospholipase C activation.

With increasing spacer length, receptor activation seems to go through an optimum and then to decrease again, suggesting that a certain amount of "tugging" at the tether may be conducive to activation.⁵ Figure prepared from original data in [37].

⁵The authors of the study cited did not interpret their findings in terms of receptor oligomerization; however, oligomerization of muscarinic receptors has since been demonstrated [36].

5.6.3 Novel receptor specificity: selective activation of $\kappa \delta$ opioid receptor hetero-oligomers by a monovalent ligand

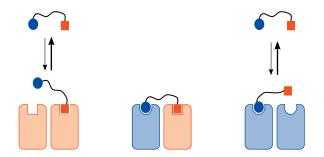


Opioid receptors are activated by endorphins, which are peptide mediators, as well as by morphine and related natural and synthetic compounds. Opioid receptor agonists are very powerful pain killers, but as is well known, they are also inebriating and addictive. Separating these two effects is a desirable but elusive target.

Opioid receptors have several subtypes that may form homo- or hetero-oligomers. Specific types of oligomers may be preferentially located in different areas of the central nervous system; for example, $\kappa\delta$ -heterodimers primarily control pain conduction in the spinal cord.

Against this background, it may be more than a curiosity to observe that the drug 6-guanidinonaltrindole (6-GNTI) preferentially activates $\kappa\delta$ -heterodimers. This was shown here through the determination of calcium release downstream of phospholipase C and IP₃, using receptors recombinantly expressed in cell culture alone and in combination. Figure prepared from original data in [38].

5.6.4 Could receptor heterodimers be targeted with heterodimeric drugs?



The discovery of the heterodimer-selective effect of 6-GNTI, while fascinating, was entirely fortuitous; it is currently impossible to rationally design monomeric drugs with such subtle specificity.

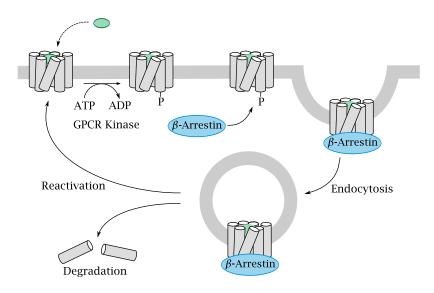
In order to rationally design heterodimer-specific drugs, one might consider the approach illustrated in this slide. While it would seem fairly obvious and straight-forward, the devil is in the details. For this approach to work, the affinities of both half-ligands would have to be tuned such that they contribute about equally, and

that divalent binding would be required for avid interaction with the receptor. An experimental study that attempts this approach is [39].

5.7 Feedback regulation of GPCR activity

A GPCR will often be inactivated simply by dissociation of the agonist. However, some agonists may bind avidly and dissociate slowly, and with protease-activated receptors, activation is entirely irreversible. Therefore, additional mechanisms are needed to control receptor activity. One widely used mechanism for GPCR inactivation consists in phosphorylation.

5.7.1 GPCR deactivation by phosphorylation and endocytosis



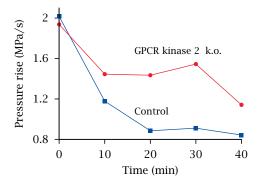
Activated GPCRs undergo phosphorylation by GPCR kinases. The phosphorylated GPCRs are then bound by β -arrestin, which prevents their further interaction with G proteins and also mediates their endocytosis. Endocytosed receptors may dissociate from β -arrestin and return to the cell surface, or alternatively they may undergo complete degradation within the endosome. As noted above, the latter fate always awaits protease-activated receptors.

GPCR phosphorylation and endocytosis are involved in *tachyphylaxis*, that is, a rapid partial desensitization to GPCR-activating drugs.

5.7.2 GPCR kinase 2 knockout attenuates tachyphylaxis of cardial β -receptors

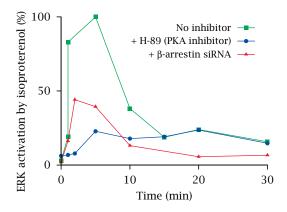
 β -Adrenergic receptors are found in the heart, where they stimulate adenylate cyclase, which then increases the heart rate and strength of contraction; this, in turn, raises the blood pressure. In the control arm of the experiment shown here, application of the β -receptor agonist isoproterenol starting at time zero caused an increase

in blood pressure, but the effect dropped by more than half within 20 minutes, despite continued application of the drug. This is an example of tachyphylaxis.



When GPCR kinase 2 is genetically knocked out, tachyphylaxis still occurs but is attenuated, indicating that this kinase participates in the inactivation of the β -receptors. Figure prepared from original data in [40].

5.7.3 Knock-down of arrestin may *reduce* GPCR-mediated signals



This experiment shows the activation of extracellular signal-regulated kinase (ERK) downstream of β -adrenergic receptors in response to isoproterenol. The experimental drug H-89 inhibits protein kinase A, which is activated downstream of β -receptors and adenylate cyclase.

ERK activation occurs in two phases. H-89 inhibits the early activation peak but not the subsequent, more shallow and protracted phase of ERK activation. This suggests that the late phase is mediated by a cAMP/PKA-independent mechanism.

Surprisingly, both the early and the late phase are inhibited when the expression of arrestin is suppressed with siRNA. This suggests that, in addition to its role in GPCR deactivation, β -arrestin may also *transduce* the signals represented by GPCR activation. There is indeed a growing body of evidence supporting this idea. Figure prepared from original data in [41].

Chapter 6

Pharmacology of cell excitation

6.1 Overview

Nerve cells, muscle cells and several other cell types are *excitable*, that is, they are activated by variations of their membrane potentials. Many of the macromolecules that control cell excitation are important drug targets.

6.1.1 Clinical applications of drugs that influence excitable cell function

- blockade of nerve conduction for local anesthesia
- reduction of nerve cell excitability in the brain in epilepsy
- stabilization of mood in the treatment of bipolar disorder
- reduction of vascular smooth muscle tone to reduce blood pressure
- suppression of aberrant excitation in cardiac arrhythmia

This list is not complete, but it suffices to illustrate that the applications are important and diverse. In order to understand how such drugs work, we must first understand cell excitation itself.

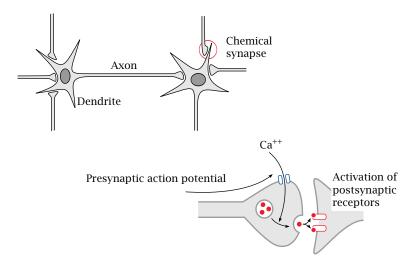
6.1.2 The nature of cell excitation

- all cells have an electrical potential across the cytoplasmic membrane, such that the cell interior is electrically negative relative to the outside (~-70 mV)
- in non-excitable cells, this membrane potential is stable; in excitable cells, it forms the *resting potential*
- cell excitation consists in transient reversals of the membrane potential, called *action potentials*, which spread rapidly across the entire cell membrane

• action potentials are spontaneously generated by some cells and transmitted between cells through chemical or electrical synapses

By definition, a negative membrane potential means that the cell interior is negative relative to the extracellular space. The negative resting potential is maintained by potassium leak channels in the cytoplasmic membrane; we will consider below how this works.

6.1.3 Neurons and synapses



Many drugs that influence cell excitation act on nerve cells. Nerve cells, or *neurons*, communicate with one another through chemical synapses. A nerve cell receives input from other nerve cells upstream through synapses on its dendrite. When stimulated sufficiently by activity of these synapses, it will generate an action potential. The action potential travels down the dendrite toward the cell body, then down the axon. When it reaches a presynaptic terminal, the action potential causes an influx of Ca⁺⁺, which in turn triggers the release of neurotransmitter into the synaptic cleft. Activation of postsynaptic receptors then transmits the signal to the next nerve cell.

A given neuron receives input from its *afferent* synapses, and it sends its output to its *efferent* synapses.

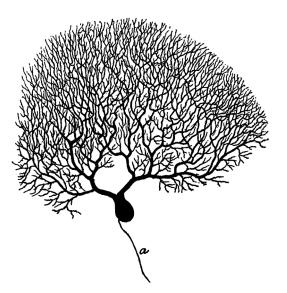
6.1.4 Some nerve cells have huge dendrites and axons

This picture (from [42]) shows a Purkinje cell from the cortex of the cerebellum. It has a large branched dendrite,¹ through which it receives input from thousands of other nerve cells.

The axon of the Purkinje cell has far fewer branches than its dendrite (and they would all be below the lower edge of this picture). However, in some other types of nerve cells, the axon may be similarly strongly branched. For example, a single

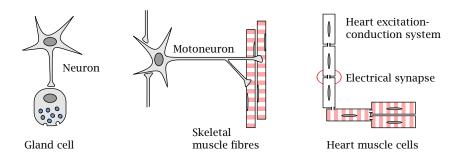
¹*Dendron* is the Greek word for tree.

 α -motoneuron may control the activity of several hundred skeletal muscle fibers; its axon must branch out and form synapses with each of these cells.



6.1.5 Other types of excitable cells

In addition to nerve cells, several other types of cells are also excitable and may contain clinically relevant drug targets. Some of these cells receive their input from nerve cells. Adrenal gland cells release norepinephrine and epinephrine when stimulated by nerve cells of the autonomic nervous system. Skeletal muscle cells contract when stimulated by α -motoneurons; vascular smooth muscle cells contract in response to stimulation by both nerve cells and circulating hormones.



The heart, while also subject to modulatory input from the autonomic nervous system, can function entirely independently.² It possesses two types of excitable cells. Those within the *excitation-conduction system* spontaneously produce the rhythm, and they then transmit it to the regular muscle cells, which we will here refer to as the "worker" cells. Communication occurs through *electrical synapses*,

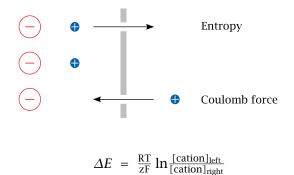
²Otherwise, heart transplants would be impossible, since the transplanted heart will be disconnected from those neuronal inputs.

in which ions can flow directly from cell to cell via cytoplasm bridges. These direct electrical connections between adjacent cells are created by so-called *gap junctions*.

6.2 The physical basis of cell excitation

Now comes the scary part—the one we all thought we had successfully dodged when choosing bioscience over real science.

6.2.1 The two driving forces that generate diffusion potentials across membranes



A diffusion potential arises if (1) the membrane is selectively permeable for some specific ion but not its counterion, and (2) there is a concentration gradient for the permeant ion. Under these conditions, entropy will induce the permeant ion to move downhill its concentration gradient, until the ensuing charge imbalance creates an equally strong Coulomb counterforce. This counterforce is reached at the equilibrium potential, E_0 , which is determined by the Nernst equation.³

Ion	Cytosolic	Extracellular	E_0 at 37°C
\mathbf{K}^+	150 mM	6 mM	- 86 mV
Na^+	15 mM	150 mM	+ 62 mV
Ca ⁺⁺	100 n M	1.2 mM	+ 126 mV
Cl-	9 mM	150 mM	- 70 mV

6.2.2 Equilibrium potentials for the major salt ions

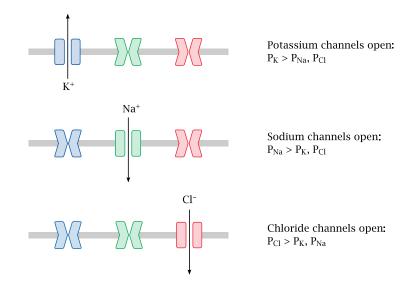
³The Nernst equation is really just an application of the Gibbs equation to electrochemical gradients—all you need to derive it is the Gibbs equation itself and the definition of the voltage: $\Delta E = \Delta G/zF$, with z as the number of charges per ion and F as Faraday's constant.

This table lists the major cations and anions that control the membrane potential and their intra- and extracellular concentrations. For each ion, the equilibrium potential was calculated using the Nernst equation.

As you can see, the equilibrium potentials are all different; those of K^+ and Cl^- are negative, while the ones for Na⁺ and Ca⁺⁺ are positive. This raises the question: How do these individual, different equilibrium potentials interact to shape the actual membrane potential? To understand this, we need to consider another parameter, namely the *permeability* of the membrane for each ion.

Pure phospholipid membranes are essentially impermeable for salt ions; therefore, in cell membranes, ion permeabilities are determined by specific transport proteins, the *ion channels*. Ion channels that open and close and thereby cause ion permeabilities to change are at the heart of cell excitation.

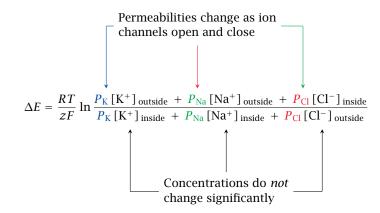
6.2.3 Specific channels control ion permeabilities



Some ion channels are continuously open, but most open transiently and then close again. Within this latter group, *ligand-gated* channels open in response to binding of a chemical agonist, while *voltage-gated* channels respond to changes in the membrane potential. Some channels may respond to both ligands and voltages. Yet others respond to changes in temperature or membrane distension; such channels are significant in sensing heat and pressure.

Many channels are very specific for individual ion species, as shown in this illustration; this applies to the major voltage-gated channels that control the action potential. Other channels have broader selectivity; for example, the nicotinic acetyl-choline receptor (slide 6.10.1) is permeable for all of the major cations (Na⁺, K⁺ and Ca⁺⁺).

In this slide, *P* represents the ion-specific membrane permeabilities that are explained in the next slide.



6.2.4 Diffusion potentials with multiple ions: the Goldman equation

The diffusion potential that prevails across a membrane with significant permeabilities for several ion species is governed by the Goldman equation, shown here for a combination of Na⁺, K⁺ and Cl⁻. In addition to the terms known from the Nernst equation, it introduces the permeability P, a scaling factor that weights each ion for its rate of diffusion across the membrane, relative to that of the other ions.

If channels open for a specific ion, its permeability goes up. This increases the weight of this ion in the Goldman equation, and thus pulls the overall membrane potential toward its own (Nernst) equilibrium potential. In resting cells, the potential is close to the K^+ equilibrium potential, because the always-open K^+ leak channels give K^+ a greater permeability than all other ions. However, all that is needed to flip the membrane potential from its negative-inside resting state to a positive-inside value is a forceful opening of sodium or calcium channels. On the other hand, opening of chloride channels or of additional potassium channels will drive the membrane potential back down toward the resting state or below.

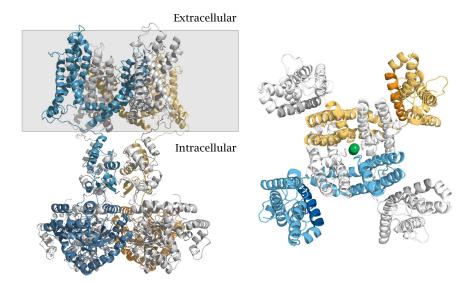
As stated above, such transient reversals of the membrane potential are referred to as action potentials. In nerve and skeletal muscle cells, an action potential lasts only 1–3 milliseconds, and the absolute number of ions that cross the membrane during this short time interval is very low. With Na⁺, K⁺ and Cl⁻, it is too low to measurably change the ion concentration on either side of the membrane.

The one important exception from this rule is calcium. Its intracellular concentration is so low (see slide 6.2.2) that the small number of ions flowing across open channels still causes a significant relative increase. The increased intracellular calcium level will cause activation of calmodulin and other calcium-binding proteins. Therefore, the opening of calcium channels constitutes both a physical and a biochemical signal. Calcium channels are important in nerve cells, and they are particularly prominent in heart and smooth muscle cells, where they replace sodium channels as the primary drivers of membrane potential reversal, or *depolarization*.

6.3 Structure and function of voltage-gated cation channels

Voltage-gated cation channels are crucial in triggering, sustaining and propagating action potentials. The channels for K^+ , Na^+ and Ca^{++} are homologous and functionally similar. We will look at the structure of voltage-gated K^+ channels as an example.

6.3.1 Structure of a voltage-gated K⁺ channel



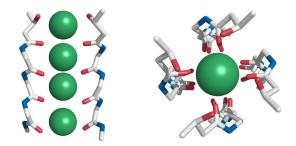
The picture on the left shows a side view. The gray rectangle delineates the membrane-embedded portion. A large part of the channel protein protrudes into the cytosol. The channel is composed of four identical subunits; one is shown in blue, the one opposite to it in yellow, and the other two in light gray. The conformationally flexible N-terminal inactivation domain that is located in the cytosolic portion (see below) is missing from this structure.

The panel on the right shows a view from the extracellular side onto the membrane-embedded domain of the channel. This domain consists of an inner layer, which contains the selectivity filter, and an outer layer that mediates voltage-dependent channel opening. In each of the four subunits, an arginine-rich helix that forms the voltage sensor is rendered in a darker shade. A potassium ion inside the central channel pore is shown as a green ball. While most of the channel protein is α -helical, the selectivity filter that enwraps the potassium ion has β -structure. Structure rendered from 2r9r.pdb.

6.3.2 Structure of the K⁺ selectivity filter

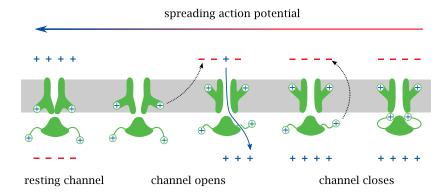
Side view (left) and top view (right) of the K^+ selectivity filter that is in the center of the membrane-embedded portion. Backbone oxygen atoms tightly enclose the

dehydrated K⁺ ions. Each of the four subunits contributes one backbone segment, but for clarity only two segments are shown in the side view.



Similar selectivity filters are also found in other types of K^+ channels, for example the leak channels that maintain the resting potential, as well as the K_{ATP} channels (slide 6.6.1). The selectivity filters found in sodium and calcium channels work according to the same principle, although they differ in diameter and architectural detail. In each case, the filter fits the ion like a glove. Ions that are too large are simply excluded; those that are too small are rejected because they cannot bind avidly to the filter, and therefore cannot compensate the energetic cost of their dehydration.

6.3.3 Voltage-gated sodium channels sustain and spread the action potential

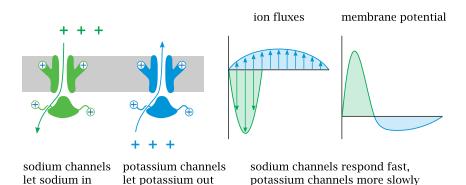


On nerve and skeletal muscle cells, the action potential is carried and sustained mostly by voltage-gated Na^+ (Na_V) channels. As stated above, all voltage-gated channels have the same general structure as the K_V channel, so we will now see how those structural features operate in practice.

When the membrane potential in the vicinity of a sodium channel is reversed by a spreading action potential, the membrane-embedded voltage sensor moves outward, which decompresses the inner layer and opens the channel. This allows Na^+ to enter the cell, enlarging the depolarized membrane area, causing more Na_V channel molecules to open and the action potential to propagate further. After the channel has been open for a short time,⁴ a separate inactivation gate, which is flexibly attached to the cytosolic portion of the channel and also carries a positive net charge—and which, as stated, is missing from the K_V channel structure shown in slide 6.3.1—responds to the same electric field reversal and moves to plug the channel. The channel is now inactivated, in spite of the continued depolarized state of the membrane. Before the channel becomes ready for opening again, both gates must revert to their initial conformations, which happens only after the membrane has been repolarized.⁵

While the explanation given here is mostly quite accurate, we must add one caveat: the membrane potential does not have to be fully reversed in order to induce channel opening; instead, the channels begin to open once the membrane has been partially depolarized to approximately –50 mV. The threshold potentials or "firing levels" of different channels do vary, but they are at negative values for all voltage-gated channels I'm aware of.

6.3.4 Voltage-gated potassium channels extinguish the action potential



This slide illustrates the interplay of Na_V and K_V channels in shaping the action potential. Both channels open in response to depolarization. Because of the opposite orientations of the two concentration gradients, this causes Na^+ influx but K^+ efflux.

The Na_V channels open and inactivate faster and therefore dominate the early (depolarization) phase of the action potential. The slower response of the K_V channels dominates the later phase and causes a transient hyperpolarization of the membrane.

As long as the composition of the channel population remains the same, the shape and amplitude of each action potential will also be the same.⁶ Hence, infor-

⁴This time is variable; both opening and inactivation are stochastic processes, with separate rate constants; the rate constant for opening is greater than that for inactivation.

⁵This reactivation again takes a bit of time; therefore, the membrane will not be excitable for a short period immediately after repolarization. This time interval is the *refractory period*.

⁶Note, however, that we do find different channel populations on different cell types, and this gives rise to action potentials that differ in shape and duration. On nerve and skeletal muscle cells, action potentials last only a few milliseconds; on heart muscle cells, half a second, and several seconds on smooth muscle cells. In skeletal muscle, a contraction is sustained by a repetitive burst of action

mation cannot be encoded in the properties of individual action potentials; instead, it is encoded in their frequency.

Sinoatrial node $\begin{array}{c} & & \\$

6.3.5 Voltage-gated channels and action potentials in the heart

The interplay of sodium and potassium channel controls the action potentials in nerve and skeletal muscle fibers, which are of short duration and often occur with high repetition rates. In contrast, action potentials in the heart last much longer and occur with lower frequency, and their control involves voltage-gated channels for calcium in addition to those for sodium and potassium.

The heart contains two types of excitable cells: those in the excitation-conduction system generate action potentials spontaneously and periodically, whereas the worker muscle cells stay put until they are ordered otherwise, much like the muscle fibers in skeletal muscle. The latter is true at least of a healthy heart; however, in certain forms of cardiac arrhythmia, worker cells create havoc by firing autonomously, and drug treatment will then aim at silencing them again.

The various parts of the excitation-conduction system are hierarchically organized. In a healthy heart, action potentials are generated in the sino-atrial node, which is the topmost center of the excitation-conduction system. From there, the action potentials travel to the atrioventricular node and then spread across the remainder of the system and to the worker cells.⁷

In the sinoatrial node, T type Ca_V channels open slowly and spontaneously at the resting potential, causing a gradual depolarization. Once this depolarization reaches the firing level of the faster and more abundant L type Ca_V channels, these open rapidly and trigger an action potential.

In the worker muscle cells, the action potential is initiated by Na_V channels and sustained for the duration of the contraction through Ca_V channels. Repolarization is mediated by L channel inactivation and by opening of some fairly tardily respond-

potentials; in contrast, in the heart and in smooth muscle, each action potential is sustained for the entire duration of the contraction.

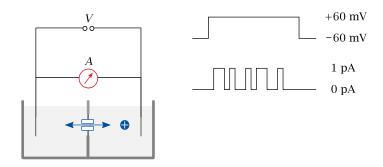
⁷The lower parts of the excitation-conduction system can also spontaneously produce action potentials, but do so at a slower rate, and their internal timers get reset each time an action potential arrives from above. However, when the sinoatrial node fails, another center—usually the atrioventricular node—kicks in with its own, somewhat slower rhythm.

ing K_V channels. Among these, the so-called hERG channels have a prominent role as drug targets and antitargets (see section 6.6).

6.4 Measuring ion fluxes across single channels

One of the allures that ion channels hold for many researchers is the ease and detail with which the behavior of individual channel molecules can be studied. Here, we take a brief look at how this is done.

6.4.1 Planar lipid bilayers

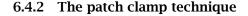


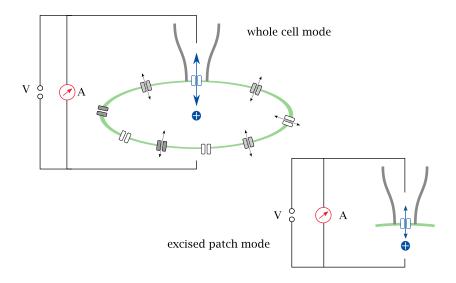
In this experimental setup, a single lipid bilayer is "painted" across a small aperture between two buffered reservoirs. Purified channel molecules, typically solubilized with some non-denaturing detergent, are introduced into one reservoir. Membrane proteins prepared in this way tend to spontaneously insert again into lipid bilayers when offered the opportunity; the right amount of protein sample that will cause just one channel molecule to find and insert into the membrane patch is determined by trial and error.

Electrodes are inserted into the buffer reservoirs on both sides of the bilayer. Voltage is applied, and the opening and closing of the channel can be observed, typically in the form of discrete jumps of constant magnitude that is proportional to the conductivity of the channel molecule.

The buffer solutions usually contain salts in high concentration (~1 M), since this will yield greater currents in response to channel opening. Even then, the currents are typically on the order of only 1 pA.⁸ Additional compounds—drugs, for example—can be introduced into either chamber to examine their effect on the channel from either end.

⁸Note that this still works out to a flow rate of $\sim 6 \times 10^6$ ions per second. Unlike glucose transporters etc., which undergo conformational changes with each molecule transported, ion channels just remain static while the ions zip through in rapid succession.





In the patch clamp technique, the channel is observed in its natural environment within the cell membrane; therefore, interactions with regulatory proteins or other effectors are preserved. Isolated observation of a single channel, or of a small number of channels, is accomplished by gently lowering a glass micro-pipette onto the cell surface, such that it forms a seal with the cell membrane. All current through the pipette must then flow through the membrane patch delineated by the pipette aperture, and through any channels this patch happens to contain.

Patch clamp experiments can be performed either in whole-cell mode or in excised-patch mode. In whole-cell mode, the current has to pass the membrane twice in order to close the circuit: once across the sealed patch, and once across the remainder of the cell surface. However, the latter contains very many channel molecules working in parallel, causing negligible ohmic resistance; therefore, the current is controlled almost exclusively by the channels in the patch. In excised-patch mode, the pipette is withdrawn after seal formation; the cell membrane ruptures, with the pipette holding on to the sealed patch of membrane. This makes it possible to experimentally vary the buffer milieu on both the extracellular side and the intracellular side of the membrane.

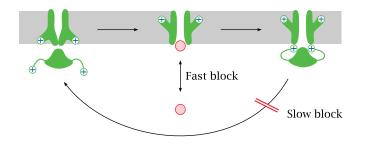
Channels of different specificities will usually be present in the membrane patch, and in order to selectively observe one species, some others may need to be suppressed, either through addition of specific inhibitors, or through elimination of their specific substrate ions from the buffer. For example, sodium and potassium channels may be suppressed by replacing Na⁺ and K⁺ with tetraethylammonium ions; this trick can be used to selectively detect calcium currents.

6.5 Drug effects on voltage-gated sodium channels

Sodium channels play a crucial role in the propagation of action potentials, and thus they are a good target when inhibition of nerve conduction is desired, as it is in local

anesthesia. In addition, inhibitors of sodium channels are also used in several other situations that require neural excitability to be dampened.

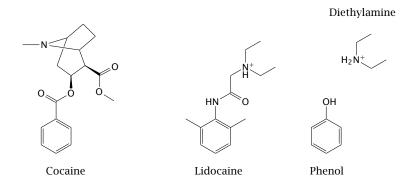
6.5.1 Fast and slow channel block



Channel-blocking drugs may cause either fast or slow blocks. A fast block occurs when a drug reversibly binds within the channel lumen and obstructs it.

A slow block is observed when a drug binds to the inactivated state of the channel and delays its reactivation. Both fast and slow blocks may be *use-dependent*, which means that the channel needs to be activated in order to allow the drugs to access their binding sites and apply the block.

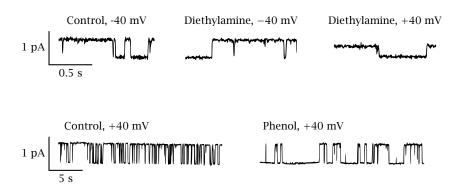
6.5.2 Diethylamine and phenol resemble parts of the lidocaine molecule



The drug lidocaine is used for local anesthesia and in the treatment of certain types of cardiac arrhythmias. It causes both slow and fast block at the Na_V channel. The model compounds diethylamine and phenol resemble parts of the lidocaine molecule; as we will see in the next slide, they also mimic partial activities of it.

Cocaine also blocks sodium channels and can be used for local anesthesia,⁹ but it is no longer used in this application. This activity of cocaine is unrelated to its psychotropic effect that is caused in dopaminergic synapses (slide 6.14.3).

⁹The discovery was facilitated by this drug's liberal recreational use in Vienna in the late 19th century. Karl Köller, who is credited with the invention, recounts [43]: "On a certain occasion, another colleague, Dr. Engel, shared some cocaine with me, on the tip of his pocket-knife, and observed: 'It really numbs the tongue!' To which I replied: 'Yes, this has been noticed by everyone who has eaten it.' At that moment I realized that I was carrying in my pocket the local anesthetic I had been searching a few years ago."

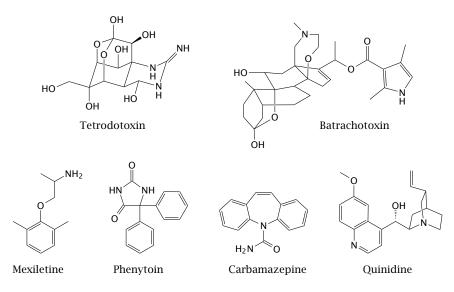


6.5.3 Effects of diethylamine and of phenol on Nav channel conductance

This slide shows a series of single channel recordings on Na_V channels in planar lipid bilayers. The channels were biased towards the open state with batrachotoxin (see next slide). Diethylamine reversibly blocks the open state; the block is more pronounced if the membrane potential pushes the drug into the channel (as is the case at +40 mV). Individual binding and dissociation events occur faster than can be resolved by the recording electronics, which therefore averages them out and reports an apparent decrease in the conductance. Such behavior constitutes a fast block.

In contrast to diethylamine, phenol measurably extends the time intervals of channel inactivity but does not alter the observed conductivity of the open state. This effect represents a slow block. Thus, it appears that diethylamine and phenol respectively account for the dual fast and slow block that was reported previously for lidocaine. Figure adapted from [44].

6.5.4 Drugs and poisons that act on Na_V channels



Batrachotoxin, which is found in certain frogs and some other animal species, interacts with the channel from within the lipid bilayer and activates it. It has no the rapeutic application but is useful in experimental studies on Na_{V} channels, such as the one illustrated in the preceding slide.

All other molecules shown here are Na_V channel blockers. Tetrodotoxin is the poison found in pufferfish. Mexiletine is a more metabolically stable analogue of lidocaine; it can be applied orally and is used in some forms of cardiac arrhythmia, as is quinidine. Carbamazepine is used to treat epilepsy and some other neurological and psychiatric diseases.

Phenytoin is also used in epilepsy. In addition to blocking sodium channels, it also affects some types of potassium and calcium channels. These latter effects may give rise to cardiac arrhythmias but also contribute to its antiepileptic efficacy.

6.6 Pharmacology of potassium channels

Among the various subtypes of voltage-gated potassium channels, the most important drug target is the so-called hERG channel, which mediates the repolarization and terminates the action potential (see slide 6.3.5). Inhibition of hERG channels with drugs such as sotalol (slide 6.6.3) and amiodarone (slide 7.3.6) extends the duration of the action potential and of the subsequent refractory period. This is useful in some forms of cardiac arrhythmia. However, hERG channel inhibitors also may trigger their own peculiar forms of arrhythmias, sometimes leading to sudden cardiac death. Blockade of hERG channels is also a remarkably frequent occurrence with drugs that primarily act on different targets entirely; an example is terfenadine [45], which accordingly has been supplanted by its metabolite fexofenadine (see slide 4.2.2). Novel drug candidates are nowadays routinely screened for this particular side effect.

In addition to voltage-gated potassium channels, there are several other families of potassium channels. K^+ leak channels, which are always open, dominate the resting potential, whereas other K^+ channel types are regulated by various ligands, including Ca⁺⁺ or G protein $\beta\gamma$ -dimers (see slide 5.3.1).

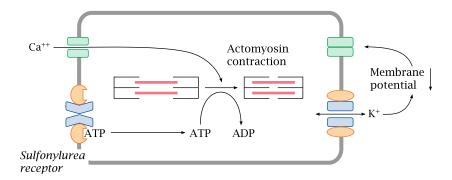
A pharmacologically important K^+ channel, which belongs to the K_{ir} family,¹⁰ is associated with and controlled by another membrane protein, the sulfonylurea receptor. This receptor functions as an ATP sensor; when bound to ATP, it closes the channel. The complex comprising the channel and the receptor is referred to as the K_{ATP} channel.

6.6.1 K_{ATP} channels regulate the tone of smooth muscle cells

In vascular smooth muscle cells, the K_{ATP} channel serves to regulate the strength of contraction. Sustained contraction of the cell will deplete ATP. This will promote dissociation of ATP from the sulfonylurea receptor and open the connected K_{ir} channel. The increase in K^+ permeability will hyperpolarize the membrane, that is,

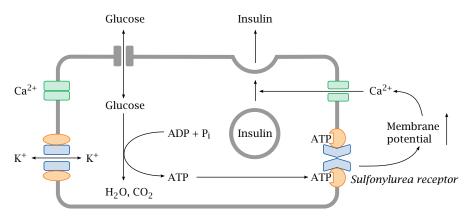
¹⁰The "ir" stands for "inward rectifier", meaning that these channels let K⁺ ions in more readily than out. However, the difference between the inward and the outward currents is not very large, and it is actually the outward permeability that is responsible for their main physiological function.

lower the membrane potential, and thus inhibit the activation of Ca_V channels; the overall effect will be inhibition of contraction. If a smooth muscle cell is exhausted and depleted of ATP, opening of the K_{ATP} channels lets it "tune out" calcium signals and suspend contraction until it has caught its breath and replenished ATP.



Drugs such as diazoxide (slide 6.6.3) that counteract the effect of ATP on the sulfonylurea receptor will keep the K_{ir} open and promote relaxation of vascular smooth muscle cells. This is an effective means to lower blood pressure.

6.6.2 K_{ATP} channels in pancreatic β cells regulate insulin secretion



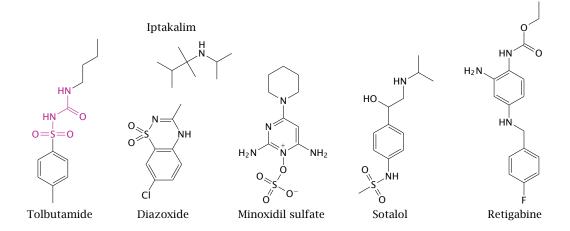
 K_{ATP} channels also occur in the β cells of pancreatic islets, where they regulate the release of insulin in response to changing blood glucose levels. While most cells require insulin for the uptake of glucose, the islet cells themselves do not; therefore, the intracellular glucose levels will rise and fall with blood glucose.

When blood glucose is high, the islet cells will take up more of it and then degrade it. This produces ATP, which binds the sulfonylurea effector, closes K_{ir}, *increases* the membrane potential and thereby promotes calcium-mediated insulin release. Pharmacological activation of the sulfonylurea receptor in order to increase insulin secretion is a useful strategy in type 2 diabetics, who, in contrast to type 1 diabetics, retain the ability to produce their own insulin.

Note that the intended effects on the K_{ATP} channel differ between the two applications: in type 2 diabetics, we want the channel to close, whereas in hypertension

we want it to open. This leads to mutual side effects of antidiabetic and antihypertensive therapy. However, the channel molecules found on β cells and smooth muscle cells are slightly different, which may be exploited by selective drugs to reduce this mutual interference.

6.6.3 Drugs that act on K⁺ channels



Tolbutamide is a sulfonylurea receptor agonist and closes the associated K_{ir} channel. It promotes insulin secretion from pancreatic β cells and is used for oral therapy in type II diabetics. (In the structure of tolbutamide, the sulfonylurea moiety is highlighted.)

Diazoxide and minoxidil sulfate are sulfonylurea receptor antagonists. They open K_{ir} channels on vascular smooth muscle cells, which induces relaxation and lowers the blood pressure. The experimental drug iptakalim reportedly opens K_{ir} channels in the vasculature but closes those in pancreatic β cells.

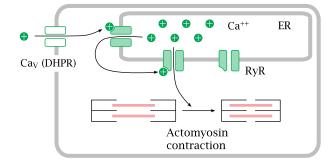
Sotalol inhibits K_V channels of the hERG type; this inhibition delays repolarization and prolongs the action potential. The drug also blocks β -adrenergic receptors. In combination, these two effects can be useful in certain forms of cardiac arrhythmias. Retigabine activates neuronal KCNQ channels, which also belong to the K_V family. It reduces neuronal excitability and is used to treat epilepsy.

6.7 Pharmacology of calcium channels

Calcium channels have a prominent role in controlling the rhythm and contraction of the heart (see slide 6.3.5). Drugs that affect T type Ca_V channels will preferentially act on the excitation-conduction system, whereas sodium channel blockers such as lidocaine will act mostly on the worker cells. Drugs that act on K⁺ channels or L type calcium channels will affect both cell types.

Calcium channels also control the activity of vascular smooth muscle and the release of neurotransmitters from presynaptic terminals (see slide 6.1.3). All of these physiological effects have found pharmacological applications.

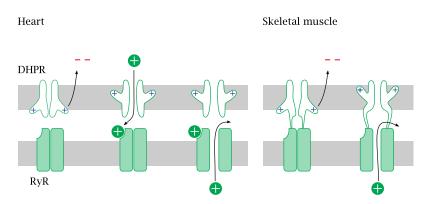
6.7.1 Two calcium channels control the contraction of striated muscle cells



Voltage-gated calcium channels of type L are also referred to as dihydropyridine receptors (DHPRs). They control contraction both in the heart and in skeletal muscle cells.

When an action potential arrives and opens the channel, calcium flows in and opens the ryanodine receptor (RyR), a calcium-activated calcium channel that sits in the membrane of the endoplasmic reticulum. The large secondary wave of calcium released by the open RyR then triggers actomyosin contraction.

6.7.2 Entry of Ca⁺⁺ through the DHPR is necessary in the heart, but not in skeletal muscle cells

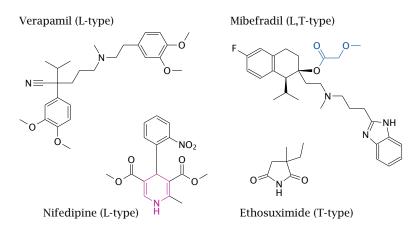


There exists a surprising difference in the interaction of the two calcium channels between heart and skeletal muscle. In the heart, activation of the ryanodine receptor (RyR) requires actual influx of Ca⁺⁺ through the dihydropyridine receptor (DHPR), which opens during an action potential.

In skeletal muscle, the two channels are hooked up directly to each other. Through this connection, the conformational change that occurs in the DHPR upon membrane depolarization is communicated to the RyR and causes it to open. Therefore, no actual influx of extracellular Ca⁺⁺ is necessary—contraction can still occur even if the DHPR channel is plugged up, as long as it still performs its conformational pantomime of gate opening. Therefore, DHPR channel blockers will not interfere with contraction of skeletal muscle.

DHPR channels are also found in vascular smooth muscle. As in heart muscle cells, they don't couple directly to ryanodine receptors. Therefore, DHPR blockers will affect the function of the heart and the blood vessels but not of the skeletal muscles.

6.7.3 Inhibitors of voltage-gated calcium channels



Nifedipine and verapamil both inhibit L type Ca_V channels. As stated above, these channels are also called dihydropyridine receptors (DHPRs), after the dihydropyridine moiety that occurs in some inhibitors. In the structure of nifedipine, the dihydropyridine group is highlighted.

Mibefradil inhibits both L type and T type calcium channels. Its effect on L type channels has been ascribed to an active metabolite in which the highlighted substituent is hydrolytically removed. Replacement of this group with a cyclopropyl moiety reportedly produces a selective T type channel blocker [46].¹¹

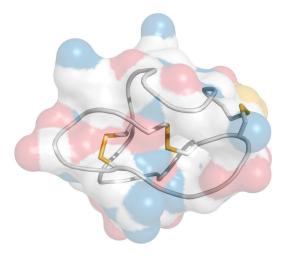
Ethosuximide selectively blocks specific T type Ca_V channel variants that are found on nerve cells in the brain. The drug is used in specific forms of epilepsy.

6.7.4 Structure of ω -conotoxin, an inhibitor of N-type Ca_V channels

N type channels are those Ca_V channels that are found on presynaptic nerve terminals and mediate neurotransmitter release (see slide 6.1.3).

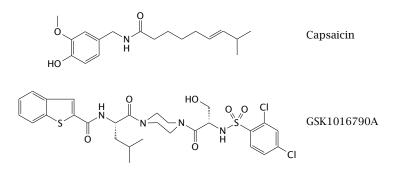
The ω -conotoxins, produced by cone snails, selectively block N type channels. Some conotoxins are of interest in the experimental study or treatment of pain [48]. The figure shows ω -conotoxin CVID. Oxygen is rendered in red, nitrogen in blue. Disulfide bonds are rendered in yellow.

¹¹Mibefradil was withdrawn from the market due to strong inhibition of cytochrome P450 3A4 [47].



6.7.5 Agonists of transient receptor potential (TRP) channels

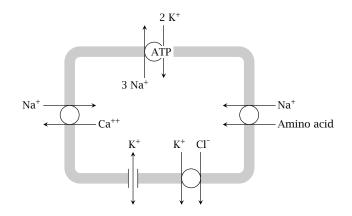
Transient receptor potential channels conduct calcium as well as other cations.¹² They respond to various types of physical stimuli, such as heat or mechanical tension, and are found in various types of sensory cells, such as those for sound waves in the inner ear and for heat in the skin and mucous membranes. They may also be activated chemically; for example, the TRPV1 receptor is activated both by heat and by capsaicin, the active ingredient of chili peppers—which validates the ambiguity of our notion of "hot" food.



The experimental drug GSK1016790A activates the mechanosensitive TRPV4 channel, which is found in the circulation and acts as a blood pressure sensor. Activation of this receptor will feign an elevation of blood pressure to the autonomic nervous system and trick it into issuing a reflex to relax the blood vessels and lower the pressure. However, activation of the same receptor also causes leakiness of capillary endothelia [49], which makes drugs of this type unsuitable for therapy.

¹²Such low specificity occurs with channels that transport hydrated ions. The same is also observed with the nicotinic acetylcholine receptor and some other ligand-gated channels.

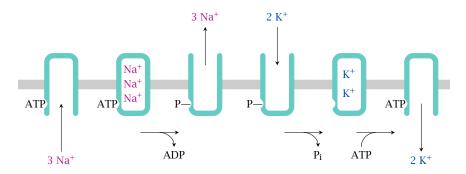
6.8 Na⁺/K⁺-ATPase maintains the ion gradients at the plasma membrane



We had seen earlier that, with the exception of calcium, the ion concentrations are not changed significantly by the fluxes that occur during single action potentials. However, over time, the repetitive firing of action potentials will wear down the ion gradients. Therefore, all ion gradients must be maintained through active transport that ultimately depends on ATP expenditure.

The key molecule in maintaining the cellular ion concentration gradients is Na^+/K^+ -ATPase, which keeps up the gradients of the two major cations; secondary active transport coupled to Na^+ or K^+ sustains the other ion gradients. The high extracellular sodium concentration drives calcium antiport as well as symport of amino acids and other metabolites. Potassium symport drives the export of chloride from the cell. Potassium leak channels maintain the negative-inside resting potential potential.

6.8.1 Functional cycle of Na⁺/K⁺-ATPase



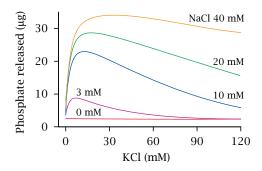
The functional cycle of Na⁺/K⁺-ATPase involves the following stages:

- 1. The ATP-bound form of the enzyme is open to the cytosol and accepts three Na⁺ ions. Bound Na⁺ becomes occluded inside the enzyme.
- 2. The occluded enzyme phosphorylates itself and releases ADP. The phosphorylated molecule everts and releases the bound Na⁺ to the extracellular space.
- 3. Two K⁺ ions bind from the outside and become occluded. Concomitantly, phosphate is hydrolyzed.

4. ATP binds, which opens the enzyme to the intracellular side. This releases K⁺ and restores the enzyme to its initial state.

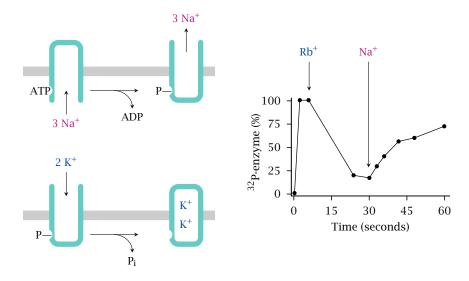
This sequence of events has been pieced together from numerous experimental studies. The following slides present some of the key experiments.

6.8.2 Na⁺/K⁺-ATPase activity as a function of KCl and NaCl concentrations



Neither NaCl nor KCl alone elicit significant ATP cleavage activity. Maximal ATP hydrolysis is observed at approximately equal concentrations of KCl and NaCl. In this experiment, the enzyme had been extracted from membranes; therefore, only ATP hydrolysis, but no transport phenomena were observed. This early result gave the enzyme its name. Figure prepared from original data in [50].

6.8.3 Effects of Rb⁺ and of Na⁺ on the phosphorylation state of Na⁺/K⁺-ATPase

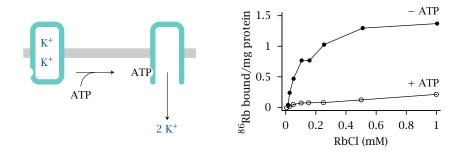


In this experiment, the enzyme was incubated with ³²P-labeled ATP. At time zero, only Na⁺ is present, and phosphorylated enzyme accumulates; therefore, sodium induces autophosphorylation of the enzyme, but not its dephosphorylation.

Addition of Rb⁺, which mimics K⁺, dephosphorylates the enzyme; Na⁺ added in excess over Rb⁺ phosphorylates it again. Therefore, binding—and, presumably,

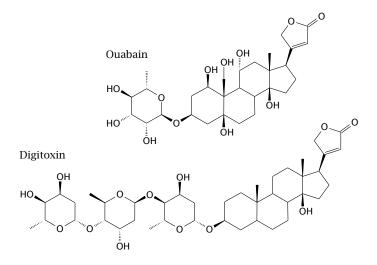
transport—of sodium is associated with phosphorylation, whereas binding of K⁺ is associated with dephosphorylation. This explains why both are required for continuous, catalytic cleavage of ATP. Figure prepared from original data in [51].

6.8.4 ATP is required to release Rb⁺ from tight binding to Na⁺/K⁺-ATPase



As stated before, Rb^+ is a substitute for K^+ .¹³ Tight binding of Rb^+ is interpreted as occlusion of the ion within the enzyme. Figure prepared from original data in [52].

6.8.5 Structures of the Na⁺/K⁺-ATPase inhibitors ouabain and digitoxin



As discussed earlier (see slide 6.8), the Ca^{++} gradient across the cytoplasmic membrane is sustained by the Na^+ gradient, which in turn is maintained by Na^+/K^+ -ATPase. Therefore, inhibition of this enzyme will increase the intracellular calcium level. In muscle cells, this will tend to increase the force of contraction.

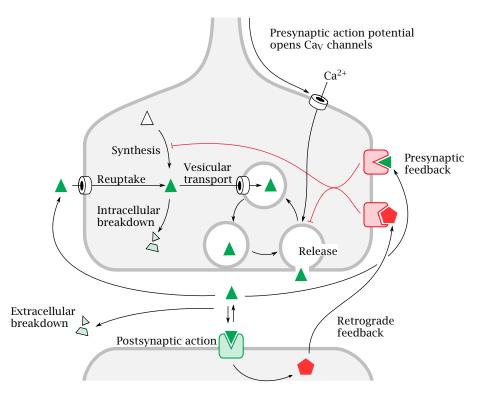
The Na^+/K^+ -ATPase inhibitors digitoxin and the related drug digoxin are isolated from the foxglove plant (*Digitalis purpurea*). They are used clinically in the treatment of cardiac insufficiency to augment the contractile force of the heart muscle. Ouabain has the same effect but is inconvenient for clinical use because of rapid elimination; it is mostly used experimentally.

¹³The rubidium isotope ⁸⁶Rb is radioactive and thus is easily quantified. It is often used instead of K^+ because none of the potassium radioisotopes have experimentally convenient half lives.

6.9 Overview of synaptic transmission

In a chemical synapse, the electrical signals traveling around the nervous system are transiently converted to chemical signals. The transmitter substances used in different types of nerve cells are quite diverse, and specific cell types or neural subsystems can be addressed in a more selective manner than is usually possible with the drug targets we have considered so far. Accordingly, most drugs that act on the central nervous system target chemical synapses.

6.9.1 Function of a chemical synapse



Synaptic transmission is initiated by N-type Ca_V channels, which are activated by action potentials arriving via the axon from upstream. Ca^{++} ions enter the nerve terminal and trigger exocytosis of vesicles loaded with neurotransmitter. Released transmitter molecules bind and activate postsynaptic receptors. Depending on the functional properties of these receptors, the postsynaptic cell is either excited or inhibited.

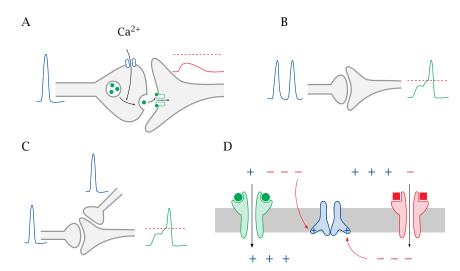
In response to stimulation by transmitters, a postsynaptic cell may release mediators that exert retrograde negative feedback on the presynaptic cell.¹⁴ The transmitter itself can also cause negative presynaptic feedback, through receptors that usually differ from those found on the postsynaptic membrane.

A short while after its release, the transmitter is inactivated; this may occur through either extracellular breakdown or presynaptic reuptake. Transmitter vesi-

¹⁴These mediators are endocannabinoids; we will discuss them in more detail later (see slide 9.7).

cles are recycled and loaded with transmitter through active transport. The pool of transmitter for loading into the vesicles is controlled by the rates of transmitter synthesis, degradation, and reuptake.

All these processes provide drug targets and create opportunities for pharmacotherapy, and we will see one or more example drugs for each of them.



6.9.2 Summation of postsynaptic potentials

We have seen before that a given nerve cell may receive input from a large number of other nerve cells upstream. If each nerve cell were to trigger an all-out action potential in response to every single stimulus from upstream, all cells would very soon be mutually stimulating each other to exhaustion. Therefore, a nerve cell does not respond all out to a single upstream stimulus but instead requires multiple input stimuli before firing.¹⁵ This slide explains how that works.

- (A) In excitatory synapses between nerve cells, a single presynaptic action potential causes a localized partial depolarization, the excitatory postsynaptic potential (EPSP). A single EPSP will remain below the firing level and not trigger a postsynaptic action potential.
- (B) An action potential may form if a single synapse fires repeatedly before the EPSP dissipates; this is known as temporal summation.
- (C) Alternatively, in spatial summation, the EPSPs caused by neighboring synapses firing at the same time may combine to trigger an action potential.
- (D) Inhibitory synapses, which often employ chloride channels, cause inhibitory postsynaptic potentials (IPSPs), which counteract the activation of voltage-gated channels by EPSPs in the vicinity.

The firing level that may or may not be reached by EPSPs is the threshold voltage at which the neighboring voltage-gated ion channels will be activated and set off a

¹⁵As an interesting aside, in neuromuscular synapses, a single presynaptic action potential *is* sufficient for triggering a postsynaptic action potential. This agrees with the fact that a skeletal muscle cell receives input only from a single nerve cell.

self-sustaining wave of depolarization, that is, an action potential (see slide 6.3.3). Also note that, unlike in this cartoon, summation of more than just two EPSPs will be required to actually trigger a postsynaptic potential.

6.9.3 Neurotransmitter receptor families

- 1. Ligand-gated channels
 - (a) Cys-loop family
 - (b) Glutamate receptors
 - (c) Purine P2X receptors
- 2. G protein-coupled receptors

The most straightforward drug targets, and also the most widely used ones, are the neurotransmitter receptors on the postsynaptic membranes. The feedback receptors on the presynaptic membranes are sometimes targeted as well. All of these receptors belong to two major functional classes:

1. Ligand-gated channels, also referred to as *ionotropic receptors*, open in response to the binding of their cognate transmitters and thereby directly change the potential at the postsynaptic membrane. This mode of action is very rapid. Ligand-gated channels fall into three distinct structural families, the members of which—in the above order, from (a) to (c)—have five, four, and three subunits, respectively.

2. G protein-coupled receptors affect the membrane potential in various and somewhat more roundabout ways. One important signaling mechanism is through adenylate cyclase, cAMP, and cyclic nucleotide-gated (CNG) channels. The CNG channels conduct all major cations; on balance, they will depolarize the membrane and promote formation of postsynaptic action potentials.

Most transmitters have multiple receptor types, and some, like glutamate and acetylcholine, have receptors among both the ligand-gated channels and the GPCRs.

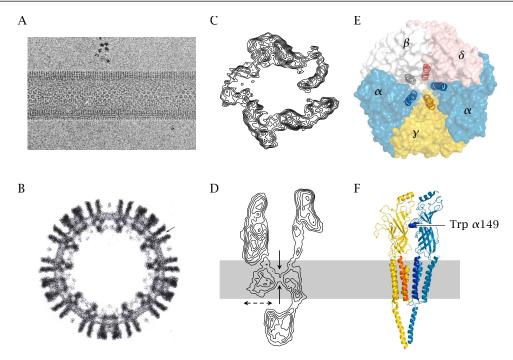
6.10 Structure, function, and pharmacology of cys-loop receptors

Among the ligand-gated channels, this family contains the most clinically important drug targets, and it also contains the most widely studied ionotropic receptor molecule, namely, the nicotinic acetylcholine receptor.

6.10.1 Structure of the nicotinic acetylcholine receptor

The nicotinic acetylcholine receptor (NAR) conducts cations, in a fairly nonselective way; Na^+ , K^+ and Ca^{++} can all get across. It depolarizes the postsynaptic membrane.¹⁶

¹⁶If we assume equal permeabilities for sodium and potassium, we would, according to slides 6.2.2 and 6.2.4, expect that the EPSP created by the NAR would top out at about -15 mV. This is still negative, so how can it trigger opening of the adjacent Na_V channels? The answer is that full

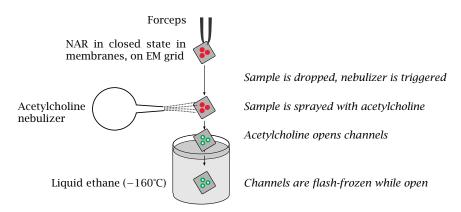


The structure shown here has been worked out with electron crystallography, which is sort of a hybrid between conventional crystallography and electron microscopy. The individual panels show the following:

- **A, B:** Electron micrographs of tubular synaptic membrane particles isolated from *Torpedo* electric organs. The cross-section in B already shows the major structural features of the receptor. The formation of dense, regularly packed arrays as seen in A allows the collection of in-phase electron diffraction patterns, from which higher resolution contour maps can be constructed.
- **C, D:** Contour maps obtained by electron crystallography. In the side view (D), a constriction within the bilayer is clearly visible. The receptor has an intracellular domain with lateral openings.
- **E:** High resolution structural model, top view. The receptor consists of five subunits, each of which contributes one helix to the gate located at the level of the lipid bilayer.
- F: Side view of the structural model. Only one of the α chains and the adjacent γ chain are shown. The tryptophan residue at position 149 of the α chain is part of one of the two acetylcholine binding sites, which are located at the α - γ and the α - δ interfaces, respectively.

The subunit composition illustrated here applies to nicotinic receptors in neuromuscular synapses. Receptors at other anatomical sites differ in subunit composition, and some drugs discriminate between these different receptor types. Panels A–D courtesy of Nigel Unwin; E and F rendered from 2bg9.pdb.

membrane depolarization is not required for those channels to respond, since their firing level is at $-55 \,\mathrm{mV}$.



6.10.2 Trapping the nicotinic acetylcholine receptor in the open state

Nigel Unwin managed to study the structure of the nicotinic acetylcholine receptor both in the open and the closed state. Now, considering that the open state has a lifetime of only a few milliseconds, how did he manage to prepare it, and then stabilize it so that it could be imaged? This slide explains his experimental setup.

- 1. The membrane vesicles containing the receptors, in 2D crystal arrangement and in the closed state, are mounted on an EM sample support and held in a forceps.
- 2. When the sample is dropped, it passes through a stream of vaporized acetylcholine. Binding of acetylcholine opens the channels. Immediately thereafter, the sample plunges straight into cold, liquid ethane, and the channels are shockfrozen in the open state.
- 3. Data acquisition for electron crystallography is performed at low temperatures, so as to preserve the receptor in its open state.

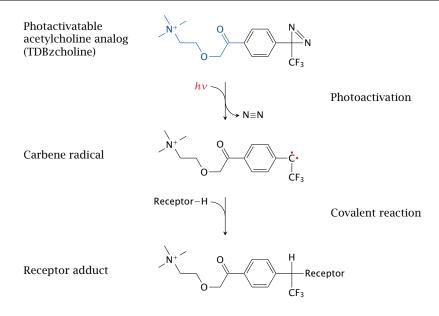
Rather brilliant, isn't it? One of my all time favorite experiments.

6.10.3 Photoaffinity labeling of the acetylcholine binding site

The binding sites of acetylcholine in the receptor have been identified not by 3Dstructural methods but by affinity labeling and protein chemistry. Multiple studies using different affinity probes have revealed different features of the binding sites; one such study is summarized here as an example.

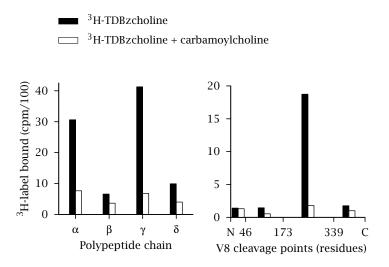
The probe used in this experiment, ³H-TDBzcholine, contains a moiety that resembles acetylcholine (highlighted in blue), which steers it toward the acetylcholine binding site of the receptor. It is radioactively labeled and also photoactivatable, which enables it to covalently react with the receptor.

After allowing time for binding of the probe to the receptor, the probe is activated by exposing the sample to UV light. The energy of a photon absorbed by the probe's aromatic ring induces the release of nitrogen. This leaves behind a highly reactive carbene radical, which immediately reacts with amino acid residues in the vicinity. To identify the labeled amino acid residues, the receptor adduct is then fragmented in a stepwise fashion, as shown in the following slides.



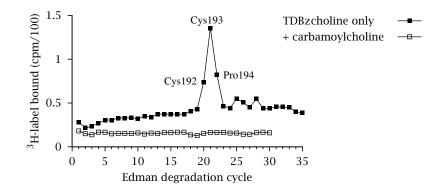
6.10.4 Isolation of affinity-labeled polypeptide chains and fragments

In the first fractionation step, the hetero-oligomeric receptor was simply boiled in SDS and subjected to SDS-PAGE (left panel). The greatest extent of radiolabel incorporation was observed with the α and the γ chain, indicating that these two chains surround the binding site.



In the second step, the labeled chains were fragmented by proteolysis. The protease used here, staphylococcal V8 protease, cleaves specifically after aspartic and glutamic acid residues. The fragments were separated by HPLC and identified by N-terminal sequencing. In the case of the α chain (right panel), the radioactivity was associated with a fragment of 20 kDa that spans residues 174–339.

In both steps, control samples were included that had been exposed to ³H-TDBzcholine in the presence of unlabeled carbamoylcholine in excess. The unlabeled ligand displaced the ³H-TDBzcholine from the acetylcholine binding site and largely suppressed labeling. This confirms that ³H-TDBzcholine indeed lodges within the regular acetylcholine binding site and not in some other, nonspecific binding crevice or pocket.

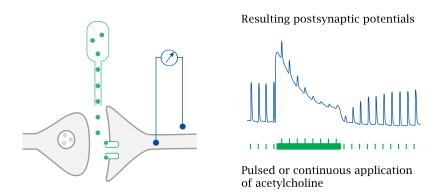


6.10.5 Identification of affinity-labeled amino acid residues

In the final stage of the experiment, the labeled proteolytic fragments were subjected to N-terminal sequencing through Edman degradation in order to identify the individual amino acid residues that had reacted with the label.

In the case of the labeled 20 kDa α -chain fragment shown in the previous slide, residues 192–194 contained the most incorporated label, indicating that they are part of the acetylcholine binding site. Figures in both above slides were prepared from original data in [53].

6.10.6 Desensitization of the nicotinic acetylcholine receptor

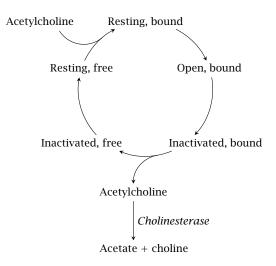


As had been mentioned earlier (slide 2.5.5), the nicotinic acetylcholine receptor undergoes inactivation when bound to its ligand for prolonged periods of time. This property is important in the action mode of the "depolarizing blocker" succinylcholine (slide 6.15.1).

In the experiment illustrated here, acetylcholine was electrophoretically applied to frog muscle neuromuscular synapses, and the resulting postsynaptic potentials were recorded. Repetitive short acetylcholine stimuli evoke postsynaptic potentials of uniform amplitude. When continuous application starts, a strong depolarization occurs, which then declines even in the face of continued transmitter application. The response to superimposed short acetylcholine pulses also declines.

After continuous acetylcholine application is stopped, the response to short acetylcholine pulses recovers over a period of several seconds. Drawn after an original figure in [54].

6.10.7 Functional states of the nicotinic acetylcholine receptor



This slide shows a kinetic scheme for acetylcholine binding, receptor activation, and desensitization. When acetylcholine binds, the receptor initially opens, but then quickly inactivates. Opening is faster than inactivation, but the inactive conformation is more stable; therefore, after sufficient time, most receptor molecules will accumulate in the refractory state.

As long as acetylcholine remains bound, the receptor remains inactive. Dissociation of acetylcholine is driven by cholinesterase, which depletes the free transmitter by cleaving it to choline and acetate. Once the bound transmitter molecule has left, the receptor reverts from the refractory state to the inactive one, from which it can again be activated by binding another acetylcholine molecule.

In contrast to a simple equilibrium reaction, which simply flickers back and forth between its two states, a unidirectional cycle like this one requires an input of energy. With the NAR, this energy is supplied by the hydrolysis of acetylcholine.

6.10.8 Ionotropic receptors in the cys-loop family

The nicotinic acetylcholine receptor occurs in the CNS, in the autonomic nervous system, and in neuromuscular synapses (motor endplates). We will see some drugs that target this receptor in section 6.15, after having examined the organization of the autonomic nervous system.

Receptor	Ion selectivity	Effect	Comments
nicotinic acetylcho- line	cations	excitatory	pharmacologically distinct sub- types, various applications
5-HT ₃ serotonin	cations	excitatory	inhibitors are used to treat emesis
GABA _A	chloride	inhibitory	major drug target in narcosis, epilepsy, psychoses
glycine	chloride	inhibitory	regulates motor activity

Inhibitors of the 5-HT₃ serotonin receptor are used to suppress emesis, particularly in cancer patients undergoing chemotherapy. The 5-HT₃ inhibitor ondansetron is shown in slide 6.12.2.

The GABA_A receptor and the glycine receptor are both chloride channels and therefore, when open, hyperpolarize the postsynaptic cells; they are the most important inhibitory neurotransmitter receptors in the brain. The GABA_A receptor is one of the preeminent drug targets in the CNS; agonists of this channel are used in conditions as diverse as epilepsy, psychoses, insomnia, and narcosis. We have already seen several GABA_A agonists, such as diazepam and oxazepam (slide 4.2.2) as well as phenobarbital (slide 4.1.4) and thiopental (slide 3.6.9). Some more drugs that target GABA_A receptors are shown in slide 6.10.9.

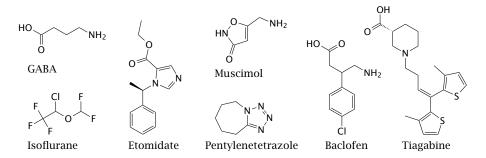
The glycine receptor is less prominent as a drug target; some drugs interacting with it are shown in slide 6.10.10. Nevertheless, it has a crucial role in maintaining a proper balance of excitation and inhibition in the CNS, which is quite strikingly illustrated by the clinical manifestations that result when its function is disrupted. The clinical picture is known as *tetanus* and consists in sustained maximal, uncontrollable contraction of skeletal muscles, to the point where tendons snap and bones break.¹⁷

6.10.9 Drugs that interact with GABA receptors and transporters

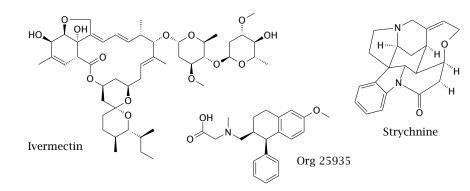
The $GABA_A$ and $GABA_C$ receptors are ligand-gated channels, whereas the $GABA_B$ receptors are GPCRs.

Isoflurane is an inhalation anesthetic, and etomidate is an intravenously applied anesthetic; both are GABA_A receptor agonists. Muscimol is an ingredient of fly agaric (a mushroom) and a GABA_A and GABA_C receptor agonist. Pentylenetetrazol is a GABA_A antagonist used to induce seizures in animal experiments.

¹⁷As you may know, tetanus is caused by tetanus toxin, a protein toxin produced by an anaerobic soil bacterium, *Clostridium tetani*. Tetanus occurs when wounds are infected with this bacterium. The toxin is a protease that cleaves synaptobrevin, a membrane-associated protein essential for transmitter exocytosis. Tetanus can be lethal because it interferes with the alternation of muscular contraction and relaxation required for breathing. Preventive immunization with tetanus toxoid, which is chemically or genetically inactivated tetanus toxin, protects against the disease.



Baclofen is a $GABA_B$ agonist that is used in the treatment of spasticity, for example in patients with multiple sclerosis. Tiagabine is an inhibitor of presynaptic GABA reuptake, which is used in the treatment of epilepsy.



6.10.10 Drugs that interact with glycine receptors and transporters

Ivermectin is an allosteric agonist of the glycine receptor. Its main target, however, is a glutamate receptor/chloride channel that occurs in non-vertebrates including some human worm parasites, but not in humans. Accordingly, the drug is used in the treatment of parasite infections.

Strychnine is an alkaloid that acts as an antagonist of the glycine receptor. Once upon a time, it was used as a stimulant, as well as an important ingredient of murder novels. The symptoms of strychnine poisoning resemble those of tetanus. Wikipedia's page on this subject is well worth a read.

Org 29535 is an inhibitor of glycine reuptake that is being investigated for the treatment of psychosis and addiction.

6.11 Glutamate receptors

Glutamate is the major excitatory transmitter in the brain. It has various receptors, both the among ligand-gated channels and GPCRs.

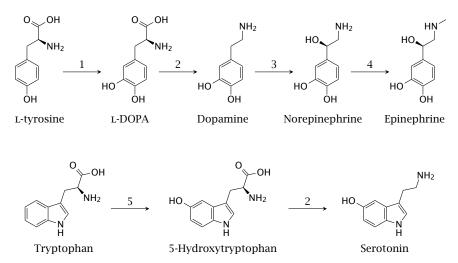
The ionotropic glutamate receptors have four subunits each and thus are structurally distinct from the receptors in the cys-loop family. Ionotropic glutamate receptors are pharmacologically diverse; different types can be selectively activated by the synthetic agonists AMPA, kainate and NMDA, and are accordingly named. Among these receptors, the NMDA receptor is unusual in that one of its four subunits actually binds glycine, not glutamate, and both transmitters are required to activate the receptor. The structures of various glutamate receptor ligands are shown in slide 15.1.1.

While experimental agonists and antagonists with specificity for different glutamate receptors are readily available, compelling clinical applications are scarce [55].

6.12 Pharmacology of dopamine and of serotonin

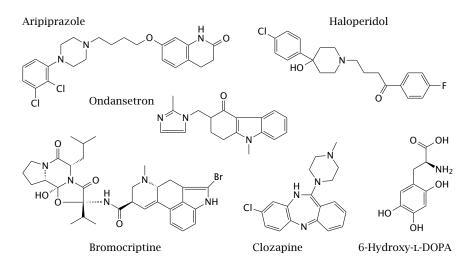
Dopamine belongs to the catecholamines, which also comprise norepinephrine and epinephrine. In their synthesis and degradation, catecholamines share similarities with serotonin. Catecholamines and serotonin also overlap in their pharmacology, and they have prominent roles in regulating mood, vigilance, and voluntary movement. Synapses that use these transmitters are therefore commonly targeted by drug therapy.

6.12.1 Biosynthesis of the catecholamines and of serotonin



The catecholamines are biosynthetically derived from tyrosine, while serotonin is derived from tryptophan. Hydroxylation and decarboxylation occur in both biosynthetic pathways.

Enzymes: 1, tyrosine hydroxylase; 2, L-aromatic acid decarboxylase or DOPA decarboxylase; 3, dopamine β -hydroxylase; 4, norepinephrine methyltransferase; 5, tryptophan hydroxylase.



6.12.2 Drugs that interact with dopaminergic and serotoninergic synapses

Dopamine and serotonin in the brain are both important in the regulation of mood. Dopamine also participates in the control of body movements, and its deficiency gives rise to Parkinson's disease (see slide 3.5.5). Accordingly, Parkinson-like symptoms—rigidity, tremor, and slowness of movement—are a common side effect of some dopamine receptor antagonists that are used as antipsychotic drugs.

Haloperidol is a first-generation antipsychotic and a D_2 dopamine receptor antagonist with a pronounced tendency to cause Parkinson-like symptoms. Bromocriptine is an agonist at the same receptor. It is used in Parkinson's disease and to suppress prolactin secretion; this latter application relates to yet another physiological function of dopamine, namely, the regulation of hormone secretion in the hypothalamus and the hypophyseal gland (see section 7.2).

Clozapine is a second-generation antipsychotic with relatively low affinity for D_2 receptors but higher antagonistic activity on D_3 and D_4 dopamine receptors, as well as on 5-HT_{2A} serotonin receptors. Aripiprazole is another second-generation antipsychotic; it is a partial agonist at the D_2 receptor and otherwise similar to clozapine. Both of these drugs are much less prone to triggering Parkinson-like symptoms than first-generation drugs like haloperidol.

6-Hydroxy-L-DOPA is not a therapeutic drug but rather a poison. It is transported into dopaminergic neurons by reuptake and damages the cells through inhibition of the respiratory chain. It is sometimes used in research to induce a Parkinson-like condition in experimental animals.

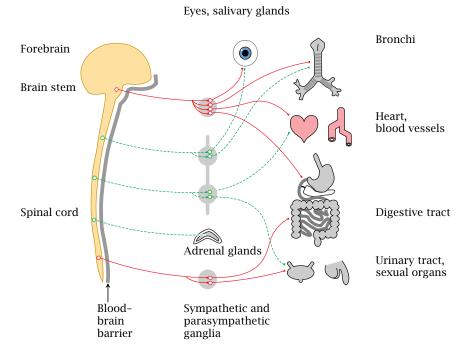
Ondansetron is an antagonist at the 5-HT₃ receptor, the sole ligand-gated channel among the serotonin receptors. It is used mostly in the treatment of chemotherapy-induced nausea in cancer patients.

Another major class of drugs that is used to increase postsynaptic stimulation in dopaminergic and serotoninergic synapses are inhibitors of transmitter reuptake. Some examples will be shown later (see slide 6.14.3).

6.13 The autonomic nervous system

The autonomic nervous system is the part of our nervous system that is not under conscious control. It controls many physiological functions, and therefore contains many targets for drug therapy. Prominent neurotransmitters in the autonomic nervous system are acetylcholine and the catecholamines epinephrine and norepinephrine.

6.13.1 Organization of the autonomic nervous system



The autonomic nervous system comprises parts of both the central and the peripheral nervous system. It contains parasympathetic and sympathetic parts (red and green lines, respectively).

Within the autonomic nervous system, each connection between the CNS and a peripheral organ is made by two neurons connected in series. The cell body of the first neuron is located inside the CNS, but its efferent synapses are located inside a sympathetic or parasympathetic ganglion, outside of the blood brain barrier. The cell body of the corresponding second neuron is also located in this ganglion.

In most organs, the sympathetic and the parasympathetic nervous system exert antagonistic regulatory influences. The sympathetic nervous system will increase blood pressure and heart rate and will dilate the bronchi, while also diverting blood flow from the intestines and other interior organs toward the skeletal muscles. The parasympathetic nervous system will slow down cardiovascular function and promote perfusion and activity of the glands and digestive organs.

Most of the drugs covered in the following act on some type of synapse or other in the autonomic nervous system. The heart, the circulation, and the bronchi are particularly important as target organs for pharmacotherapy related to the autonomic nervous system.

Subsystem	1 st Synapse	2 nd Synapse
sympathetic	nicotinic	α - or β -adrenergic
parasympathetic	nicotinic	muscarinic

6.13.2 Transmitter receptors in the peripheral autonomic nervous system

Nicotinic acetylcholine receptors are found in both the sympathetic and the parasympathetic ganglia. Inhibiting these receptors will therefore sweepingly inhibit both sympathetic and parasympathetic activity. On blood pressure, which is the single most important target parameter for drugs that act on the autonomic nervous system, the net effect will be a reduction. Nicotinic receptor antagonists can therefore be used to treat hypertension, and they were indeed so used in the past; however, because of their numerous side effects, they are now obsolete in this application.

Muscarinic acetylcholine receptors are found in the second synapses, that is, those between the second parasympathetic neurons and the effector cells such as gland and smooth muscle cells. Adrenergic receptors are found in the second synapses of the sympathetic nervous system. α -Adrenergic receptors are prominent in the blood vessels, β_1 receptors in the heart, and β_2 receptors in the bronchi and uterus. Drugs that act on these receptors have fewer side effects than nicotinic receptor agonists and antagonists, and they are more practically important in modern pharmacotherapy.

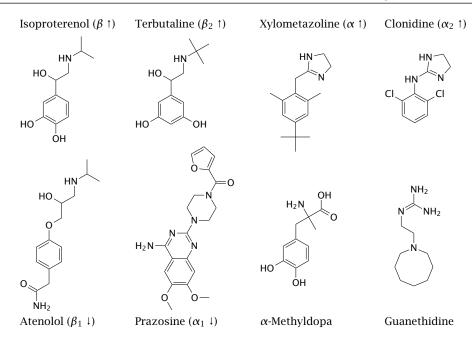
6.14 Pharmacology of adrenergic synapses

The adrenergic synapses in the sympathetic nervous system are targeted by numerous drugs with multiple action modes. We will consider some representative examples.

6.14.1 Receptor agonists or antagonists and false transmitters at adrenergic synapses

In this slide, up- and downward arrows indicate the activities of direct receptor agonists and antagonists, respectively.

Isoproterenol is used to increase the heart rate and the force of heart muscle contraction, while atenolol is used for the opposite purpose. β_2 -Selective adrenergic agonists like terbutaline relax smooth muscle in the bronchi and are used to treat asthma. They also relax smooth muscle in the uterus and are used to suppress premature labor.



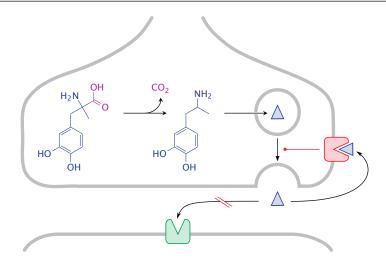
Xylometazoline induces vasoconstriction and is applied locally to the nasal mucous membranes in order to suppress congestion. Clonidine was developed with similar intention, but turned out to be an α_2 -selective agonist [56]. Since α_2 receptors prominently function in presynaptic feedback in adrenergic synapses, it actually *reduces* signaling efficiency in those synapses. It is used in the treatment of hypertension, as well as of various other conditions that range from pain to addiction and point to additional physiological roles of α_2 receptors.

 α -Methyldopa and guanethidine do not interact directly with adrenergic receptors; instead, they act as "false transmitters". This peculiar mode of action is explained in the next slide.

6.14.2 Methyldopa is a false transmitter in noradrenergic synapses

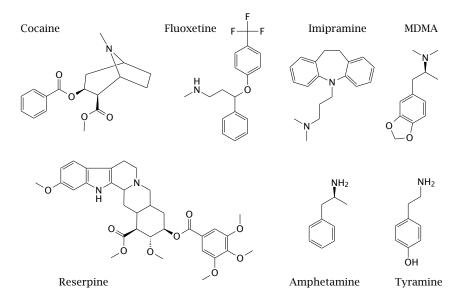
 α -Methyldopa is a prodrug; after crossing the blood brain barrier in the same way as L-DOPA (see slide 3.5.5), it is taken up into adrenergic neurons and undergoes decarboxylation to α -methyldopamine. The metabolite is transported into presynaptic transmitter vesicles. The capacity of these vesicles for storing transmitter is limited, and therefore norepinephrine is displaced. When the presynaptic cell is activated, the false transmitter is released; it fails to stimulate postsynaptic α_1 receptors but activates presynaptic α_2 receptors, which exercise feedback inhibition on presynaptic transmitter release.

 α -Methyldopa has been used for antihypertensive treatment but is no longer widely used. The drug guanethidine (shown in slide 6.14.1) acts in a manner similar to α -methyldopa, but without undergoing metabolic activation and only in the periphery, since it does not cross the blood brain barrier.



6.14.3 Drugs that act on the membrane transport of monoamine transmitters

Aside from direct stimulation neurotransmitter receptors, the inhibition of presynaptic transmitter reuptake is another important mechanism by which synaptic signals can be amplified. This mechanism is important with psychoactive drugs of abuse as well as with regular antidepressant and antipsychotic drugs.



The reuptake transporters for the different monoamine transmitters are distinct but related; some drugs target specific transporters, while others have a broader specificity. Cocaine inhibits the presynaptic reuptake of dopamine, serotonin and norepinephrine, which accounts for its powerful stimulating effect.¹⁸

Fluoxetine and imipramine inhibit the presynaptic reuptake of serotonin and are used as antidepressants. The effect of fluoxetine is specific for serotonin, whereas

¹⁸This might be the last time I'm showing you the structure of cocaine—but no guarantees.

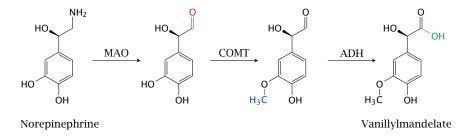
imipramine also affects dopamine reuptake. In addition to these direct effects, enzymatic demethylation of imipramine gives rise to desipramine, which is also used as an antidepressant drug and blocks the reuptake of norepinephrine.

Amphetamine and tyramine release dopamine and norepinephrine from presynaptic storage vesicles and then promote their retrograde transport from the cytosol into the synaptic cleft. *N*-Methyl-3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") does the same to serotonin. It appears that the overall effect involves both specific interaction with the transporter proteins as well as non-ionic diffusion across cell and vesicle membranes [57].

Reserpine blocks the *vesicular monoamine transporter* (VMAT), which transports monoamine transmitters from the cytosol into storage vesicles in presynaptic cells. The VMAT is shared between adrenergic, dopaminergic, and serotoninergic synapses, and accordingly the effects of all of these transmitters are inhibited by reserpine. The drug has been used for antihypertensive therapy in the past, but it has been abandoned due to strong side effects, including impotence and depression.

Reserpine binds to its target noncovalently, but its affinity is so high that it will virtually never dissociate from its target, which illustrates that irreversible and covalent binding do not always coincide. It has recently attracted some renewed interest as an inhibitor of ABC transporters such as P-glycoprotein.¹⁹

6.14.4 Degradation of norepinephrine



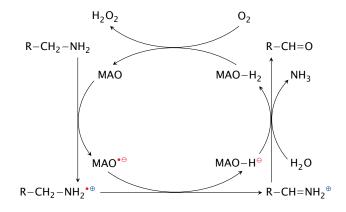
Synaptic signaling can also be influenced by inhibiting the degradation of transmitters. This slide illustrates the degradation pathway of norepinephrine; the pathways for dopamine and epinephrine are analogous. Either monoamine oxidase (MAO) or catechol-*O*-methyltransferase (COMT) may act on the substrate first, which gives rise to different intermediates but the same end product. The aldehyde group formed by monoamine oxidase can either be oxidized to the acid by aldehyde dehydrogenase (ADH) as shown here, or it can be reduced to the alcohol by aldehyde reductase. Both end products are excreted in the urine.

In the case of the catecholamines, both MAO and COMT can be targeted in order to increase synaptic transmitter levels. MAO is also involved in the degradation of serotonin. Monoamine oxidase inhibitors have been used in the past as antidepres-

¹⁹Aside from reserpine, verapamil and quinidine also inhibit ABC transporters. Intriguingly, the primary targets of all three drugs are transport proteins other than ABC transporters.

sants, but are now obsolete in this application. However, inhibitors of both MAO and COMT are still of interest as adjuvant treatments in Parkinson's disease.

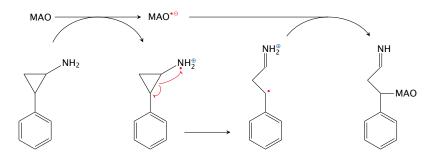
6.14.5 Reaction mechanism of monoamine oxidase (MAO)



Monoamine oxidase contains a flavin coenzyme, which abstracts two electrons from the substrate in two successive steps. Abstraction of the first electron converts both the substrate and the enzyme to radicals. On the enzyme, the unpaired electron may remain with the flavin or migrate to a cysteine residue in the active site.

The abstraction of the second electron and of a proton converts the substrate to a Schiff base, which is then hydrolyzed to an aldehyde and free ammonia. MAO itself is reoxidized by molecular oxygen, which forms H_2O_2 in the process.

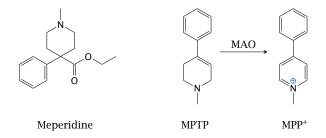
6.14.6 Mechanism-based inhibition of MAO by tranylcypromine



Tranylcypromine covalently inhibits monoamine oxidase by trapping the radical reaction intermediate. In the drug molecule, the amino group is attached to a reactive cyclopropyl ring, which upon the initial electron withdrawal opens up to produce a carbon radical. This carbon radical then combines with the radical intermediate of the enzyme.

MAO occurs not only in the brain but also in the liver. Its fairly broad specificity means that it can engage in some oxidative reactions in the metabolism of drugs and xenobiotics (see next slide). Liver MAO also scavenges aromatic amines such as tyramine, which are found in cheese and other fermented foods. Like amphetamine, tyramine releases norepinephrine and epinephrine from synaptic storage vesicles (see slide 6.14.3) and also from adrenal glands [58]. In patients treated with MAO inhibitors, the release of catecholamines by unscavenged tyramine can evoke an episode of hypertension, the so-called "cheese reaction."

6.14.7 MAO-induced toxicity of MPTP (N-methyl-4-phenyl-tetrahydropyridine)



A xenobiotic metabolized by MAO is *N*-methyl-4-phenyl-tetrahydro-pyridine (MPTP). This is not a drug; instead, it has been found as a by-product in the synthesis of meperidine, a synthetic opioid. It enters dopaminergic neurons via the dopamine reuptake transporter. Inside the cells, it is converted by MAO to methyl-phenyl-pyridinium (MPP⁺), which inhibits complex I of the respiratory chain and causes cell death.

In the 1980s, a batch of MPTP-contaminated meperidine that had been made in an illicit lab in Vancouver caused several drug addicts in California to develop symptoms of Parkinson's disease. MPTP has since been used to model Parkinson's in experimental animals.

6.15 Pharmacology of cholinergic synapses

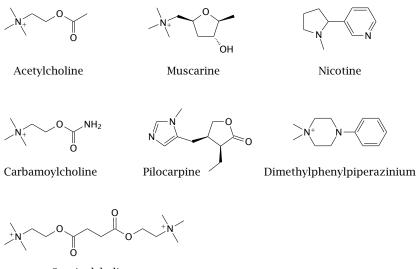
As discussed above, acetylcholine has two major classes of receptors, which are selectively activated by muscarine and by nicotine, respectively. While nicotinic ace-tylcholine receptors are ionotropic, the muscarinic receptors are GPCRs. Muscarinic receptors occur in several subtypes, but most clinically used agonists or antagonists are not subtype-selective.

Both nicotinic and muscarinic receptors occur in the brain in various functions. In the periphery, nicotinic receptors occur in motor endplates and in autonomic ganglia, whereas muscarinic receptors occur in the second synapses of the parasympathetic nervous system. There are multiple clinical uses for drugs that target these receptors.

6.15.1 Structures of cholinergic receptor agonists

Like muscimol (slide 6.10.9), muscarine is found in fly agaric; it is not clinically used. Pilocarpine is a muscarinic agonist that is used in ophthalmology to promote the discharge of fluid from the eyeball in order to reduce intraocular pressure in

glaucoma patients.²⁰ Carbamoylcholine is active on both muscarinic and nicotinic receptors, but more so on the former. It is used occasionally following abdominal surgery for making a drowsy, hung-over intestine sit up and take notice.



Succinylcholine

The stimulating effect of nicotine is mediated by nicotinic receptors in the brain. In the periphery, the drug stimulates the first synapses in both the sympathetic and the parasympathetic nervous system (see slide 6.13.1); apart from increased blood pressure, consequences also include accelerated bowel movements, which in former, less pedagogically wholesome times could be nicely observed in small boys partaking of their grandfathers' cigars. Dimethylphenylpiperazinium is a nicotinic agonist that is excluded by the blood brain barrier and is useful in physiological experiments.

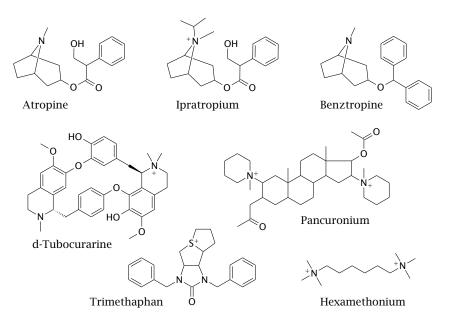
As stated earlier, the nicotinic acetylcholine receptors in the motor endplates differ in their subunit composition from those in the autonomic ganglia. Succinylcholine is a selective agonist for the subtype found in motor endplates. By a mechanism that involves receptor desensitization (see slide 6.10.6), it renders the muscle cells unresponsive to presynaptically released acetylcholine; the effect is muscle relaxation. This is used as an adjuvant to systemic narcosis. Succinylcholine is cleaved and inactivated within minutes by a cholinesterase in blood plasma.

6.15.2 Structures of cholinergic receptor antagonists

Atropine, ipratropium bromide and benztropine are all muscarinic antagonists. Atropine is used to suppress bronchial secretion in preparation of intubation for inhalation narcosis. Ipratropium is excluded by the blood brain barrier and therefore more suitable than atropine for sustained treatment; it is used to relax bronchi

²⁰The decompression of the tiny *canal of Schlemm*, which drains surplus fluid from the eyeball, occurs as a side effect of the contraction of the ciliary muscle that modulates the shape and refractive power of the lens. Muscarinic receptors induce contraction of this muscle.

in asthma patients and, with cardiac patients, to set a little fire to the pants of a sluggish sinoatrial node.



In contrast to ipratropium, benztropine has been designed for *increased* penetration of the blood brain barrier. It targets muscarinic synapses in the brain that are functionally antagonistic to dopaminergic ones and is used as an adjuvant treatment in Parkinson's disease.

Pancuronium and d-tubocurarine are antagonists at nicotinic receptors in motor endplates. While their effect on the receptor is opposite to that of succinylcholine, the end result is the same—neuromuscular blockade and muscle relaxation, and they are used for the same purpose.²¹

Trimethaphan and hexamethonium are "ganglion blockers," that is, antagonists selective for the nicotinic receptor subtype found in autonomic ganglia. They have powerful antihypertensive effects but are no longer commonly used in that application.²²

6.15.3 The catalytic mechanism of cholinesterase

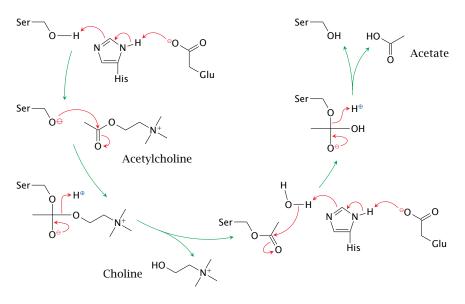
In both nicotinergic and muscarinergic synapses, acetylcholine is inactivated by cholinesterase; inhibitors of the enzyme will thus affect both types of synapses. They are of limited use in medicine but are more widely used as poisons.

The catalytic mechanism of cholinesterase resembles that of serine proteases. In the active site, a glutamate and a histidine residue cooperate in deprotonating the catalytic serine, which then performs nucleophilic attack on the substrate. This

²¹Muscle relaxants obviate the need for suppressing pain reflexes in the spinal cord. The dosage of inhalation narcotics can then be reduced to the lower amount required for suppressing consciousness in the brain.

²²An orally applicable ganglion blocker is mecamylamine (slide 3.4.7).

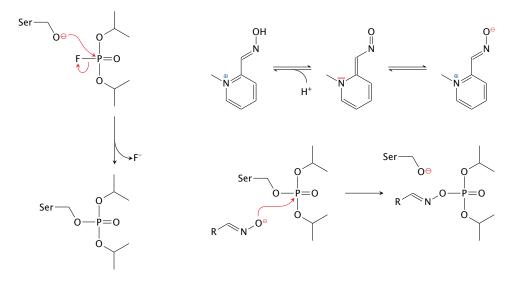
results in a tetrahedral transition state, which rapidly converts to an acylated form of the enzyme; the latter is then hydrolysed. Cholinesterase is very efficient; because of this, the chance of any single acetylcholine molecule binding to a receptor more than once is negligible.



6.15.4 Covalent inactivation of cholinesterase by DFP, and its reactivation by pralidoxime

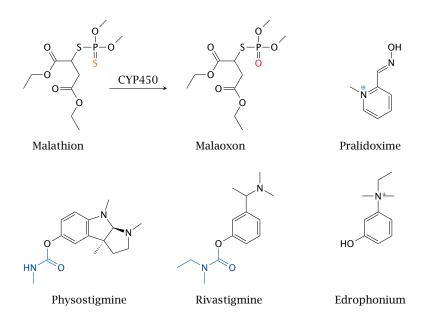
Organophosphate inhibitors such as diisopropylfluorophosphate (DFP) are sterically similar to the tetrahedral transition state of the cholinesterase reaction and therefore bind the enzyme very avidly, before reacting with it covalently (top). The modification can be reversed with oximes (bottom) such as pralidoxime (see slide 6.15.5).

DFP and several structurally similar compounds are extremely toxic to humans; they are taken up by inhalation and also penetrate intact skin. They were massproduced for chemical warfare by all major parties in World War II but never used in that war. However, Iraq's Saddam Hussein used them in his war on Iran in the 1980s.



6.15.5 Structures of cholinesterase inhibitors and of a reactivator

Organophosphates are also widely used as insecticides. In malathion, the fluoride leaving group found in DFP has been replaced by a substituted thiol. In addition, one of the doubly bonded oxygens has also been replaced by sulfur. Before reacting with cholinesterase, this second sulfur atom has to be replaced with oxygen by cytochrome P450, which yields malaoxon. The sulfur-substituted precursor form is deployed because it is more environmentally stable.



The two ester groups in the side chains of the leaving group constitute a safety feature. They are susceptible to cleavage by esterases in human blood plasma. Esterase cleavage competes with activation by cytochrome P450, and the cleavage

product no longer reacts with cholinesterase. Malathion is thus much less toxic to humans than other, older organophosphates.

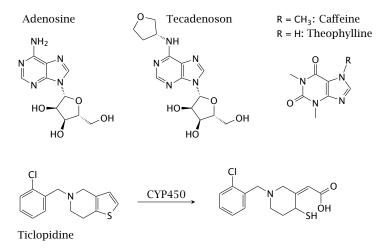
Edrophonium is a noncovalent inhibitor, whereas rivastigmine and physostigmine are covalent ones; the groups that end up bound to the active site serine are highlighted in both. Pralidoxime is a cholinesterase reactivator that is used in the treatment of organophosphate poisonings.

Clinical uses of cholinesterase inhibitors concern the reduction of intraocular pressure in glaucoma (see the footnote to section 6.15.1), and the amplification of cholinergic signaling in motor endplates, which is used in *myasthenia gravis*. In the latter disease, nicotinic acetylcholine receptors in motor endplates are inactivated by autoantibodies, which leads to muscle weakness; extending the lifetime of acetylcholine can improve the compromised synaptic function.

6.16 Drugs that interact with purine or opioid receptors

There are many more neurotransmitters; we will mention just two more classes that have considerable importance in pharmacology.

6.16.1 Purine receptor agonists and antagonists

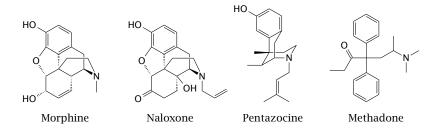


Purine receptors fall into two structural families. The P2X receptors constitute an independent family of ligand-gated channels; all others belong to the GPCR family. The receptors serve in different roles inside and outside the nervous system.

Adenosine receptors are often found alongside catecholamine receptors in the same synapses, where they tend to counteract the effects of the latter. Accordingly, the effect of inhibiting these adenosine receptors resembles that of stimulating the catecholamine receptors, and vice versa. As an aside, adenosine receptors have also been reported to form hetero-oligomers with GPCRs specific for various other neuro-transmitters. Caffeine and theophylline are A_{2A} adenosine receptor antagonists that are used as stimulants. Theophylline is also effective in the treatment of asthma. Tecadenoson is an adenosine receptor agonist that is used in the treatment of some

forms of cardiac arrhythmia. Ticlopidine blocks ADP receptors on thrombocytes, which inhibits their aggregation; this is useful in the treatment of atherosclerosis (see slide 10.6.7). It requires metabolic activation by cytochrome P450.

6.16.2 Opioid receptor agonists and antagonists



Opioid receptors are very effective at suppressing pain perception; their endogenous agonists are endorphins. Morphine is an agonist at μ , κ and δ -opioid receptors. Naloxone is an antagonist at the same receptors and is used in opioid poisonings. Pentazocine acts as a partial agonist primarily at μ -opioid receptors; it is less potent than morphine yet still a strong analgesic. Methadone is a full μ -receptor agonist and is used as a legal substitute for heroin; more recently, a combination of pentazocine and naloxone has come into use as a milder alternative to methadone.

We had previously considered the formation of $\kappa\delta$ -receptor heterodimers and their selective activation by 6-guanidinonaltrindole (see slide 5.6.3).

Chapter 7

Hormones

7.1 Major types of hormone receptors

- · G protein-coupled receptors
 - Many peptide hormones: glucagon, hypothalamic and hypophyseal hormones
 - Epinephrine, norepinephrine
- Receptor tyrosine kinases
 - Insulin
 - Growth hormone and growth factors
- Nuclear hormone receptors
 - Steroid hormones
 - Thyroid hormones

Hormones are fundamental to metabolic and other physiological regulation. Accordingly, hormone receptors, as well as enzymes involved in hormone synthesis, are important drug targets.

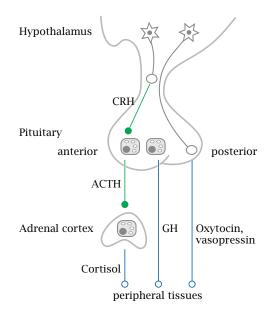
Hormone receptors fall into different functional classes. We have already encountered numerous GPCRs, and we have met some nuclear hormone receptors in the context of drug metabolism, namely the pregnane X receptor and the retinoid X receptor.

The receptor tyrosine kinases (RTKs) are another major receptor class. Like GPCRs, these receptors sit in the cytoplasmic membrane, bind their ligands on the extracellular side, and transmit the signal to the cell interior through a conformational change. However, unlike GPCRs, the RTKs do not transmit their signal through noncovalent interaction with downstream adapters. Instead, the activated receptors themselves function as protein kinases. RTKs typically phosphorylate multiple sub-

strate proteins, which then noncovalently bind and activate various downstream regulatory proteins.

Important examples of the RTK class are the receptors for insulin and for growth hormone. Several other receptor tyrosine kinases are involved in tumor initiation and growth. We will learn a bit more about these in Chapter 12.

7.2 The hypothalamic-pituitary axis



Hormone-secreting glands are also referred to as *endocrine* glands; this name reflects the fact that secretion occurs into the body interior, that is, into the blood-stream. In contrast, *exocrine* glands are the ones that secrete their products across the skin or the mucous membranes.

Many of the endocrine glands are under control by the hypophyseal gland or pituitary, which in turn is controlled by the hypothalamus. These two are located next to each other at the base of the brain. The hypothalamus is itself part of the brain, whereas the hypophyseal gland extends downward from it. Hypothalamic neurons extend their axon endings into the hypophyseal gland, which peeks out of the blood brain barrier and is able to release hormones into the systemic circulation; one could say that the hypophyseal gland is an outlet for the brain through which it releases hormones to the body.

The hypophyseal gland consists of an anterior and a posterior lobe. The posterior lobe contains just the axon endings of hypothalamic nerve cells, which secrete two related peptide hormones, oxytocin and vasopressin, directly into the circulation.

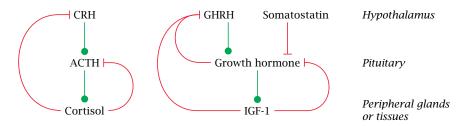
The anterior pituitary lobe contains gland cells that are controlled by specific hypothalamic peptide hormones, for example corticotropin releasing hormone (CRH). In response to these hypothalamic hormones, the cells of the anterior pituitary release peptide hormones that stimulate peripheral glands. One of these is adrenocorticotropic hormone (ACTH), which stimulates the cortex of the adrenal glands; another one is thyroid-stimulating hormone (TSH), which controls the thyroid gland. In addition to hormones that control peripheral endocrine glands, the anterior pituitary also produces prolactin, which stimulates the mammary glands, as well as growth hormone (GH).¹

Gland	Stimulated by	Hormones produced
adrenal glands (cortex)	adrenocorticotropic hormone (ACTH)	gluco-, mineralocorti- coids; androgens
thyroid gland	thyroid-stimulating hormone (TSH)	tri-, tetraiodothyronine (T ₃ , T ₄)
testicles / ovaries	follicle-stimulating hor- mone (FSH), luteinizing hormone (LH)	androgens, estrogens, progestins
diffuse	growth hormone	growth factors
mammary gland	prolactin	_

7.2.1 Peripheral glands and hormones controlled by the anterior pituitary

7.2.2 Regulatory patterns in the hypothalamic-pituitary axis

This scheme illustrates two different regulatory patterns that occur in the hypothalamic-pituitary axis. The production of cortisol by the adrenal glands is stimulated by adrenocorticotropic hormone (ACTH), which in turn is stimulated by hypothalamic corticotropin-releasing hormone (CRH). Cortisol exercises negative feedback on both. The control of the thyroid gland by thyroid-stimulating hormone (TSH) and thyrotropin-releasing hormone (TRH) works analogously, as does the regulation of sexual hormone secretion by endocrine cells in the ovaries and testicles (see later).



The secretion of growth hormone (GH) by the anterior pituitary is controlled by two hypothalamic hormones; it is activated by growth hormone releasing hormone

¹The cells in the anterior pituitary are specialized for the production of individual hormones; accordingly, since tumors originate from single cells, most hypophyseal tumors also secrete only individual hormones. On the other hand, damage to the pituitary usually causes lack of several or all hypophyseal hormones at once.

(GHRH) and inhibited by somatostatin. GH itself stimulates the production of insulinlike growth factor 1 (IGF-1) in the liver and other tissues. Both GH itself and IGF-1 exert negative feedback on GH secretion.

Keep in mind that these homeostatic feedback loops are only part of the story. Spontaneous variations in hormone levels, such as the early-morning high of cortisol, the spike of growth hormone secretion shortly after onset of sleep, or the variations of estrogens and progestins that occur during the menstrual cycle, involve additional control mechanisms. This is where the brain comes in—for simple homeostatic feedback alone, we wouldn't need the brain; peripheral glands such as the parathyroids are perfectly capable of it (see section 7.5).

7.2.3 The posterior lobe of the hypophyseal gland produces oxytocin and vasopressin

Oxytocin
$$H_2N-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-L-Leu-Gly-CO-NH_2$$
Vasopressin $H_2N-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-L-Arg-Gly-CO-NH_2$ Desmopressin $H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-CO-NH_2$

Both oxytocin and vasopressin are derived from precursor polypeptides; the amido groups at their C-termini are derived from a glycine residue that gets oxidatively truncated after proteolytic cleavage of the precursor. Each of the two hormones acts on cognate GPCRs that couple prominently to the phospholipase C cascade (see slide 5.3.2).

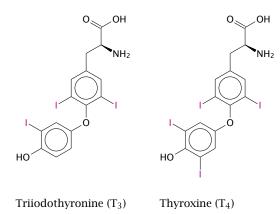
Oxytocin drives the contraction of smooth muscle in the uterus during labor; it is also used as a drug to induce labor when its spontaneous onset is delayed.² Moreover, it is released during breast feeding and induces contraction of smooth muscle in the milk ducts of the mammary glands, which assists in the release of the milk.

Vasopressin, also known as antidiuretic hormone, activates smooth muscle in the circulation; it also acts on cells in the distal tubules of the kidney, where it promotes the preservation of water. It is used as a drug in individuals with deficient endogenous production, a fairly rare condition that is known as *diabetes insipidus*.

Desmopressin is a synthetic vasopressin analogue in which the N-terminal amino group has been removed, and the L-arginine residue has been replaced by the Denantiomer. Both of these modifications slow the degradation of the molecule by circulating peptidases. Like the native hormone, it can be in the treatment of diabetes insipidus.

²Birth should proceed within one day after the amniotic sac has burst; if it does not, oxytocin may be used. My impression from anecdotal evidence is that this is not too successful and quite often still ends with a Cesarean section—performing the latter right away seems to me the more sensible option.

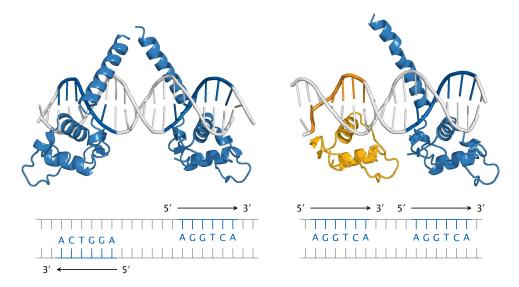
7.3 Thyroid hormones



The thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (thyroxine, T_4) are produced by the thyroid gland, which is under the control of the thyroid-stimulating hormone from the anterior pituitary. These hormones have broad and diverse activating effects on human physiology, which are mediated through transcriptional regulation. For example, they increase the expression of β -adrenergic receptors, boosting heart rate and ejection volume, and of respiratory chain-uncoupling proteins, which cause metabolic substrates to be burned without ATP production.³

Lack of thyroid hormones in children due to defective thyroid gland development causes delayed and stunted physical and mental development. If diagnosed in time, this can be corrected by hormone substitution treatment.

7.3.1 Thyroid hormones activate cognate nuclear hormone receptors



³From these biochemical effects, it is understandable that patients with excessive thyroid hormone secretion will experience tachycardia, weight loss, and sweating.

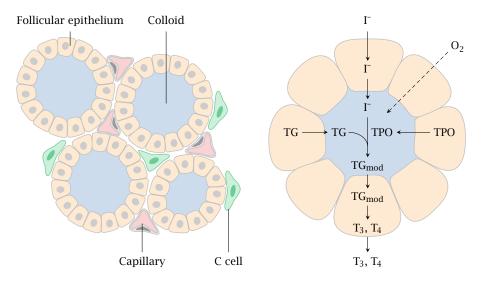
The thyroid hormones activate cognate nuclear hormone receptors; these are similar to the pregnane X receptor (see slide 4.3) but bind to different recognition elements. The thyroid hormone receptors come in α and β versions, and further diversification arises from the formation of various receptor homo- or heterodimers, which then bind to different recognition sequences on the DNA.

This slide illustrates the recognition of specific DNA sequences (thyroid hormone response elements) by thyroid receptor (TR β , blue) and retinoid X receptor (RXR α , yellow). Both monomeric receptors recognize the same sequence element, namely, the hexanucleotide AGGTCA. However, different receptor dimers recognize different target sequences nevertheless; this is due to variations in the relative orientations of the DNA-binding domains within these dimers.

Left: The TR β homodimer recognizes two instances of the consensus sequence that occur on opposite strands and point away from one another; such sequence motifs are referred to as *inverted palindromes*.

Right: The TR β -RXR α heterodimer recognizes two instances of the consensus sequence that are located on the same strand (direct repeats). In both panels, only the DNA-binding domains of the receptor molecules are shown; the remaining domains would attach to the tips of the upward pointing helices.

7.3.2 Tissue structure of the thyroid gland, and localization of hormone synthesis



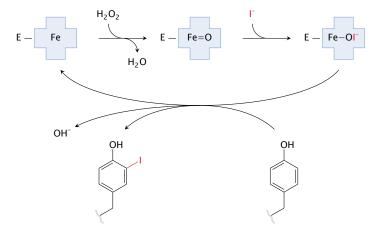
The thyroid gland has a peculiar tissue structure that is related to its likewise unusual biochemical function. The crucial and characteristic steps in thyroid hormone synthesis occur in confined extracellular compartments, little "reactors", which are referred to as thyroid follicles.

Left: Follicular epithelial cells enclose the follicle lumen, which is filled with protein-rich colloid. C-cells are located outside the follicles; they are unrelated to T_3 and T_4 synthesis but instead produce the peptide hormone calcitonin (see slide 7.5.2).

Right: The cells of the follicular epithelium cells synthesize the proteins thyroglobulin (TG) and thyroid peroxidase (TPO) and secrete them into the follicle lumen. They also take up iodide and transport it into the follicle lumen, where it is used by thyroid peroxidase for the iodination of tyrosine side chains of thyroglobulin. Thyroid peroxidase also catalyzes the subsequent step, in which it joins two iodinated tyrosine residues (see slide 7.3.4).

The peroxidase-modified thyroglobulin (TG_{mod}) is taken up into the follicle cells again via endocytosis and proteolytically degraded, which yields the free hormones triiodothyronine (T_3) and thyroxine (T_4).

7.3.3 Tyrosine side chain iodination by thyroid peroxidase



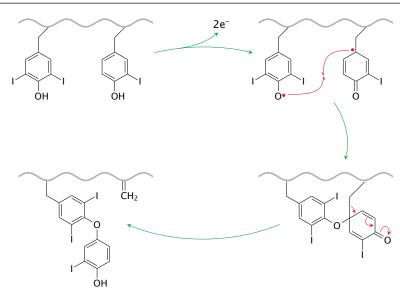
In the biosynthesis of the thyroid hormones, the first step is the iodination of tyrosyl residues in thyroglobulin. Thyroid peroxidase contains a heme moiety that generates hypoiodite from iodide and H₂O₂. The hypoiodite then reacts with the hydroxyphenyl side chain of tyrosine.

The slide shows the formation of monoiodotyrosine; diiodotyrosine is formed from monoiodotyrosine in another round of the same reaction. Both mono- and diiodotyrosine can participate in the subsequent coupling reaction (see next slide).

7.3.4 Coupling of two iodinated tyrosine side chains

The coupling reaction between two iodinated tyrosyl side chains is also catalyzed by thyroid peroxidase. The enzyme abstracts an electron from each of the substrate side chains, which then undergo radical recombination. The reaction leaves a dehydroalanine residue in the protein backbone.

The slide shows the reaction of a diiodotyrosine residue with a monoiodotyrosine side chain; this will produce a precursor of T_3 . The same reaction can also occur between two diiodotyrosine side chains, which will produce a T_4 precursor. After reuptake of the modified thyroglobulin by the follicular epithelial cells, T_3 and T_4 are liberated simply by proteolytic breakdown of the protein.



Both T_3 and T_4 are secreted into the bloodstream. Both are able to activate thyroid hormone receptors; however, T_3 is much more active. T_4 can be deiodinated to T_3 and thus functions mostly as a reservoir of the latter. Drugs that inhibit the enzyme that carries out this deiodination step—the antiarrhythmic drug amiodarone (see slide 7.3.6) in particular—may cause a functional thyroid hormone deficit, although the combined serum levels of T_3 and T_4 may be normal.

7.3.5 Thyroid hormones and pharmacotherapy

- Goiter: iodide supplementation
- Hyperthyroidism due to anti-TSH-receptor autoantibodies or hormone-producing tumors:
 - thyroid peroxidase inhibitors
 - radioiodine (¹³¹I)
- Lowering blood lipid levels: TR- β -selective agonists (KB-141)
- Interference with thyroid hormone release or conversion: lithium and amiodarone

The hypophyseal thyroid-stimulating hormone (TSH) stimulates the activity of existing gland cells, driving iodine accumulation and hormone synthesis. In addition, TSH also drives the growth of gland tissue. This dual effect is important for both pathogenesis and therapy.

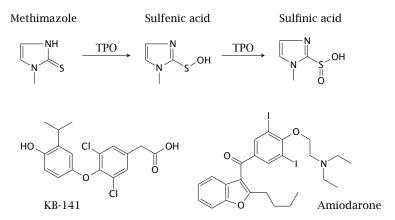
A shortage of iodine will hamper thyroid hormone synthesis. The hypophyseal gland will produce more TSH, which will prompt excessive growth of the thyroid gland (goiter). The enlarged thyroid gland usually manages to supply enough thyroid hormones, so that symptoms other than goiter itself are usually absent. Goiter due to iodine deficiency used to be common but is now largely controlled by the iodide supplementation of cooking salt.

Both autoantibodies and tumors—benign or, less commonly, malignant—are common causes of hyperthyroidism. Autoantibodies directed at the TSH receptor, a GPCR, may activate it and trigger nonphysiological growth of the thyroid gland, as well as excessive thyroid hormone production. Increased metabolic turnover results in weight loss, accelerated heart rate, lowered blood lipids and other symptoms.

Treatment options for hyperthyroidism include inhibitors of thyroid peroxidase as well as surgery and radioactive iodide ($^{131}I^-$). The latter form of treatment takes advantage of the fact that only thyroid gland cells actively accumulate iodide and are therefore selectively destroyed. Importantly, in thyroid cancer, $^{131}I^-$ will also affect metastatic tumor cells, as long as these retain the ability to accumulate iodide. The accumulation of $^{131}I^-$ can be enhanced by simultaneous application of TSH.

Lithium inhibits the release of thyroid hormones from gland cells. While this can cause side effects when lithium is used to treat depression, it can be used to advantage in radioiodide treatment, since it will cause the hormone-incorporated ¹³¹I⁻ to be retained longer inside the gland cells.⁴

7.3.6 Drugs that influence thyroid hormone function



This slide shows some of the drugs whose modes of action were discussed above. Methimazole is a mechanism-based inhibitor of thyroid peroxidase that is used in hyperthyroidism. Shown next to methimazole are the putative oxidation products that are formed by thyroid peroxidase (TPO) and inactivate the enzyme by binding to its heme group.

KB-141 is a TR β -selective thyroid receptor agonist. Such agonists lower blood lipid concentrations but avoid some other effects of nonselective receptor agonists; they are of interest in the treatment of hyperlipidemia. Amiodarone has been discussed in section 7.3.4.

⁴Radioactive iodine isotopes, both ¹³¹I⁻ and ¹²⁹I⁻, are formed by uranium fission in nuclear reactors. In case of reactor disasters, people are supposed to swallow large amounts of non-radioactive iodide in order to saturate their thyroids and prevent accumulation of radioactive iodine. Thyroid cancer induced by ¹³¹I⁻ was the most common form of cancer observed after the Chernobyl reactor meltdown in 1986.

7.4 Steroid hormones

Class	Major members	Glands
Glucocorticoids	Cortisol, cortisone	Adrenal glands
Mineralocorticoids	Aldosterone	Adrenal glands
Androgens	Testosterone, dihydro- testosterone	Testicles
Estrogens	Estradiol, estriol	Ovary
Progestins	Progesterone	Ovary, placenta

Steroid hormones are all structurally related and biosynthetically derived from cholesterol. Glucocorticoids are involved in metabolic regulation, but their application as drugs is owed to their antiinflammatory and immunosuppressive activity. Mineralocorticoids regulate secretion and retention of sodium and potassium in the kidneys; this also has effects on blood pressure. Sexual steroids sustain the function of sexual organs and, in women, gestation.

Pharmacological applications exist for all functional classes of steroid hormones or related synthetic agonists and antagonists.

7.4.1 Effector mechanisms

Mechanism/target	Receptor binds to	Example
Transcriptional induc- tion (transactivation)	DNA directly	Induction of enzymes of glu- coneogenesis by cortisol
Transrepression	Other proteins that regulate transcription	Inhibition of transcription factors AP-1 and NF-κB by cortisol

There is a cognate nuclear hormone receptor for each class of steroid hormones. All of these receptors act as transcription factors, much like the thyroid hormone receptors; this mode of action is referred to as *transactivation*. Glucocorticoids can also act via *transrepression*, which is mediated by direct protein-protein interaction between the ligand-bound glucocorticoid receptor and other transcriptional regulators.

Additional signaling mechanisms exist for some steroids. G protein-coupled receptors have been characterized that are activated by estrogens [59]. While conceptually intriguing, this has not yet resulted in pharmacological applications. So-called *neurosteroids* activate the GABA_A receptor in the brain [60]; synthetic analogues such as alfaxalone can be used to induce narcosis.

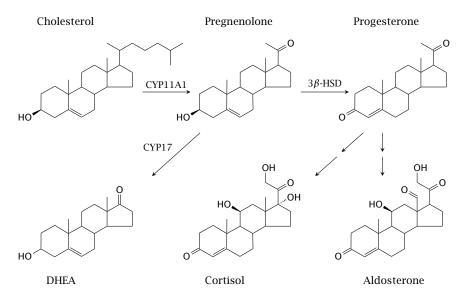
Class	Physiological function
Glucocorticoids	Metabolic regulation, strong anti- inflammatory action
Mineralocorticoids	Control of sodium and potassium elimination in the kidneys
Androgens	Precursors for gonadal androgen and estrogen synthesis

7.4.2 Adrenal steroid hormones: functional classes

The adrenal glands sit atop each of the two kidneys. They have two distinct zones, the outer *cortex* ("bark") and the inner *medulla* ("marrow"). The steroid hormones are all synthesized in the cortex, while the medulla produces epinephrine and nor-epinephrine.

Glucocorticoids and mineralocorticoids are produced entirely in the adrenal gland and are released as finished products. In contrast, the adrenal androgens, which are formed in substantial amounts in the adrenal glands of both men and women, serve mostly as precursors for the gonadal synthesis of sexual hormones. Compared to the gonadal androgens testosterone and dihydrotestosterone, the adrenal androgens have low specific activity.

7.4.3 Synthesis of adrenal steroids



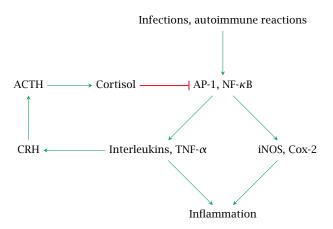
Enzymes: CYP11A1, cholesterol side-chain cleavage enzyme; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; CYP17, steroid 17 α -hydroxylase.

Aldosterone and cortisol are each derived from progesterone in three successive enzyme reactions; most of the enzymes involved are cytochrome P450 enzymes.

The key hormones released from the adrenal gland are cortisol, aldosterone, and dehydroepiandrosterone (DHEA), which are the major glucocorticoid, mineralocorticoid, and adrenal androgen, respectively. Progesterone is the most important progestin hormone; however, in the adrenal glands, it is not released to a significant extent but mostly serves as a synthetic intermediate, as does pregnenolone.

One of the steps in the synthetic pathway from progesterone to cortisol—the introduction of the hydroxyl group on the ring—is carried out by $11-\beta$ -hydroxylase (CYP11B). This enzyme also activates the anticancer drug mitotane, which therefore selectively targets tumors in the adrenal gland (see slide 12.4.3).

7.4.4 Glucocorticoids control inflammation via transrepression

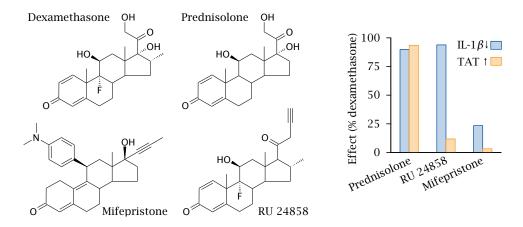


Cortisol and other glucocorticoids are powerful antiinflammatory agents, and most of their therapeutic applications are based on this activity. While direct transcriptional regulation participates, it seems that transrepression is responsible for much of the antiinflammatory activity.

The proinflammatory transcription factors AP-1 and NF- κ B are active in inflamed tissue. They increase the expression of the pro-inflammatory enzymes cyclooxy-genase 2 (Cox-2) and inducible nitric oxide synthase (iNOS),⁵ and also of inflammatory peptide mediators such as interleukins and tumor necrosis factor α , TNF- α .

Interleukins 1, 2 and 6 as well as TNF α stimulate the secretion of corticotropinreleasing hormone (CRH) in the hypothalamus. Cortisol released downstream of CRH activates the glucocorticoid receptor, which then inhibits AP-1 and NF- κ B through transrepression. Glucocorticoids therefore are part of a negative feedback loop that keeps overall inflammatory activity in check.

⁵The roles of iNOS and of cyclooxygenase in inflammation are discussed in chapters 8 and 9, respectively.



7.4.5 Glucocorticoid receptor agonists and antagonists

Dexamethasone and prednisolone are synthetic glucocorticoid receptor agonists that, like cortisol, exercise both antiinflammatory and metabolic effects. Mifepristone is a glucocorticoid receptor antagonist. In addition, it is also a progestin receptor antagonist; its more common medical applications relate to this latter activity.

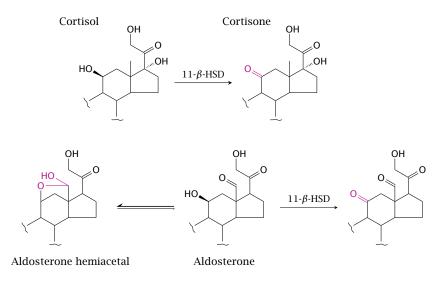
In the experiment shown on the right (plot redrawn from [61]), the activities of these conventional drugs were compared to those of the experimental drug RU24858. Downregulation of interleukin-1 β (IL-1 β) measures antiinflammatory activity and is mediated by transrepression. The induction of tyrosine transaminase (TAT) serves as a parameter of metabolic regulation via transcriptional induction. This enzyme participates in amino acid degradation and bolsters the supply of substrate carbon to gluconeogenesis. With dexamethasone, prednisolone and mifepristone, the antiinflammatory and metabolic effects are similarly weak or strong. In contrast, with RU 24858, they are clearly distinct, suggesting that this drug can selectively trigger the antiinflammatory glucocorticoid effect, without triggering the side effects on metabolic regulation.

The metabolic effects of glucocorticoids include increased protein breakdown and excessive glucose formation, leading to loss of muscle mass, osteoporosis, and symptomatic diabetes, all of which are prominent side effects of prolonged systemic corticosteroid treatment. Therefore, the discovery of agonists that separate the two action mechanisms of glucocorticoids is really quite exciting!

More detailed studies on RU 24858 found that some of its antiinflammatory effects are, in fact, mediated by transactivation, not transrepression [62, 63]. Like the thyroid hormone receptors, the glucocorticoid receptor can form homodimers as well as heterodimers with other nuclear hormone receptors; it seems possible that RU 24858 promotes formation of some, but not all of these receptor dimers.

While the term "dissociated steroids" has been used to describe the activity of compounds like RU 24858, we already know a better name for it—this is really just another case of agonist-specific coupling, and indeed a more compelling and practically relevant one than the examples we have seen with G protein-coupled receptors.

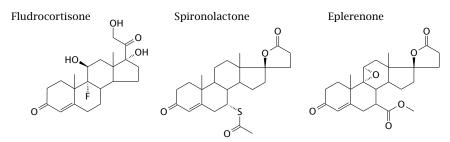
7.4.6 11-β-Hydroxysteroid dehydrogenase prevents non-specific mineralocorticoid receptor activation by cortisol



The mineralocorticoid receptor (MR) is expressed in the tubular epithelia of the kidney (see slide 3.7.4). When activated, the receptor induces the expression of transporters that mediate excretion of potassium and reuptake of sodium; it therefore plays an important role in the homeostasis of these two electrolytes that are of paramount importance in cell excitation. Mineralocorticoid activity may become excessively high downstream of other physiological dysregulation in chronic heart disease, and as a side effect of excess glucocorticoid activity, since glucocorticoids may also have some activity on the MR.

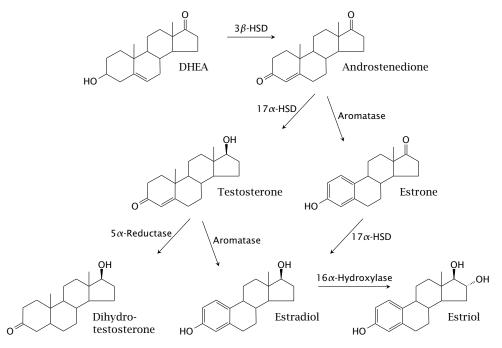
When studied *in vitro*, cortisol and aldosterone activate the MR to a similar degree. However, *in vivo*, activation of the MR by cortisol is inhibited by the enzyme $11-\beta$ -hydroxysteroid dehydrogenase ($11-\beta$ -HSD), which is strongly expressed in kidney cells along with the MR. The enzyme dehydrogenates cortisol to cortisone, which is inactive at the MR. Aldosterone is protected from dehydrogenation by $11-\beta$ -HSD because it mostly exists in the hemiacetal form.

7.4.7 Mineralocorticoid receptor ligands



Fludrocortisone is a mineralocorticoid receptor agonist; it may be used in substitution treatment in patients who lack normal aldosterone synthesis. The antagonists spironolactone and eplerenone are used in cardiac insufficiency and other states of excessive endogenous mineralocorticoid activity.

7.4.8 Gonadal biosynthesis of androgens and estrogens



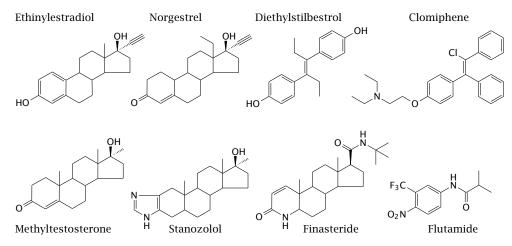
Abbreviations: DHEA, dehydroepiandrosterone; 3β -HSD and 17α -HSD, 3β - and 17α -hydroxysteroid dehydrogenase, respectively. Aromatase and 16α -hydroxylase are cytochrome P450 enzymes. Estradiol, estriol and estrone are estrogens; all others are androgens.

Gonadal synthesis of androgens and estrogens starts with dehydroepiandrosterone, which is supplied by the adrenal glands but can also be locally formed in the testicles and ovaries. Note that all estrogens are derived from androgens, and that aromatase is required for their synthesis. Accordingly, aromatase inhibitors are used in the treatment of estrogen-dependent gynecological tumors (see slide 12.4.2).

Dihydrotestosterone is the most potent androgen; its specific activity is five times higher than that of testosterone. Therefore, inhibition of 5α -reductase, which converts testosterone to dihydrotestosterone, significantly reduces the level of androgen activity. This is exploited in the treatment of androgen-dependent prostate tumors.

7.4.9 Synthetic analogues of gonadal steroids

Natural gonadal steroids are subject to strong first-pass effects in the liver and therefore cannot be used orally. Ethinylestradiol, norgestrel and methyltestosterone are orally available estrogen, progestin and androgen analogues, respectively. Diethyl-



stilbestrol was an early synthetic estrogen but was withdrawn after it was found to induce cancer.

The main use of synthetic estrogens and progestins is for contraception in females. As with glucocorticoids or thyroid hormones, the production of endogenous estrogens and progestins is controlled by the hypothalamic-pituitary axis, and these steroids exercise feedback inhibition on the hypophyseal secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Introducing extrinsic estrogens and progestins suppresses the secretion of FSH and LH, which deprives the ovaries of their hormonal stimulus and thus prevents ovulation.

In men, FSH and LH support sperm cell formation (*spermatogenesis*). Most efforts devoted to male contraception follow the same general idea as that used in females, namely, the suppression of FSH and LH secretion through application of synthetic androgens. While this works in principle, most clinical studies so far have concluded that behavioral or medical side effects were not acceptable.

Clomiphene is a "selective estrogen receptor modulator;" this class of compounds acts variously agonistic and antagonistic in different tissues, which must ultimately again arise from some sort of agonist-specific coupling. It inhibits the negative feedback exercised by gonadal steroids on the hypothalamus and the hypophyseal gland, and therefore increases the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and subsequently LH and FSH from the hypophyseal gland. It is used in fertility treatment in women, sometimes resulting in multiple pregnancies. Stanozolol is an oral androgen that is popular among body builders.⁶

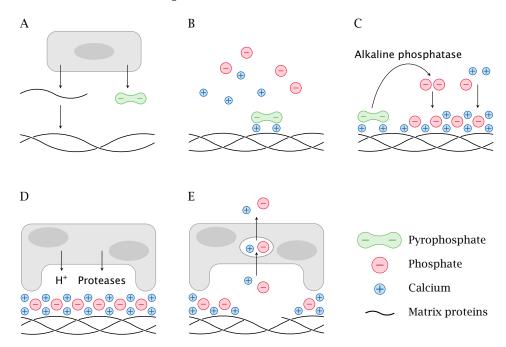
Finasteride is a 5α -reductase inhibitor; it prevents conversion of testosterone to the considerably more active dihydrotestosterone and therefore reduces androgen activity. Flutamide is an androgen receptor antagonist. Both drugs are used in the treatment of hormone-dependent prostate cancer.

⁶It also used to be popular among weight lifters and other competitive athletes, but it no longer is because it can readily be detected by blood tests.

7.5 Endocrine control of bone mineralization

Bone matrix consists of structural proteins, mostly collagen, and bone mineral, which is mostly hydroxyapatite ($Ca_5(PO_4)_3OH$). In addition to its structural role, bone mineral also serves as a reservoir for calcium and phosphate. Its deposition and mobilization are controlled by several hormones.

The most widespread affliction of bone matrix is osteoporosis. In this condition, which is most common among elderly women, both the matrix proteins and the bone mineral are depleted. The most effective treatment is substitution of estrogens, the endogenous levels of which recede after menopause; estrogens are required for sustaining bone mass.⁷ However, estrogen substitution is fraught with a considerable risk of tumor promotion that increases with the cumulative duration of therapy. Other forms of treatment address the bone mineral balance, with limited success.



7.5.1 Formation and resorption of bone matrix

While bone matrix is evidently very durable after death, it is quite dynamic in life. Bone matrix is formed by one specialized cell type, the osteoblasts, and dissolved by another one, the osteoclasts.

- (A) Osteoblast cells secrete bone matrix proteins that self-assemble into fibrils. They also produce and release pyrophosphate ions.
- (B) The bone matrix proteins provide nucleation sites for the deposition of bone mineral crystals. Pyrophosphate binds to incipient bone mineral crystals and inhibits their premature growth.

⁷Interestingly, this even seems to apply to males—see [64].

- (C) Alkaline phosphatase, which is also produced by osteoblasts, cleaves the pyrophosphate. This triggers the deposition of bone mineral (hydroxyapatite).
- (D) Osteoclasts dissolve bone matrix. They attach to the surface of the bone matrix and seal off a patch of surface underneath. Into the occluded pocket, they secrete acid that dissolves the bone mineral, as well as proteases that digest the embedded matrix proteins.
- (E) Calcium and phosphate ions are taken up by the osteoclasts via endocytosis and then released into the circulation.

Osteoblasts and osteoclasts are both continuously active. Their relative levels of activity are under hormonal control and determine whether there will be a net accumulation or dissolution of bone matrix and minerals.

7.5.2 Hormones that affect bone mineralization and bone matrix

- Parathyroid hormone (PTH)
 - mobilizes calcium and phosphate from the bone
 - promotes calcium retention and phosphate elimination in the kidneys
 - promotes activation of vitamin D by hydroxylation
- Calcitonin: promotes deposition of calcium and phosphate in the bone
- Calcitriol (activated vitamin D)
 - promotes intestinal calcium and phosphate uptake
 - inhibits PTH secretion
- Estrogens: sustain bone matrix

Parathyroid hormone (PTH) is a peptide hormone that is secreted by the parathyroid glands, which are four little cell clusters attached to the thyroid gland. Calcitonin is secreted by the C cells in the thyroid gland itself (see slide 7.3.2). Both hormones are regulated by the blood calcium level; low blood calcium stimulates release of PTH and inhibits calcitonin secretion, and high calcium does the opposite.

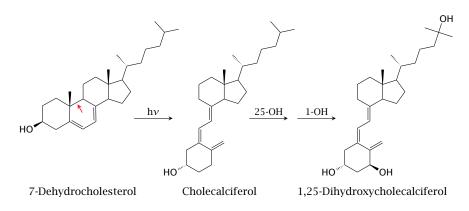
While PTH and calcitonin manage the short term calcium homeostasis, calcitriol is important in long term control. Estrogens affect not only the bone mineral but also the bone matrix density.

7.5.3 Endogenous biosynthesis of calcitriol

While conventionally referred to as a vitamin and often used as a supplement, calcitriol can actually be synthesized from scratch in human metabolism. The pathway branches off from cholesterol biosynthesis at 7-dehydrocholesterol, which is also the immediate precursor of cholesterol. The initial reaction is *not* enzyme-catalyzed but requires a UV photon.

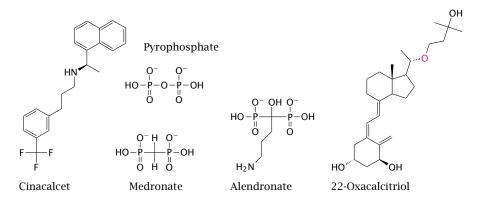
The slide summarizes the synthetic pathway. Photochemical breakage of one bond in the ring (red arrow) in 7-dehydrocholesterol produces cholecalciferol.

Two subsequent enzymatic hydroxylations yield 1,25-dihydroxycholecalciferol (calcitriol).⁸



7.5.4 Drugs that influence calcium balance and bone mineralization

The secretion of PTH by the parathyroid gland is regulated by an unusual G protein-coupled receptor that senses the extracellular calcium level. Cinacalcet is an allosteric agonist of this receptor; its effect is to downregulate PTH secretion and thereby preserve bone minerals.



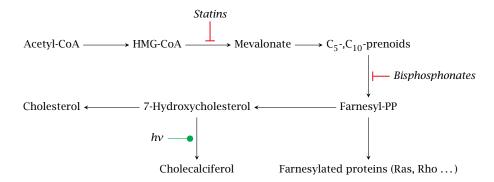
The bisphosphonates medronate and alendronate inhibit the resorption of bone matrix by osteoclasts. Like pyrophosphate—which is not a drug, but shown just for comparison—they bind to the surface of bone mineral particles. When mobilized by acid secreted from an osteoclast, they undergo endocytosis; in this way, they are preferentially targeted to the osteoclast cells. Within the cells, they inhibit the synthesis of farnesylpyrophosphate (see next slide).

22-Oxacalcitriol is an analog of calcitriol. Like calcitriol, the drug inhibits parathyroid hormone secretion and thereby reduces calcium mobilization from the

⁸The photochemical formation of cholecalciferol proceeds only in the skin. Photons absorbed by skin pigment are lost to cholecalciferol formation; this likely created the selective pressure for *Homo sapiens* to lose most of the skin pigment after leaving Africa for more Northern climates. Persons with dark skin who live in less sunny regions should be diligent about vitamin D supplementation.

bone matrix. Unlike calcitriol, however, 22-oxacalcitriol does not greatly increase intestinal calcium uptake.⁹

7.5.5 Sites of action of statins and bisphosphonates



Bisphosphonates inhibit the formation of farnesylpyrophosphate, which is not only an intermediate in sterol synthesis but also a cosubstrate in the posttranslational modification of some membrane-associated proteins. Inhibited protein farnesylation causes apoptosis of the osteoclast cells.¹⁰

In the sterol synthesis pathway, both statins and bisphosphonates act upstream not only of protein farnesylation but also of cholecalciferol synthesis. While the bisphosphonates affect mainly osteoclasts, the statins indeed inhibit cholesterol synthesis in the liver, and one would therefore expect them to lower vitamin D levels. However, with patients on atorvastatin, the opposite has been reported [66]; and moreover, clinical studies support a beneficial effect of statins in osteoporosis [67]. This example clearly shows that, as the saying goes, the difference between theory and practice is greater in practice than in theory.

⁹This appears to be another example of agonist-specific coupling, this time by the vitamin D receptor; however, I have not been able to find detailed experimental studies on this point.

¹⁰Bisphosphonates also have useful antimicrobial activity with some parasites such as *Trypanosoma* species; this effect seems to be caused by inhibition of the hexokinase enzymes of these parasites [65].

Chapter 8

Pharmacology of nitric oxide

8.1 Physiological significance of nitric oxide (NO)

- Powerful vasodilator—NO-releasing drugs are used in the treatment of cardiovascular disease
- Neurotransmitter—signaling in the CNS and the autonomic nervous system
- Inflammatory mediator—inhibition of NO synthesis is of interest as a therapeutic strategy in infection and chronic inflammation

Nitric oxide differs in many ways from other signaling molecules, and because of its unusual nature was discovered relatively late. Its first physiological function to be discovered was the relaxation of blood vessels. While this function remains the focus of its application in pharmacotherapy, a range of other effects with at least potential applications in clinical practice were characterized subsequently.

The three listed physiological functions correspond to three different isoforms of nitric oxide synthase, the enzyme that produces NO (see slide 8.3).

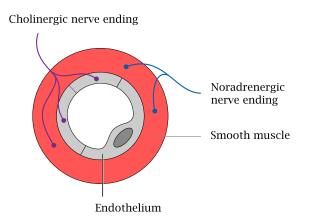
8.2 Identification of "endothelium-derived relaxing factor" as nitric oxide

Vasorelaxation mediated by nitric oxide is controlled by the autonomic nervous system. The physiological experiments that uncovered the role of the endothelium, and subsequently of NO, in vasorelaxation were simple yet ingenious. In order to appreciate them, we need a little more anatomy.

8.2.1 Cholinergic and adrenergic nerve endings in a blood vessel wall

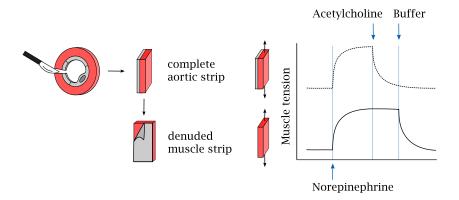
We had seen earlier that both the sympathetic and the parasympathetic nervous system innervate blood vessel walls and regulate their contraction and wall tension (slide 6.13.1). The adrenergic nerve endings of the sympathetic nerve fibers are

found in the muscular layer of the vessel walls. In contrast, cholinergic nerve endings are found both in the muscular and the endothelial layers.



The wall tension of the blood vessel is sustained by the muscular layer, not the endothelium; therefore, the cholinergic innervation of the endothelium may be surprising. As it turns out, however, it is needed for the vasorelaxation and vasodilation induced by the parasympathetic system.

8.2.2 The endothelium is required for vascular relaxation in response to acetylcholine



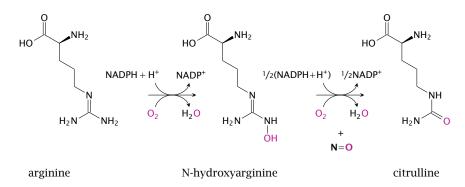
This slide illustrates the experiment that led to the discovery of nitric oxide-mediated vasorelaxation.

Contraction and relaxation of the vascular smooth muscle cells in response to norepinephrine and acetylcholine were studied with aortic strips. In this technique, slices are cut from an animal's aorta and then opened with a radial incision. The endothelium can be left in place or peeled away in order to study its effect on the muscular layer. The resulting intact or denuded aortic strips were mounted between two distending hooks to measure their contractile force.

Application of norepinephrine induced contraction in strips with or without attached endothelium, as would be expected from the innervation pattern of the sympathetic nerve fibers. In contrast, norepinephrine-induced contraction could be reversed by acetylcholine only if the strip retained the endothelium (top) but not when the endothelium had been peeled away.

Interestingly, relaxation of a denuded muscular strip could be restored if the endothelial side of an intact strip was strapped onto it (not illustrated). These observations showed that, in response to cholinergic stimulation, the endothelium releases a diffusible substance that enters the muscular layer and induces its relaxation. This "endothelium-derived relaxing factor" was subsequently isolated and identified as NO.

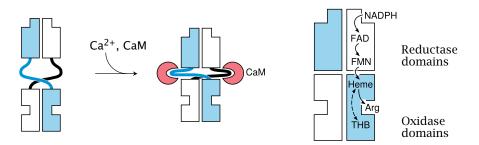
8.3 The nitric oxide synthase reaction



Nitric oxide is synthesized intracellularly by nitric oxide synthase (NOS). This reaction is rather complex and involves two successive monooxygenase steps. In the first step, arginine is converted to N-hydroxyarginine (NOHA), which is cleaved in the second step to NO and citrulline.

NOS occurs in several variations. Endothelial NOS (eNOS) and neuronal NOS (nNOS) are found in the eponymous cell types. Inducible NOS (iNOS) is found mainly in inflammatory cells. All three enzymes are homologous and perform the same reaction, but they differ in their regulatory properties.

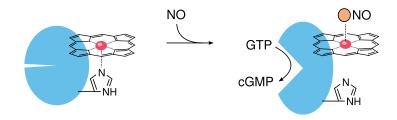
8.3.1 Activation of NOS by calcium and calmodulin



Nitric oxide synthase exists as a dimer. Each subunit contains a reductase domain and an oxidase domain. The reductase domain accepts electrons from NADPH and hands them over to the oxidase domain of the other subunit. The reductase and oxidase domains are loosely connected by flexible hinges that, when extended, prevent the flow of electrons between them. The two domains are brought into close, productive interaction when calmodulin (CaM) binds to the hinges and changes their conformations. In the case of eNOS and nNOS, CaM binding only happens if the cytosolic level of Ca^{++} is elevated, which occurs only transiently when triggered by action potentials or through activation of GPCRs, such as for example the muscarinic ones in endothelial cells.

In contrast, inducible NOS binds calmodulin very avidly regardless of the calcium concentration, and therefore is active even at the low resting level of Ca⁺⁺. Its activity is regulated not by short-term calcium signals but instead by transcriptional induction.

8.4 NO activates soluble guanylate cyclase (sGC)



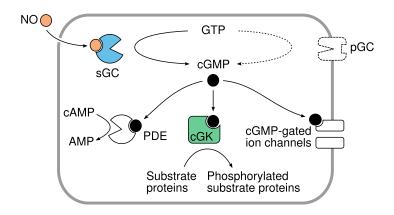
Between the last slide and this one, we have skipped an important step, namely the diffusion of NO out of the cell of origin, for example an endothelial cell, and into another one, such as a vascular smooth muscle cell. Like other small gas molecules, nitric oxide passes through cell membranes with ease, which enables it to directly and rapidly interact with intracellular targets.

Within its target cell, NO binds to the heme group of soluble guanylate cyclase (sGC), dislodging a strategic histidine residue. This causes a conformational change that activates the enzyme, which then begins to make cyclic GMP (cGMP). The production of cGMP as a second messenger is the most important signal downstream of NO.

8.4.1 Signaling effects of cGMP

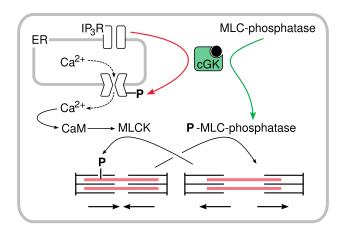
Like cAMP, cGMP targets multiple effector molecules inside the cell. The activation of cGMP-dependent protein kinases (cGK) is the most important single mechanism. Phosphodiesterase 5 (PDE) is activated, too, reducing the levels of both cAMP and cGMP. Actuation of cyclic nucleotide-gated cation channels affects the membrane potential and the cellular calcium level.

Membrane-bound receptor or "particulate" guanylate cyclases (pGC) provide an alternate means of cGMP production that is independent of NO but instead is controlled by several peptide hormones.



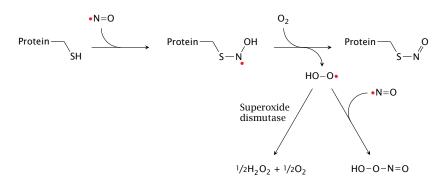
8.4.2 NO-induced relaxation of smooth muscle cells is mediated by cGK

The relaxation of smooth muscle that occurs downstream of endothelial NO release is mediated by cGMP-dependent protein kinase. This kinase phosphorylates and thereby activates myosin light chain phosphatase. The phosphatase dephosphorylates myosin, which interrupts interaction of myosin with actin and induces relaxation.



cGK also phosporylates a regulatory subunit of the IP_3 receptor channel. As we have seen before (slide 5.3.2), this channel mediates the outflow of Ca⁺⁺ from the ER to the cytosol. Phosphorylation of the IP_3 channel reduces Ca⁺⁺ flow and therefore the calmodulin-dependent activation of myosin light chain kinase (MLCK).

It is worth noting that the effects of NO cut in below the GPCRs that mediate the effects of major vasoconstrictors such as angiotensin and norepinephrine. Therefore, NO-releasing drugs offer a means to interrupt the out-of-control signaling by such mediators that occurs in hemodynamic shock or hypertensive crisis.

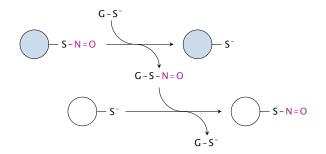


8.5 S-nitrosylation of cysteine residues in proteins by NO

In addition to the cGMP-mediated signaling effects, nitric oxide can also influence protein function through reacting covalently with them. The reaction involves single cysteine residues in proteins, which become *S*-nitrosylated. The reaction also requires molecular oxygen, which is converted to superoxide. While the latter has been observed *in vitro* to react with a second molecule of NO to form peroxynitrite, it seems likely that *in vivo* most superoxide would be scavenged by superoxide dismutase.¹

One might expect this modification to be too indiscriminate to be of use in selective and directed control of cell physiology. However, it has been demonstrated experimentally that *S*-nitrosylation is indeed quite selective *in vivo*.

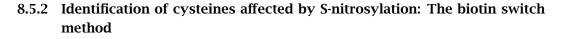
8.5.1 Transfer of nitrosyl groups between proteins by glutathione

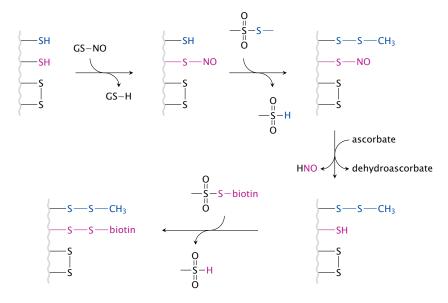


One mechanism that may contribute to the selectivity of protein-*S*-nitrosylation *in vivo* is the transfer of nitrosyl groups between proteins, which is mediated by glutathione. In this way, the nitrosyl groups can travel around the cell before settling down on the proteins with the thermodynamically most favorable cysteine residues.

Since glutathione is the most abundant thiol in the cell, this also means that nitrosylated glutathione functions as a reservoir of S-N=O (nitrosothiol) groups in the cell. In fact, many *in vitro* studies on the regulatory effects of protein-*S*-nitrosylation have used nitrosylated glutathione as the source of nitrosyl group instead of free NO for simplicity.

¹Note, however, that peroxynitrite is indeed formed by phagocytes in order to kill ingested microbes (see section 8.6).





To understand the regulatory effects of protein-*S*-nitrosylation, it is important to identify the proteins, and the specific cysteine residues within them, that actually become *S*-nitrosylated *in vivo* upon NOS activation. The biotin switch method is an experimental protocol that permits the identification of these cysteines by selectively attaching biotin to them. This procedure involves the following steps:

- 1. After NOS has been activated and protein-*S*-nitrosylation has run its course, proteins are extracted from cells. Their remaining free SH groups are converted to disulfides using *S*-methyl-methanethiosulfonate.
- 2. Ascorbic acid is used to selectively reduce S–NO groups, while leaving native or synthetic disulfide bonds intact.
- 3. The reduced SH groups are labeled with a biotinylation reagent, via disulfide formation (as shown here) or other coupling chemistries.

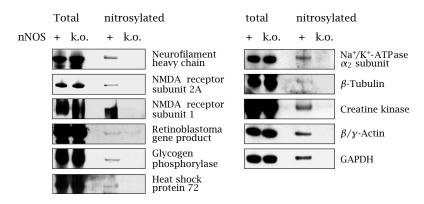
After this procedure, biotin will be attached specifically to those cysteine residues that had been nitrosylated before. The biotinylated proteins can then be purified or selectively detected on a blot using the very strong and specific interaction between biotin and streptavidin.

8.5.3 Identification of proteins subject to nNOS-dependent S-nitrosylation

This experiment illustrates the selectivity of protein-*S*-nitrosylation in brain tissue from mice due to activation of neuronal NOS. The role of nNOS was ascertained by comparing the extent of nitrosylation between wild-type and nNOS k.o. mice.

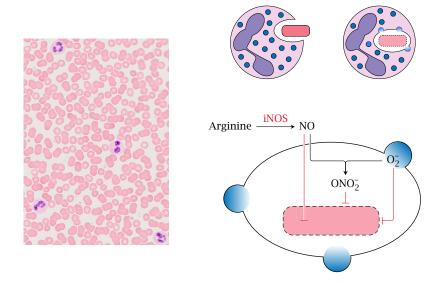
After extracting the proteins from homogenized tissue, *S*-nitrosyl groups were converted to biotin derivatives as outlined above, and selectively detected on blots using labeled streptavidin. The total amount of each protein—nitrosylated or not—

was imaged with antibodies and seems indistinguishable between wild-type and nNOS k.o. mice in every case. With each protein, the extent of S-nitrosylation is represented by the intensity of the third sample relative to the first one.



In all cases, *S*-nitrosylation is caused by nNOS, as shown by its absence in the knockout mice. The extent of nitrosylation is particularly strong with the NMDA receptor, and indeed this receptor—one of the ligand-gated glutamate receptors (see section 6.11)—is known to be inhibited by *S*-nitrosylation. Glyceraldehyde-3-dehydrogenase (GAPDH) is also quite strongly affected. Intriguingly, this protein does not only serve in its catalytic role in glycolysis but moonlights as a signaling molecule in the control of apoptosis (programmed cell death). Figure adapted from [68].

8.6 Role of NO and iNOS in the killing of microbes by phagocytes



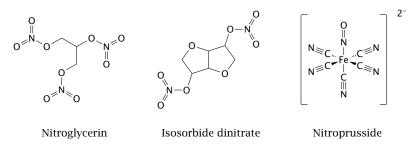
NO released by eNOS and nNOS serves signaling roles and is subject to rapid, calcium-dependent regulation. In contrast, NO produced in cells of the immune system primarily serves as an inflammatory effector. Its production doesn't need to

occur as rapidly but must be sustained for longer, and accordingly it is regulated by transcriptional induction of the enzyme rather than calcium and calmodulin. This form of the enzyme is therefore called inducible NOS (iNOS).

The antimicrobial effect of NO is strongest in combination with reactive oxygen species. In phagocytosis, microbes are ingested by granulocytes or macrophages and then end up inside intracellular vesicles called phagosomes. Peroxisomes then fuse to the phagosomes, releasing $O_2^{\bullet-}$ (superoxide; produced by NADPH oxidase) and H_2O_2 (produced by superoxide dismutase). Concomitantly, iNOS is activated in the cytosol. NO enters the phagosomes, where it may combine with superoxide to form peroxynitrite (ONOO⁻), which is strongly microbicidal. Alternatively, NO itself may diffuse across the microbial cell wall to wreak havoc inside.²

As with reactive oxygen species, the production of NO by inflammatory cells is a mixed blessing—important in antimicrobial defense, but destructive in rheumatic or other autoimmune disease. In severe, systemic infection (septicemia), the massive release of NO by immune cells causes relaxation of the vasculature, leading to a dangerous drop in blood pressure (septic shock). Therefore, pharmacological inhibition of iNOS is of great medical interest.

8.7 NO-releasing drugs



The drugs shown in this slide are used in the treatment of hypertension and of stenotic coronary artery disease. The first drug to be so employed was nitroglycerin, which is more commonly known as an explosive. Its therapeutic properties were discovered when workers exposed to nitroglycerin fumes at Mr. Alfred Nobel's factory complained about headaches recurring at the beginning of each work week (headaches are often caused by dilating blood vessels in the skull).

Nitric oxide is released from nitroglycerin by various enzymes. The drug is promptly absorbed across mucous membranes, and sublingual application of spray or droplets is used by patients with acute attacks of angina pectoris. Isosorbide dinitrate is used by the same group of patients, but has a slower and longer lasting effect than nitroglycerin and is used orally for sustained therapy.

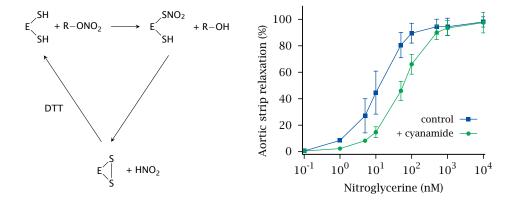
Sodium nitroprusside releases NO spontaneously, without enzymatic catalysis. It is the most powerful NO-releasing drug and is used, by intravenous infusion, in

²The acidic pH that prevails inside phagosomes will promote protonation of $O_2^{\bullet-}$ and $ONOO^-$. This enables them to enter and traverse cell membranes, which should enhance their microbicidal activity.

hypertensive crisis and other emergencies involving dangerously high blood pressure. Somewhat counterintuitively, it is also used in hemodynamic shock, which involves extremely low blood pressure. In this condition, perfusion of the kidneys and other interior organs is reduced by autonomic reflexes to a dangerous extent. After stabilizing blood pressure with other means, nitroprusside is used to break through this reflex blockade and restore perfusion to the endangered organs.

In addition to NO, nitroprusside also releases cyanide. This is potentially toxic but can be neutralized by the simultaneous application of sodium thiosulfate, which reacts with cyanide to form thiocyanate.

8.7.1 Bioactivation of nitroglycerin by mitochondrial aldehyde dehydrogenase



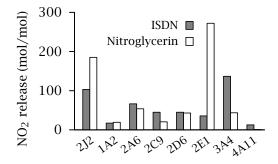
Nitroglycerin and isosorbide dinitrate can be activated by multiple enzymes. In mice and several other species, an important enzyme is mitochondrial aldehyde dehydrogenase (ALDH). This slide shows the reaction mechanism—in vitro, the reaction requires the addition of a thiol-reducing agent such as dithiothreitol (DTT), and the reaction produces *S*-nitrosylated intermediates—as well as the effect of inhibiting ALDH with cyanamide on the relaxation of aortic strips ([69]; compare slide 8.2.2). Genetic knock-out of ALDH suggests an even greater contribution of ALDH to nitroglycerine bioactivation [70]; however, this varies with the drug and the animal species in question [71, 72].

8.7.2 Bioactivation of nitroglycerin and ISDN by human cytochrome P450 isoforms

While both nitroglycerin and isosorbide dinitrate are metabolized by cytochrome P450 enzymes, a more detailed analysis shows that the enzyme isoforms with the strongest activity toward each are somewhat different: CYP2E1 is highly active with nitroglycerin, whereas CYP3A4 is more active with ISDN. Both drugs are converted efficiently by CYP2J2, which is strongly expressed in blood vessels and is likely important in the prompt clinical effect of nitroglycerin.

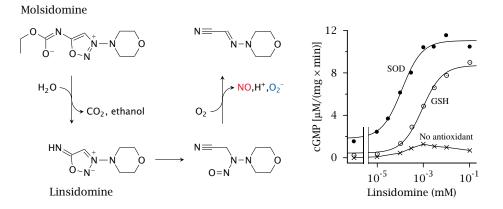
As we have seen, cytochrome P450 enzymes contain heme. Like the heme in sGC, the one in cytochrome P450 may bind NO; this will inactivate the enzyme. Inac-

tivation of cytochrome P450—and/or aldehyde dehydrogenase, which is subject to protein-*S*-nitrosylation—by NO released from drugs contributes to nitrate tolerance, which develops upon prolonged drug application and can only be broken by pausing the use of these drugs.³



In the experiment shown, the CYP isoforms were recombinantly expressed in yeast. Experimental systems such as this one are often used to study the susceptibility of novel drug molecules to metabolism during preclinical development. Figure prepared from original data in [73].

8.7.3 NO release by molsidomine



Molsidomine is an alternative NO-releasing drug that does not require metabolism by cytochrome P450 or aldehyde dehydrogenase, and therefore is less prone to the development of tolerance. The left panel shows the mechanism of NO release: Enzymatic hydrolysis of molsidomine yields linsidomine, which then decomposes spontaneously. The final step depends on O_2 and yields both NO and superoxide.

As we had seen above (slide 8.6), superoxide can combine with NO to form peroxynitrite. Accordingly, in order to render the NO released by linsidomine available for the activation of guanylate cyclase, superoxide must be scavenged. This is illustrated in the experiment in the right panel, which shows that appreciable

³Abatement of nitrate tolerance during the weekend was the basis of the Monday headache in those who worked in Mr. Nobel's nitroglycerin manufacture.

amounts of cGMP are formed downstream of linsidomine decay only in the presence of scavenging agents such as superoxide dismutase (SOD) or glutathione (GSH). Figure prepared from original data in [74].

Foot swelling (mL) wild type 0.5 NH2 iNOS k.o. L-NAME 74150 0 22 24 26 28 32 30 NH2 Days after collagen injection GW274150

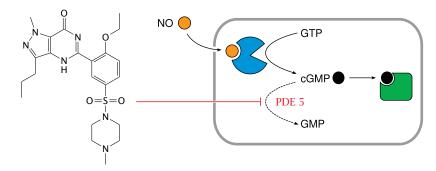
8.8 Antiinflammatory effect of iNOS inhibition

The activity of iNOS in inflammation is potentially destructive and contributes to tissue damage in rheumatism and other autoimmune diseases; therefore, inhibiting iNOS is potentially useful. To make this work, it is important to avoid inhibition of eNOS and nNOS. While most available NOS inhibitors, such as L-*N*-nitroarginine-methyl ester (L-NAME) inhibit all NOS isoforms, some are selective for iNOS. One such drug the experimental inhibitor GW274150.

In the experiment on the right, the effectiveness of GW274150 was studied in a mouse model of inflammation. Collagen-induced arthritis can be provoked in mice by injecting their paws with bovine collagen type II, to which the mice then mount an immune reaction that leads to swelling and inflammation.

After injection on day 0, the mice take three weeks to produce antibodies and then develop inflammation. The intensity of this inflammation can be measured by the extent of paw swelling. Genetic knock-out of iNOS and the experimental drug are equally effective in mitigating inflammation. Figure prepared from original data in [75].

8.8.1 Structure and mode of action of sildenafil (Viagra[™])



While NO-releasing drugs influence the contractile activity of smooth muscle cells by increasing cGMP, an equally viable approach should be the inhibition of cGMP degra-

dation by phosphodiesterase. This rationale led to the development of sildenafil, which was originally intended as yet another cardiovascular drug for the elderly.

As it turned out, the drug proved very popular with the old folks alright, but not entirely for the intended reasons. Nevertheless, in addition to its well-known effect on male potency, the drug also does promote vasorelaxation, which can prove dangerous when used in combination NO-releasing drugs or similar.

Prompted by the risks of sildenafil use in cardiac patients engaging in strenuous exercise, some administrative districts (Kantons) in Switzerland have mandated the training of prostitutes in the use of defibrillators.

Chapter 9

Eicosanoids and related drugs

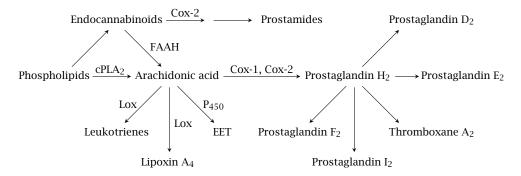
9.1 Overview

- "Local hormones"—sphere of action often limited to same anatomical site or tissue
- Involved in control of inflammation, fever, blood coagulation, pain perception, labor
- Derived from arachidonic acid and other polyunsaturated fatty acids, which occur in membrane phospholipids
- Most effects mediated by G protein-coupled receptors
- Some effects mediated by ion channels and nuclear hormone receptors

Eicosanoids are mediators that are derived from arachidonic acid several other polyunsaturated fatty acids. The precursor fatty acids are stored as parts of the phospholipid molecules that make up the membranes of the endoplasmic reticulum and the nucleus. From these phospholipids, the fatty acids are mobilized on demand by phospholipase A_2 and then converted to the various mediators by a sequence of enzymatic modifications.

Among the many physiological functions that are regulated by eicosanoids, inflammation, pain perception and blood coagulation are of the greatest interest in pharmacotherapy.

9.1.1 Pathways and key enzymes in eicosanoid synthesis



This slide introduces the major mediator classes and biosynthetic pathways. Release of arachidonic acid¹ from membrane phospholipids by cytoplasmic phospholipase A_2 (cPLA₂) feeds cyclooxygenases 1 and 2 (Cox-1, Cox-2). These enzymes both form prostaglandin H_2 , which is then converted to other prostaglandins and to thromboxanes by specific synthases downstream.

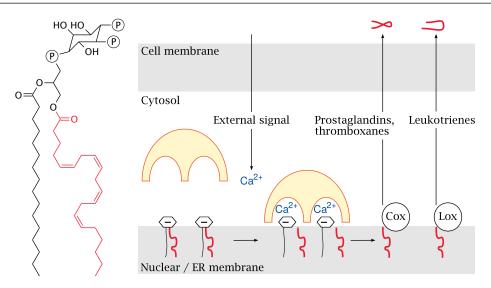
Arachidonic acid is also the substrate of various lipoxygenases (Lox) and of some cytochrome P450 enzymes (P450), which produce leukotrienes, lipoxins, and epoxytrienoic acids (EET), respectively. Endocannabinoids, which are formed from arachidonate-containing phospholipids through separate pathways, are cleaved to arachidonic acid by fatty acid amide hydrolase (FAAH). Alternatively, they may be metabolized first by Cox-2 and subsequently by one of the prostaglandin synthases, giving rise to prostamides.

9.1.2 Calcium signals activate cPLA₂ and initiate the synthesis of prostaglandins and leukotrienes

The major storage lipid for arachidonic acid is phosphatidylinositol-bis-phosphate (PIP₂). In the structure of PIP₂, the arachidonyl residue is highlighted.

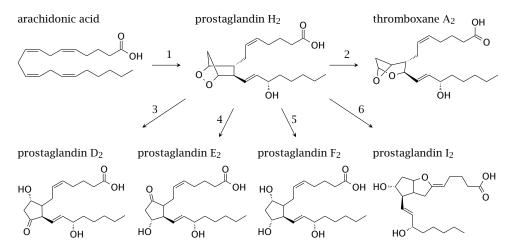
Synthesis of prostaglandins and leukotrienes is triggered by increased levels of cytosolic calcium, which may for example be induced by the phospholipase C pathway downstream of GPCR activation. Calcium allows cytosolic PLA₂ to bind and attack the negatively charged PIP₂. The arachidonic acid thus released is converted to precursors of prostaglandins and thromboxanes by the membrane-associated cyclooxygenases (Cox), or alternatively to leukotriene precursors by lipoxygenases (Lox). These enzymes are found on the membranes of the nucleus and of the endoplasmic reticulum.

¹Arachidonic acid is eicosatetraenoic acid; it is the most important precursor. Eicosapentaenoic acid, docosahexanoic acid and other ω -3 fatty acids contribute, but will not be considered here any further.



9.2 Biosynthetic pathways of prostaglandins and thromboxanes

Among the mediators synthesized downstream of cyclooxygenase and prostaglandin H_2 , thromboxane A_2 triggers thrombocyte aggregation, whereas prostaglandins E and I inhibit it (see slide 9.5.1). Prostaglandins D, E, F, and I are involved in pain perception, inflammation, and other physiological effects.

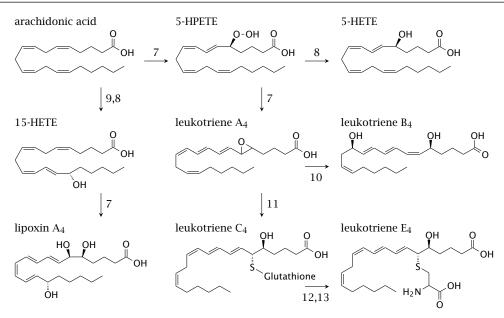


Enzymes: Cyclooxygenase (1), thromboxane A synthase (2), prostaglandin D, E, F and I synthases (3–6).

9.2.1 Eicosanoids synthesized by lipoxygenases

Leukotrienes are proinflammatory mediators; they play a major role in the pathogenesis of asthma. Lipoxins are important in the resolution of inflammation, that is, they are antiinflammatory.

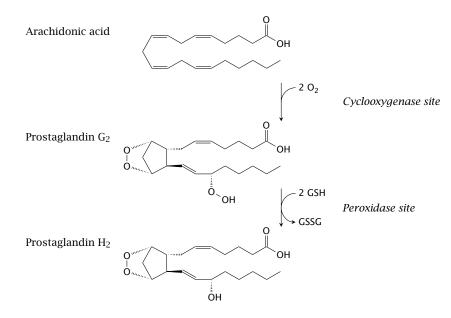
9.3 The two steps of the cyclooxygenase reaction



Enzymes: 5-lipoxygenase (7), HPETE peroxidase (8), 15-lipoxygenase (9), leukotriene A4 hydrolase (10), glutathione-S-transferase (11), peptidases (12,13).

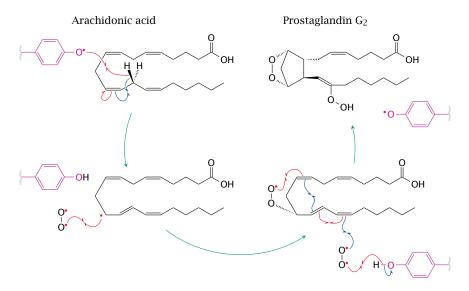
9.3 The two steps of the cyclooxygenase reaction

The cyclooxygenase reaction occurs in two stages, which are catalyzed in two separate active sites. In the cyclooxygenase site, arachidonic acid reacts with two molecules of O_2 to acquire an endoperoxide and a hydroperoxide group; this yields the intermediate prostaglandin G_2 . The hydroperoxide is then reduced to a simple hydroxyl group in the peroxidase site, which gives prostaglandin H_2 .



While the two active sites are quite close in space, there is no direct pathway for the substrate to migrate from one to the other. Prostaglandin G_2 must therefore dissociate from the cyclooxygenase site and then rebind to the peroxidase site of the same or another enzyme molecule.

9.3.1 Reactions in the cyclooxygenase site

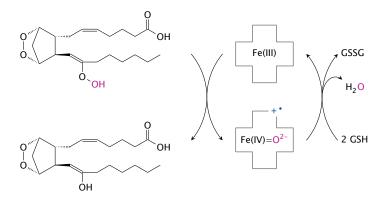


The key feature of the cyclooxygenase active site is a tyrosine residue (Tyr385 in Cox-1; highlighted in purple) which, in order to start the reaction, must be a radical. This radical abstracts a single hydrogen from arachidonic acid, which thereby itself turns into a radical. The radical electron then migrates and combines with an O_2 π -diradical.

A cascade of further radical reactions and bond migrations lead to cyclization and to radical combination with a second O_2 molecule, which then reclaims the hydrogen from the tyrosyl residue and thus restores the enzyme to its active state. The product, prostaglandin G_2 , then leaves the cyclooxygenase site to find a peroxidase site.

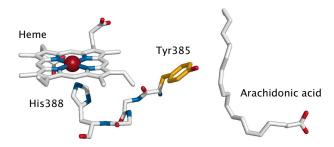
9.3.2 Reduction of prostaglandin G₂ to prostaglandin H₂

The peroxidase site contains heme as a prosthetic group. This heme "advances" one electron to each of the oxygen atoms in the peroxy group of the substrate, reducing their formal charges from -1 to -2 and retaining one of them. The heme itself is reduced in turn using two molecules of glutathione.



9.3.3 Interaction between the cyclooxygenase and peroxidase sites

The two active sites in the cyclooxygenase molecule are close in space, and an interaction between them is required to convert the tyrosine in the cyclooxygenase site to its active radical form. In this interaction, an electron is abstracted from the reduced form of the tyrosine and consumed in the peroxidase reaction, replacing one of the electrons derived from glutathione in the regular peroxidase reaction.



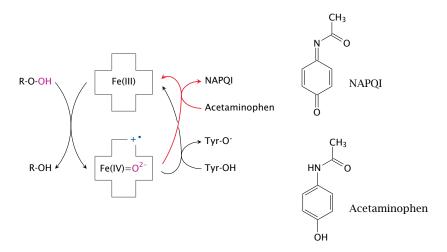
In the picture, arachidonic acid is shown to indicate the location of the cyclooxygenase site; Tyr 385 is the catalytic tyrosine residue in that site. It is connected to the heme in the peroxidase site via a short stretch of three amino acid residues, which reportedly acts as the electron conduit between it and the heme.

9.3.4 Priming of the active site tyrosine radical, and the action mode of acetaminophen

The activation of tyrosine 385 starts with the oxidation of heme by prostaglandin G_2 or another hydroperoxide (ROOH). The oxidized heme then obtains one electron from tyrosine 385 via the conduit shown in the preceding slide, thereby activating it; another electron is presumably obtained from glutathione (not shown). Acetaminophen intercepts the activation of tyrosine 385 by reducing heme itself.

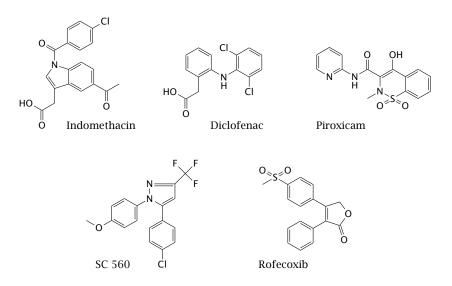
The inhibition of Cox by acetaminophen is countered by high cellular levels of hydroperoxides. Such high concentrations exist in leukocytes, which produce hydrogen peroxide and other reactive oxygen species as antimicrobial effectors (see slide 8.6). Therefore, while acetaminophen has good activity in neurons or sensory

cells and therefore suppresses pain and fever, it has little effect on the synthesis of proinflammatory prostaglandins in leukocytes. Better antiinflammatory activity is observed with drugs that inhibit the enzyme in a redox-independent fashion.



9.4 Noncovalent cyclooxygenase inhibitors

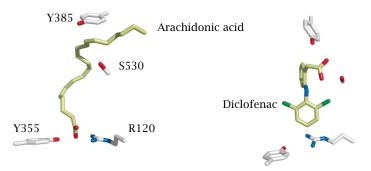
The drugs shown in this slide all inhibit the cyclooxygenase site of the enzyme. The three drugs in the top row all inhibit both Cox-1 and Cox-2, whereas SC560 inhibits only Cox-1, and rofecoxib only Cox-2. All drugs bind to the enzyme non-covalently.



Cox-2 is prominently expressed by inflammatory cells, and its selective inhibition was once believed to avoid potentially serious side effects that may arise from the simultaneous blockade of physiological "housekeeping" functions, which were credited to Cox-1. However, the opposite has turned out to be true: a significant increase in the frequency of myocardial infarctions under treatment with rofecoxib and other selective Cox-2 inhibitors has prompted their withdrawal from the market.

Indeed, experiments with genetic knockouts of Cox-1 [76] and Cox-2 [77] had thrown the assumed "good cop, bad cop" story into question before the Cox-2 inhibitors had even been introduced into the clinic; it is a bit perplexing that after these reports those efforts continued unabated.

9.4.1 Conformation of arachidonic acid and of diclofenac in the active site



Like arachidonic acid, both indomethacin and diclofenac have a carboxylic acid group, and one might reasonably surmise that all three carboxylates interact with the same amino acid residues in the active site. However, while this is true for arachidonic acid and indomethacin, crystallography shows that diclofenac sits in the active site upside down.

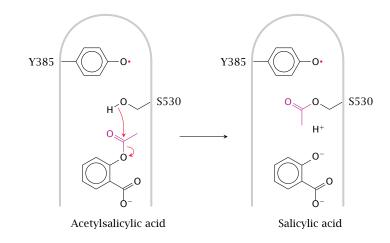
The carboxyl groups of arachidonic acid (left) and of indomethacin (not shown) bind to tyrosine 355 and arginine 120. In contrast, the carboxyl group of diclofenac (right) is hydrogen-bonded to serine 530 and to the catalytic tyrosine 385. Structures rendered from 1diy.pdb and 1pxx.pdb.

Inhibitor	Relative IC ₅₀		
	R120A	Y355F	S530A
Indomethacin	>240	>240	1.1
Diclofenac	3.3	1.8	>650
Piroxicam	>109	>109	>109

9.4.2 Cox inhibitors and Cox mutants

The above crystallographic findings agree with those from site-directed mutagenesis experiments. Arginine 120, tyrosine 255, or serine 530 were replaced with alanine or phenylalanine, respectively, whose side chains cannot engage in hydrogen bonding. An increase in the inhibitor concentration required for 50% inhibition (IC₅₀) relative to the wild type enzyme indicates that the mutant has become less susceptible to the inhibitor.

Mutants that lacked arginine 120 or tyrosine 355 were virtually resistant to indomethacin. In contrast, their inhibition by diclofenac was hardly affected by these changes, but was very sensitive to replacement of residue serine 530. Interestingly, piroxicam was susceptible to all three changes, indicating that avid binding of this inhibitor involves all three wild type residues. Data from [78].

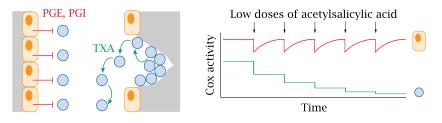


9.5 Acetylsalicylic acid is a covalent Cox inhibitor

Acetylsalicylic acid covalently modifies the side chain of serine 530 in the active site of Cox-1. This prevents access of arachidonic acid to the catalytic tyrosine and therefore inactivates the enzyme. Note that, in contrast to cholinesterase for example, the serine residue itself has no catalytic function; the interference of its modification with Cox activity is purely due to steric obstruction.

The drug also acetylates the homologous serine residue within Cox-2. The active site of Cox-2 has a larger interior volume, which means that access of arachidonic acid to the catalytic tyrosine is not completely prevented, and the reaction not completely suppressed. However, the reaction is "derailed", such that the modified enzyme no longer makes prostaglandin H_2 but instead now converts arachidonic acid to 15-*R*-HETE, which through additional enzymatic steps gives rise to epi-lipoxin A₄. Like the physiological mediator lipoxin A₄ (see slide 9.2.1), this compound has antiinflammatory activity and likely contributes to the therapeutic action of acetylsalicylic acid [79].

9.5.1 Rationale of low-dose acetylsalicylic acid treatment



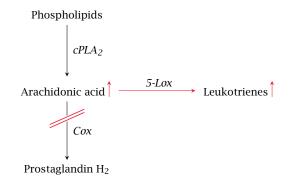
Patients with advanced atherosclerosis are at risk of myocardial infarction or stroke, which develop when atherosclerotic lesions in the vascular endothelium trigger the

formation of blood clots (see slide 10.6). Thrombocyte activation and aggregation is a key factor of blood clot formation; therefore, drugs that inhibit thrombocyte aggregation² play an important part in the preventive treatment of such patients. An early form of such treatment was low-dose acetylsalicylic acid. The rationale for this treatment is not obvious but rather subtle.

Eicosanoids have a dual role in thrombocyte aggregation (left). The vascular endothelium inhibits aggregation through sustained secretion of PGE and PGI. Where the endothelium is damaged, exposure of collagen sets off thrombocyte adhesion and aggregation, which is amplified through the secretion of thromboxanes by the platelets themselves. Both the thrombocyte-activating thromboxanes and the inhibitory prostaglandins E and I are synthesized downstream of prostaglandin H₂. Acetylsalicylic acid inhibits the synthesis of this common precursor by cyclooxygenase; why should this result in a net inhibition of aggregation?

The answer lies in the different lifetimes of the enzyme molecules in endothelial cells and in thrombocytes, respectively (right). Endothelial cells are nucleated and can, after each dosage of the drug, replace covalently inactivated cyclooxygenase molecules with newly synthesized ones. In contrast, thrombocytes lack nuclei and protein synthesis, and therefore cannot replace the modified enzyme molecules; the effect of repeated doses will thus be cumulative. Over time, cyclooxygenase activity will therefore be diminished much more in thrombocytes than in endothelial cells.

9.5.2 Cox inhibition can promote the synthesis of leukotrienes

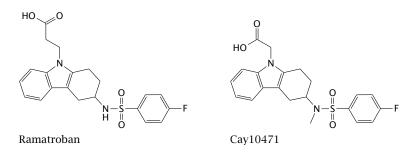


As we have seen in slide 9.1.2, the cyclooxygenase and lipoxygenase enzymes draw from the same pool of arachidonic acid substrate. Therefore, if cyclooxygenase is inhibited, more substrate may feed into the synthesis of leukotrienes which, like several prostaglandins, have proinflammatory activity. A well-known clinical consequence of this effect is the exacerbation of bronchial asthma in patients who take acetylsalicylic acid.

²Inhibitors of thrombocyte aggregation or plasmatic blood coagulation are sometimes referred to as "blood thinners." This metaphor is overly simplistic and misleading. As Einstein remarked, we should strive to make things as simple as possible, but not simpler. We here have a case of "too simple."

The production of surplus leukotrienes illustrates that inhibition of Cox as an antiinflammatory strategy has its limitations, and that more selective strategies for controlling eicosanoid action are desirable.

9.6 Inhibitors that act downstream of Prostaglandin H₂



Thromboxane A synthase is the enzyme that converts prostaglandin H_2 to thromboxane A_2 (see slide 9.2). The enzyme, as well as the receptor for thromboxane A_2 , are inhibited by the drug ramatroban.

Prostaglandin D is involved both in inflammation and pain. The experimental drug Cay 10471, which is structurally similar to ramatroban, inhibits prostaglandin D receptors.

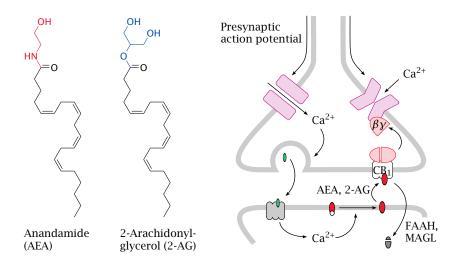
9.7 Endocannabinoids

- Arachidonate-containing, membrane-derived mediators
- Synthesis on demand, activated by calcium
- Mediate negative synaptic feedback
- CB₁ receptors involved in pain perception both in the peripheral and the central nervous system
- CB2 receptors found on immune cells

We have seen before that postsynaptic cells exercise negative feedback on presynaptic cells (see slide 6.9.1). The mediators that perform this role are endocannabinoids. Two major endocannabinoids are arachidonylethanolamine (AEA, anandamide) and arachidonylglycerol (2-AG). These mediators act on cannabinoid receptors, which are also the targets of the major active ingredients of cannabis.

Cannabinoid receptors are GPCRs and have two subtypes. CB₁ receptors dominate in the nervous system and are the major drug target. CB₂ receptors modulate immune cell function, but this observation has not yet translated into pharmacotherapy.

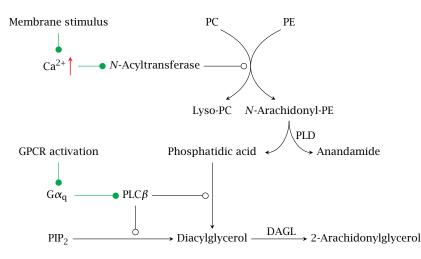
9.7.1 Feedback inhibition of synaptic transmission by endocannabinoids



Anandamide and arachidonylglycerol both contain arachidonic acid attached to a polar moiety that is biosynthetically derived from a phospholipid headgroup. Both mediators are synthesized in the postsynaptic cell in response to calcium signals; as we have seen, several neurotransmitter receptors will either directly or indirectly raise calcium levels.

The mediators are released by the postsynaptic cell into the synaptic cleft. Stimulation of presynaptic CB₁ receptors activates G proteins; the released $G\beta\gamma$ dimers inhibit cognate Ca⁺⁺ channels and also activate K⁺ channels, thereby reducing membrane excitability and transmitter release. The mediators are inactivated by cellular uptake and subsequent degradation by lipases (fatty acyl amide hydrolase, FAAH; monoacylglycerol lipase, MAGL; see slide 9.7.3).

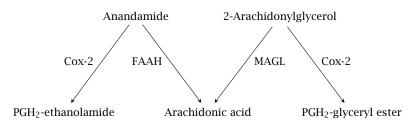
9.7.2 Biosynthesis of endocannabinoids



This slide shows the biosynthetic pathways that produce anandamide (*N*-arachidonylethanolamine) and 2-arachidonylglycerol (2-AG).

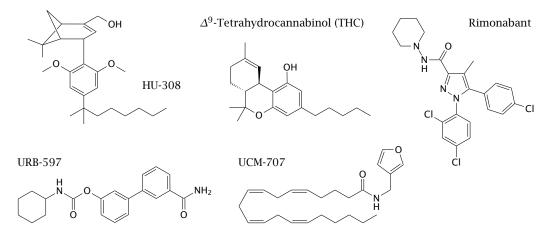
Increased Ca⁺⁺ levels activate an N-acyltransferase that transfers arachidonic acid from another phospholipid molecule to the amine in the headgroup of phosphatidylethanolamine (PE). Anandamide is then released by phospholipase D (PLD). 2-AG is produced by diacylglycerol lipase (DAGL) downstream of the phospholipase C pathway.

9.7.3 Degradation of endocannabinoids



Anandamide is hydrolyzed by fatty acid amide hydrolase (FAAH) and 2-arachidonylglycerol by monoacylglyerol lipase (MAGL). Both mediators can also be metabolized by cyclooxygenase 2 to produce prostamides, which have additional signaling roles that are not yet well characterized.

9.7.4 Drugs that interact with the endocannabinoid system



 Δ^9 -tetrahydrocannabinol (THC) is the main active ingredient of cannabis and stimulates both CB₁ and CB₂ receptors. HU-308 is a CB₂-selective agonist. URB-597 inhibits fatty acyl amide hydrolase. UCM-707 is usually described as an inhibitor of cellular uptake of endocannabinoids. However, it is not clear at present that there indeed exists a specific transporter for this reuptake; this compound, too, might inhibit the hydrolase. The functional effect of both inhibitors is to amplify endocannabinoid signaling.

Rimonabant is an inverse agonist at CB_1 receptors. It has been used to reduce appetite in order to treat obesity, but was withdrawn because depression was a common side effect.

Chapter 10

Intermediate metabolism, diabetes, and atherosclerosis

10.1 Overview

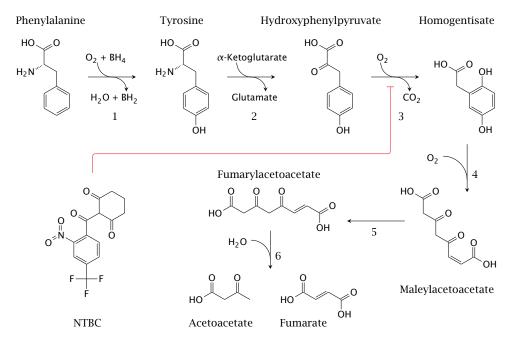
- **Genetic enzyme defects** rare; comparatively little effort spent on targeted drug development; only a few defects can be treated with drugs
- Gout more common; multiple causes, similar manifestation and treatment
- **Diabetes mellitus** very common; treatment with insulin injections (types 1 and 2) and oral antidiabetics (type 2)
- Atherosclerosis exceedingly common; drug therapy targets underlying metabolic conditions, other risk factors, and consequences of advanced disease

Intermediate metabolism involves numerous enzymes, and inheritable defects for many of them may cause disease. Only in a few cases can these genetic diseases be treated with drugs, and therefore, it does not make sense here to treat the entirety of metabolism in a systematic manner. Instead, we will look at a few selected defects for which some form of pharmacological treatment is available.

Diabetes is caused by endocrine dysregulation rather than primarily by enzyme deficiencies, so it could as well have been covered in chapter 7. It was included here because its manifestations are mostly metabolic. Also somewhat arbitrarily, atherosclerosis is covered here because of its important connections to cholesterol metabolism.

10.2 Enzyme defects in amino acid metabolism

The two hereditary enzyme defects that we will discuss here, phenylketonuria and tyrosinemia, both affect the same pathway, namely the degradation of phenylalanine and tyrosine. Their clinical manifestations are quite different, however, and so are their treatments.



10.2.1 Degradation of phenylalanine and tyrosine

BH₂ and BH₄, di- and tetrahydrobiopterin, respectively. Enzymes: 1, phenylalanine hydroxylase; 2, tyrosine transaminase; 3, *p*-phenylpyruvate dioxygenase; 4, homogentisate dioxygenase; 5, maleylacetoacetate isomerase; 6, fumarylacetoacetate hydrolase.

This slide shows the degradation pathway for phenylalanine and of tyrosine, and the structure of NTBC (2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione), an inhibitor of *p*-hydroxyphenylpyruvate dioxygenase (3) that is used in tyrosinemia type I. The enzyme defect in phenylketonuria concerns phenylalanine hydrolase (1), and the one in tyrosinemia affects fumarylacetoacetate hydrolase (6).

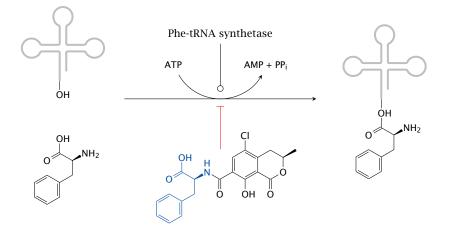
10.2.2 Phenylketonuria (PKU)

- Homozygous defect of phenylalanine hydroxylase
- Frequency: 1 newborn among 10,000 in Caucasians; lower frequency in other races
- Possible *heterozygote advantage*: reduced fetal susceptibility to ochratoxin A
- Symptomatic excess of phenylalanine manifest only after birth; intrauterine development normal
- Cognitive and neurological deficits, probably due to cerebral serotonin deficit
- Treated with phenylalanine-restricted diet
- Some cases due to reduced affinity of enzyme for cofactor tetrahydrobiopterin (BH₄), can be treated with high dosages of BH₄

As with most genetic enzyme defects, the clinical disease is manifest only in homozygous individuals. Dietary phenylalanine that is not used for protein synthesis accumulates and causes toxicity. It appears that the excess phenylalanine crowds out tryptophan at the L-aromatic amino acid transporter in brain capillaries, which is the same transporter that also transports tyrosine and L-DOPA to the brain (see slide 3.5.5). Since tryptophan is the precursor of serotonin (see slide 6.12.1), this results in a lack of cerebral serotonin [80], which is believed to cause the observed cerebral deficits.

In addition to phenylalanine itself, some aberrant metabolites derived from it also occur at increased levels, and the appearance of ketone derivatives such as phenylpyruvic acid in the urine has given the disease its name. These metabolites have no proven connection to the pathogenesis of the disease.

10.2.3 Ochratoxin A inhibits phenylalanyl-tRNA synthetase



The variation of the gene frequency for PKU between races and geographical areas suggests that, in some parts of the world, environmental conditions may confer a selective advantage of the heterozygous state; accepted examples of such *heterozygote advantage* are sickle cell anemia and glucose-6-phosphate dehydrogenase deficiency, which endow heterozygotes with greater resistance to malaria. It has been proposed that the heterozygote advantage in PKU consists in protection from the fungal toxin ochratoxin A, which is produced by some *Aspergillus* molds that cause food to rot [81].

Ochratoxin A competitively inhibits the coupling of phenylalanine to its cognate tRNA and thereby disrupts protein synthesis. It is more toxic to fetuses than to adults, most likely because fetuses are short of enzymes required for detoxifying xenobiotics. Mothers who are heterozygous for PKU will have a somewhat higher level of phenylalanine, which will be shared with the fetus via the placenta. This will counter the inhibition of tRNA aminoacylation in the fetus and thereby afford it a degree of protection.

One of the places with the highest abundance of PKU is Ireland. This country is also known for its repeated historic episodes of famine. Starving people will more likely eat rotten food rather than discard it. Indeed, reference [81] reports that lowered rates of abortion were found in Irish women who were heterozygous for PKU. I have not tried to ascertain whether these quoted statistics applied to periods of actual famine.

10.2.4 Tyrosinemia

- Homozygous defect of fumarylacetoacetate hydrolase
- Prevalence: 1 in 100,000 people worldwide; 1 in 1,850 in the Saguenay region (Quebec)
- Fumarylacetoacetate and preceding metabolites back up
- Fumaryl- and maleylacetoacetate react with glutathione and other cellular nucleophiles, causing liver toxicity, cirrhosis, carcinoma
- The drug NTBC inhibits *p*-hydroxyphenylpyruvate dioxygenase, intercepting the degradative pathway upstream of the toxic metabolites

Tyrosinemia is comparatively common in Quebec. In this case, there seems to be no heterozygote advantage; instead, the high incidence is due to the so-called "founder effect", that is, the common descent of the afflicted population from a small group of founding settlers that happened to contain one or several carriers of the gene. (Refer to slide 10.2.1 for the relevant pathway and enzyme reactions.)

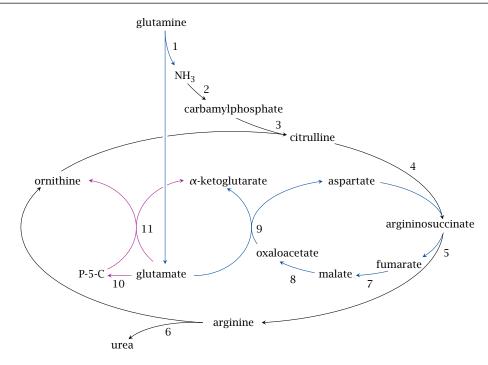
10.2.5 The urea cycle and related pathways

The urea cycle runs in the liver and disposes of ammonia that accrues in the degradation of amino acids. It incorporates two molecules of ammonia into one molecule of urea, which is excreted in the urine.

While amino acid degradation occurs to a large part in the liver, other organs also participate; for example, the branched-chain amino acids leucine, isoleucine and valine are mostly degraded in skeletal muscle. Surplus ammonia is transported from these organs to the liver in the form of alanine or glutamine.

In the slide, reactions 2–6, shown in black, form the urea cycle. Reactions 1 and 7–9, shown in blue, supply the urea cycle with nitrogen from glutamine. Reaction 10 yields Δ^1 -pyrroline-5-carboxylate (P-5-C), and together with reaction 11 replenishes ornithine.

Enzyme defects occur in various reactions of the urea cycle; all of them will cause the accumulation of surplus ammonia. The enzyme defects are treated with protein-restricted diet, alternate pathway therapy, and supplementation of arginine or citrulline.



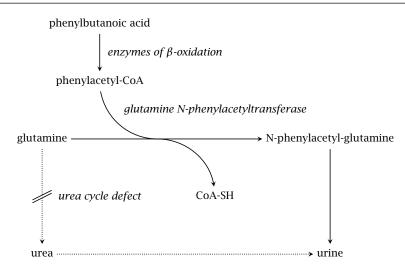
Enzymes: 1, glutaminase; 2, carbamylphosphate synthetase; 3, ornithine transcarbamylase; 4, argininosuccinate synthase; 5, argininosuccinase; 6, arginase; 7, fumarase; 8, malate dehydrogenase; 9, aspartate aminotransferase; 10, Δ^1 -pyrroline-5-carboxylate synthase; 11, ornithine amino-transferase.

10.2.6 Alternate pathway therapy in urea cycle enzyme defects

Alternate pathway takes advantage of the amino acid conjugation reactions that occur as part of phase II drug metabolism (see slide 4.4.9). In this application, the usual physiological role of amino acid conjugation is turned on its head: While in normal metabolism amino acids are expended in order to inactivate and eliminate unwanted organic acids, we here supply innocuous organic acids in order to induce their conjugation with amino acids. The elimination of these conjugates then becomes an alternate means for the disposal of surplus nitrogen. Brilliant!

The substrate acid covered in this scheme is phenylbutanoate, which undergoes β -oxidation before conjugation to glutamine and excretion in the urine. Another useful substrate acid is benzoate, which undergoes conjugation with glycine and further drains the liver's pool of nitrogen.

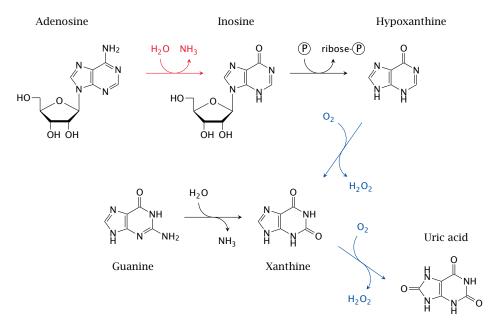
Alternate pathway therapy is combined with the application of arginine or citrulline. If the urea cycle is functional, arginine can be diverted to protein synthesis as required, and so is not an essential amino acid; however, disruption of the urea cycle may cause a shortage of arginine. This may induce protein catabolism, thereby exacerbating the symptoms. If the enzyme defect is *not* between citrulline and arginine, it is possible to supply citrulline instead, which has the advantage of picking up another surplus nitrogen en route to arginine.



10.3 Metabolic diseases related to purine nucleotide metabolism

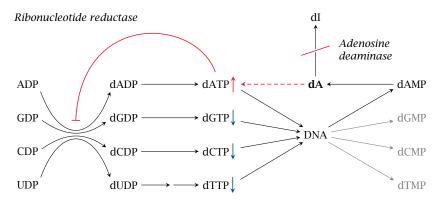
In this section, we will consider adenosine deaminase deficiency and gout. Adenosine deaminase deficiency is a hereditary enzyme defect and, as such, a rare condition. In contrast, gout can arise from multiple causes and thus is much more common.

10.3.1 Overview of purine degradation



This slide shows some of the reactions involved in purine nucleotide degradation. The reaction catalyzed by adenosine deaminase is shown in red; those catalyzed by xanthine oxidase, which is a drug target in gout, are shown in blue. Uric acid is the final degradation product that is excreted in the urine. Note that adenosine deaminase degrades both adenosine (shown here) and deoxyadenosine. Accumulation of deoxyadenosine is crucial for pathogenesis in this disease (see next slide).

10.3.2 Adenosine deaminase deficiency causes dysregulation of DNA synthesis



Adenosine deaminase (ADA) degrades not only adenosine but also deoxyadenosine (dA), which it converts to deoxyinosine (dI). If ADA is lacking, dA accumulates and is converted back to dATP by salvage pathway nucleoside and nucleotide kinases. Excess dATP inhibits ribonucleotide reductase and thereby throttles the supply of deoxyribonucloside triphosphates other than dATP. This interferes with DNA synthesis and promotes apoptosis.

The apoptotic stimulus created by ADA deficiency has limited strength and, in most cell types, falls short of triggering manifest apoptosis. However, it does induce apoptosis in lymphocytes, which are very susceptible to apoptotic stimuli in general. Apoptosis depletes both T- and B-lymphocytes, which gives rise to severe combined immunodeficiency (SCID).¹

10.3.3 Therapy of adenosine deaminase deficiency

- 1. Bone marrow transplant
- 2. *Ex vivo* gene therapy
- 3. Enzyme replacement therapy—PEGylated bovine ADA
- 4. Experimental in vitro approach: Inhibition of salvage kinases

The current standard treatment of ADA is bone marrow transplant. If the bone marrow of the patient is replaced with that of a healthy donor, the lymphocytes derived from the new bone marrow will express an intact ADA gene, and therefore be able to protect themselves from dATP accumulation and apoptosis. Gene therapy is based on the same principle, but uses replacement of the defective gene within

¹In a combined immunodeficiency, both *cellular* immunity, which is mediated by T-killer cells and other effector lymphocytes, and *humoral* immunity, that is, formation of antibodies, are compromised. The drug *pentostatin*, an inhibitor of adenosine deaminase, is used to selectively kill lymphocyte-related tumor cells in hairy cell leukemia and other lymphatic malignancies.

the patients' own bone marrow stem cells. It is transitioning from experimental to routine treatment [82].

Enzyme replacement therapy with ADA enzyme isolated from cattle is effective but inferior to bone marrow transplant. It is used mostly to bridge the time interval between diagnosis and the identification of a suitable bone marrow donor. Modification of the enzyme with polyethyleneglycol (PEG) serves to reduce its immunogenicity and extend its lifetime in the circulation.

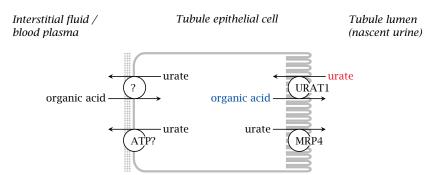
A plausible pharmacological approach to prevent the pathology caused by deoxyadenosine accumulation would be to inhibit the salvage kinases that convert deoxyadenosine to dATP, the actual inhibitor of ribonucleotide reductase. While this approach has been demonstrated *in vitro* [83], I have not seen any reports describing its further development *in vivo*.²

10.3.4 Gout

- Genetic or dietary factors promote increased production or retention of uric acid
- Uric acid has low solubility, and excess levels form crystalline deposits, preferentially in joints and soft tissue
- Urate crystals promote inflammation and lead to arthritis that is painful and destructive

Gout results from excess levels of uric acid, the end product of adenine and guanine degradation (see slide 10.3.1). The disease is not restricted to patients with mutations in a specific gene and can arise from different causes; it thus is far more common than any of the enzyme defects covered so far. Nevertheless, the clinical symptoms remain the same and are addressed by similar therapeutic measures.

10.3.5 Transport of uric acid in the kidneys



Urate is subject to glomerular filtration as well as to tubular reuptake and secretion. The key transporter in tubular reuptake is URAT1, which exchanges uric acid for

²In this context, we should note that an enzyme defect in another nucleotide salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPRT), is associated with severe pathology (Lesch-Nyhan syndrome).

other organic acids. Metabolic conditions that increase the supply of exchange substrates for URAT1 promote gout; conversely, inhibitors of URAT1 can be used to treat the disease.

10.3.6 Dietary factors that promote gout

- Overly purine-rich food
- Drugs that contain purines: dideoxyadenosine
- Alcoholic beverages—but not all kinds: beer yes, wine no
- Anorexia nervosa (!)
- Drugs that interfere with uric acid excretion: pyrazinamide, salicylic acid
- Excessive fructose or sucrose

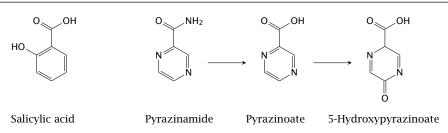
It seems that dietary purines are mostly *not* utilized via salvage pathways but instead are converted to uric acid and excreted [84]. Therefore, a diet that is overly rich in purines—typically, due to a high content of meat—offers a straightforward way to contract gout. Drugs can be a source of excess purine, too, such as for example 2,3-dideoxyadenosine (didanosine), an inhibitor of retroviral DNA synthesis that is used in HIV patients (see slide 11.10.5). The drug undergoes degradation like other purine nucleotides and may occasionally trigger gout [85].

There are several conceivable connections between alcohol and gout, of which no single one has unequivocally been shown to be the dominant one. Degradation of alcohol via alcohol dehydrogenase and then aldehyde dehydrogenase produces NADH, which shifts the equilibrium of the lactate dehydrogenase reaction from pyruvate to lactate. Lactate may then serve as an exchange substrate at URAT1 and thereby inhibit renal elimination of urate (see slide 10.3.5). However, a recent statistical study found an association of beer but not wine consumption with gout [86]. Beer is higher in calories and in purines than wine, suggesting that alcohol itself, at moderate levels of consumption, is not an important determinant.

Considering that gout is often brought on by an overly rich diet, it may be surprising that *anorexia nervosa*, an eating disorder in which patients eat only the bare minimum required to ward off death, and sometimes less, may also lead to gout [87]. This is probably due to the formation of ketone bodies, which as organic acids may also increase tubular reuptake of uric acid.

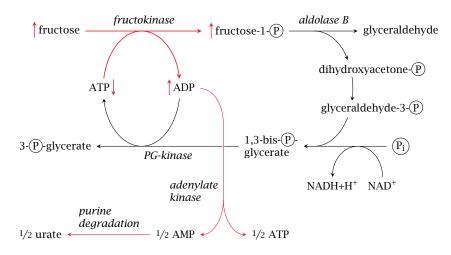
10.3.7 Drugs that may promote gout by promoting tubular reuptake of urate

Salicylic acid as well as pyrazinoic acid and 5-hydroxypyrazinoic acid, which are formed in the metabolism of the tuberculostatic drug pyrazinamide, also act as exchange substrates for uric acid at URAT1 and thereby reduce its renal elimination. Pyrazinamide may be used in dosages of grams per day and continuously for several months, and the correspondingly large accruing amount of metabolites may result in gout.



10.3.8 High dietary fructose promotes gout

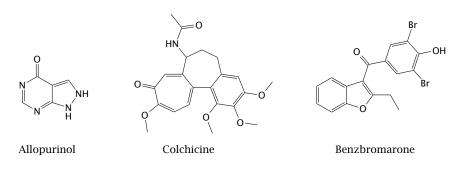
Fructose has been linked to increased uric acid production both experimentally [88, 89] and statistically [90]. The mechanism is as follows: Fructokinase produces fructose-1-phosphate more rapidly than it can be turned over by aldolase B. Accumulating fructose-1-phosphate ties up phosphate, which is then no longer available for the regeneration of ATP. ADP rises, and adenylate kinase causes AMP to rise in turn; the latter enters degradation to uric acid.



In keeping with this mechanism, a fructose challenge causes a greater increase of urate synthesis in heterozygous carriers of a deficient aldolase B gene than in individuals with two intact gene copies. In the homozygous state, aldolase B deficiency causes *hereditary fructose intolerance*, which is also due to phosphate becoming tied up in fructose-1-phosphate, but to a much greater extent and with more immediate and potentially disastrous consequences for the liver cell.

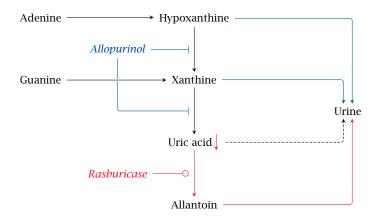
10.3.9 Drugs used in the treatment of gout

Allopurinol inhibits xanthine oxidase and thereby the formation of uric acid. Colchicine is an alkaloid that inhibits actin polymerization and has anti-inflammatory effects; it is used for symptomatic treatment in acute attacks of gout. Benzbromarone is an *uricosuric* drug, that is, it inhibits the tubular reuptake of uric acid by URAT1 and so increases its renal elimination.



10.3.10 Complementary effects of allopurinol and "rasburicase"

In purine degradation, adenine and guanine are converted by deamination to hypoxanthine and xanthine, respectively. Xanthine oxidase converts hypoxanthine to xanthine, as well as xanthine to uric acid. Uric acid is the final product of purine degradation and is excreted.



Uric acid has limited solubility, and increased levels of uric acid lead to its precipitation or crystallization in joints or in the kidneys. The latter occurs most acutely and dramatically in tumor lysis syndrome during chemotherapy of malignant lymphoma or leukemia.³ Tumor lysis syndrome is itself an acutely life-threatening condition.

In both gout and tumor lysis syndrome, inhibition of xanthine oxidase prevents the final oxidation steps, leading to the excretion of hypoxanthine and xanthine of uric acid. These are somewhat more soluble than uric acid and therefore less prone to precipitation prior to excretion. However, xanthine in excessive amounts can again give rise to calamitous precipitate formation.

A way out of this dilemma is the application of urate oxidase. This enzyme, which occurs in animals other than primates and in many other organisms, converts urate to allantoin, which is considerably more soluble and forms the end product

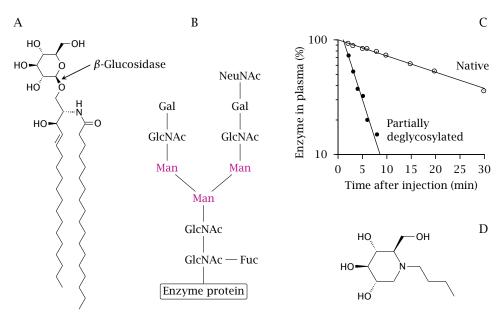
³Most solid tumors are treated first with conventional surgery, which removes most of the malignant cell mass. In contrast, lymphomas and leukemias are treated with cytotoxic drugs right away; large numbers of malignant cells are thus killed simultaneously, and the nucleic acids contained within them enter degradation all at once.

of purine degradation in these organisms. "Rasburicase" is a recombinant urate oxidase preparation used to cope with excess urate in chemotherapy patients.

10.4 Lysosomal storage diseases

- Acidic hydrolases in lysosomes break down many cellular macromolecules, including lipids and mucopolysaccharides
- Enzyme defects cause accumulation of undegraded substrates, often in liver, spleen, and bone marrow, leading to organ enlargement and loss of function
- Some enzyme defects can be treated with enzyme substitution therapy

Lysosomal storage diseases are due to homozygous enzyme defects; they are rare, often clinically serious conditions. Out of the considerable variety of diseases, we will pick Gaucher disease as an example.



10.4.1 Biochemistry of Gaucher disease

This slide summarizes the biochemical aspects of Gaucher disease. The gene defect concerns the enzyme lysosomal β -glucosidase or glucocerebrosidase, which cleaves the glucose moiety from glucocerebroside (glucosylceramide), a sphingolipid found in cell membranes (A). In Gaucher patients, the lipid accumulates in macrophages in the liver, spleen, and bone marrow. Liver and spleen become vastly enlarged and functionally deficient. Only a small fraction of the extra mass actually consists of glucosylceramide; the exact cause of the surplus enlargement is not understood.

 β -Glucosidase is a glycoprotein; the structure of its glycosyl moiety is shown in panel B.⁴ Partial enzymatic removal of the oligosaccharide moiety up to the

⁴Abbreviations: NeuNAc, N-acetylneuraminic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Man, mannose; Fuc, L-Fucose.

dotted line exposes mannose residues, which facilitate uptake of the enzyme by macrophages via a mannose-binding protein on the cell surface.

Panel C shows that the partially deglycosylated enzyme is removed from blood plasma much faster than the unmodified enzyme, which reflects the increased efficiency of uptake by macrophages.⁵ Replacement therapy with such enzyme preparations has very good clinical effectiveness and has become the standard treatment. Figure prepared from original data in [91].

Panel D shows the structure of miglustat, an inhibitor of glucocerebroside synthesis that is used in the treatment of Gaucher disease. Since glucocerebroside is an essential membrane constituent, inhibition of its synthesis cannot be complete, so this principle can only be used as an adjunct treatment.

10.5 Diabetes mellitus

- Lack of insulin activity due to
 - destruction of pancreatic island β cell (type 1)
 - loss of insulin sensitivity in peripheral organs (type 2)
 - excessive levels of hormones antagonistic to insulin
- Blood glucose accumulates and causes acute and chronic pathology
- Treated with insulin substitution (type 1 and 2) and oral drugs (type 2)

Insulin is a peptide hormone that directs the utilization of dietary glucose. In most tissues, uptake of glucose by facilitated diffusion must be activated by insulin. In addition, insulin promotes the expression of enzymes that degrade glucose and convert it to fatty acids. The insulin receptor is a membrane-associated receptor tyrosine kinase. The release of insulin from the β cells in the pancreatic islets is induced by the blood level of glucose itself (see slide 6.6.2).

The destruction of the pancreatic islet cells in type I diabetes is due to an autoimmune reaction that is triggered by coxsackievirus infections. These infections are typically contracted at young age, which is the reason why type I diabetes is also known as juvenile diabetes.

Type II diabetes typically sets in during middle or advanced age; it is more common in overweight patients. The underlying mechanism of diabetes type II is still not well understood. In contrast to type I diabetes, the capacity of the β cells for producing insulin is preserved, and one therapeutic approach is to use drugs that amplify the secretion of insulin. In advanced stages, endogenous insulin production declines, and insulin substitution becomes necessary, as it is right from the beginning in type I diabetes.

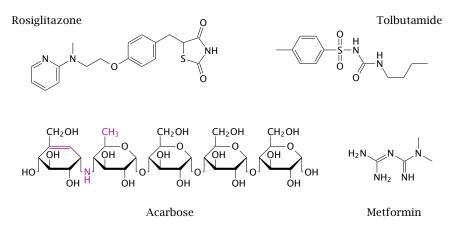
Symptomatic diabetes often arises from an excess of glucocorticoid hormones, which may be due either to their use as drugs or to their formation by tumors in

⁵Various sugar-binding proteins, or *lectins*, are found on macrophages. They serve to recognize polysaccharides on microbial cell surfaces, which then mediates phagocytosis and destruction of the microbes.

10 Intermediate metabolism, diabetes, and atherosclerosis

the adrenal gland. Excessive activity of thyroid or catecholamine hormones can also give rise to symptomatic diabetes.

10.5.1 Oral antidiabetic drugs



Oral antidiabetic drugs are a viable option only with type II diabetics in whom glucose regulation still works but is functionally inadequate. The therapeutic rationale is variously to slow glucose uptake, to increase insulin secretion, or to improve the diminished insulin sensitivity in peripheral tissues.

Acarbose is an inhibitor of intestinal maltase, the enzyme that cleaves maltose and other amylose digestion fragments to glucose. Since only monomeric glucose can be taken up from the intestinal lumen, maltase inhibition slows down glucose uptake, thereby reducing the peak load on glucose regulation.

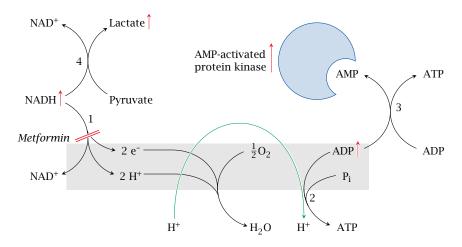
The action mode of tolbutamide has already been discussed earlier (see slide 6.6.2). Rosiglitazone, a thiazolidinedione, reduces insulin resistance in type 2 diabetes. It is an agonist of the PPAR γ , a nuclear hormone receptor that regulates the transcription of numerous enzymes and transporters involved in glucose and fat metabolism. but it has numerous side effects including the promotion of heart failure that have led to its withdrawal from the market in Europe. More recently, thiazolidinediones were found to specifically inhibit the mitochondrial pyruvate transporter [92, 93]. This should reduce ATP and increase AMP levels in the cell. The next slide, which considers metformin, discusses how this might translate into an antidiabetic effect.

10.5.2 Hypothetical mode of action of metformin

Metformin promotes activation of AMP-dependent protein kinase, which improves insulin-independent glucose utilization. This slide illustrates a hypothetical mechanism for this activation.

NADH dehydrogenase (1) is partially inhibited by metformin. This slows the entire respiratory chain and therefore the regeneration of ATP from ADP by ATP synthase (2). Adenylate kinase (3) converts ADP to AMP, which binds to AMP-activated

protein kinase. The activated kinase stimulates glucose transport, even in the absence of insulin, as well as catabolic pathways. Another potentially relevant effect of AMP is the inhibition of adenylate cyclase [94], which would counteract the effect of glucagon and epinephrine, and so help to restore the balance between insulin and its antagonists.



Accumulated NADH promotes the reduction of pyruvate by lactate dehydrogenase (1). Depletion of pyruvate inhibits gluconeogenesis, and accumulation of lactate promotes lactate acidosis, which is a known side effect of metformin.

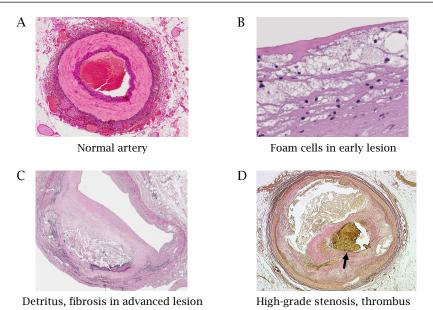
10.6 Atherosclerosis

- Degenerative and inflammatory disease of the arteries
- Promoted by hypercholesterolemia, hypertension, diabetes, smoking
- Damaged arteries subject to chronic or acute obstruction, hemorrhage
- Most common cause of death, ahead of all cancers and leukemias combined
- Treatment strategies address cholesterol levels, blood pressure, blood coagulation

Atherosclerosis is not a purely metabolic disease; hypertension is another very important causal factor, and we have already noted several types of drugs that are used to treat it. Here, we will focus on the metabolic aspects of atherosclerosis and the related pharmacology.

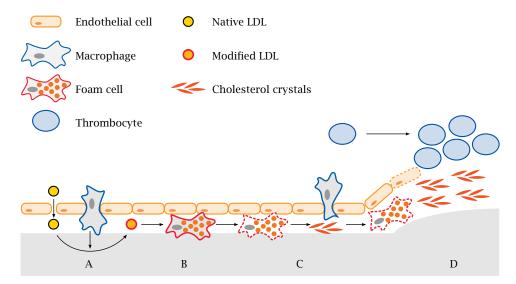
10.6.1 Appearance of atherosclerotic lesions

This slide shows cross sections of a normal artery (A) as well as those of arteries with atherosclerotic lesions in different stages of advancement (B-D). The normal artery displays inner and outer layers of connective tissue, stained in dark purple, as well as a strong intermediate muscular layer that shows up in a lighter tone. The endothelium is too thin to be discerned in this low power magnification view. The blood clot in the lumen is a post-mortem artifact.



Panel B shows a higher power view of an early lesion. The bubbly appearance is due to *foam cells*, which are macrophages stuffed chock-full with lipids. Panel C shows an advanced lesion with connective tissue proliferation and accumulation of detritus; the lumen of the blood vessel is considerably reduced. Panel D shows an artery that was already almost completely obliterated by a proliferating lesion that had spread around the entire circumference; the small residual lumen is blocked by an acutely formed thrombus (stained yellow-brown). Images reproduced with permission from pathorama.ch.

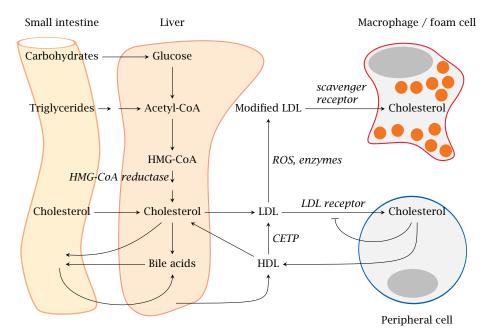
10.6.2 Development of an atherosclerotic lesion



This slide summarizes the developmental stages of an atherosclerotic lesion. LDL is low density lipoprotein, a specific type of plasma lipoprotein particles that is enriched in cholesterol.

- (A) Small defects in the endothelium cause seepage of LDL into the subendothelial tissue, followed by transmigration of macrophages. Reactive oxygen species and enzymes released by macrophages modify the LDL.
- (B) Modified LDL is taken up by macrophages via scavenger receptors. Unlike the uptake of unmodified LDL via regular LDL receptors, the uptake mediated by scavenger receptors is not subject to feedback inhibition and thus causes lipid overload, which turns macrophages into foam cells.
- (C) Foam cells perish, disintegrate and release accumulated cholesterol, which forms crystalline deposits. These crystals activate new macrophages and cause them to release inflammatory cytokines that further incite and amplify the inflammation.
- (D) In an advanced lesion, cells in the muscular layer proliferate, progressively constricting the artery. When the endothelium that covers the lesion becomes eroded, thrombocytes and plasmatic coagulation factors are activated and initiate blood clotting, causing acute thrombus formation and obstruction.

As an alternative to thrombus formation, acute failure of a damaged artery can also occur through rupture. Approximately 20% of all cerebral infarctions are caused by rupture and hemorrhage rather than thrombotic occlusion.



10.6.3 Transport and metabolism of cholesterol

Cholesterol is the key lipid that accumulates in atherosclerotic lesions and sustains the inflammation and subsequent degeneration. Lowering cholesterol is one of the most important goals in the treatment of atherosclerosis. To devise proper strategies, we first need to understand how cholesterol is formed, transported and eliminated.

The pool of cholesterol in the liver is replenished by uptake of dietary cholesterol and by synthesis from other foodstuffs via acetyl-CoA and hydroxymethylglutaryl-CoA (HMG-CoA). It is transported from the liver to peripheral tissues packaged into low density lipoprotein (LDL) particles. Cells in the peripheral organs take it up by endocytosis via LDL receptors (LDL-R). Uptake via this receptor is subject to feedback inhibition by intracellular cholesterol.

Excess cholesterol is returned from peripheral cells to the liver via HDL, but on the way some of it is transferred back from HDL to LDL by cholesterol-ester transfer protein (CETP). The liver also uses cholesterol to synthesize bile acids, which are secreted into the bile along with some excess cholesterol. Bile acids undergo reuptake at the end of the small intestine and return to the liver.

As discussed above, within atherosclerotic lesions, LDL undergoes modification by reactive oxygen species (ROS) or degradative enzymes. Enzymatically or chemically modified LDL enters macrophages via scavenger receptors, overloads them with cholesterol and turns them into foam cells.

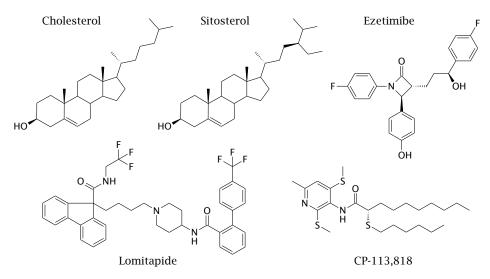
ABCA5/8 Chylomicron ABCA5/8 Precycling Precycling Chylomicron ACAT ACAT Cholesterol ester Cholester

10.6.4 Intestinal cholesterol uptake

Uptake of dietary cholesterol into the cells of the intestinal mucous membrane occurs through endocytosis and is controlled by the membrane protein NPC1L1. From the ingested vesicles, cholesterol is shuttled to the endoplasmatic reticulum by the microsomal triglyceride transport protein (MTTP; obviously a bit of a misnomer). In the ER, cholesterol is esterified by acyl-CoA-cholesteryl-acyltransferase (ACAT) and incorporated into nascent *chylomicrons*, which are large lipoprotein particles that also carry triacylglycerol.

The chylomicrons are released on the basolateral side and make their way to the liver via the lymphatics and the blood circulation. Plant sterols such as sitosterol will also be taken up by endocytosis but are ejected back into the intestinal lumen through an ABC5/8, an ABC transporter.

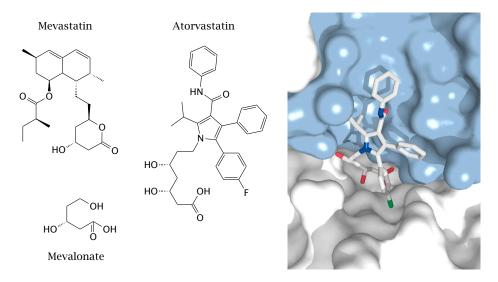
10.6.5 Inhibitors of intestinal cholesterol uptake



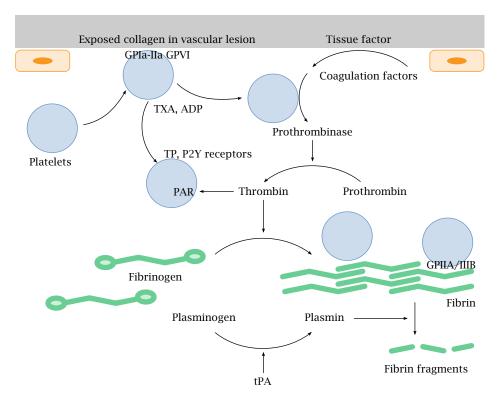
Inhibition of intestinal cholesterol uptake is, on its own, not sufficient for controlling cholesterol, but it is useful in combination with inhibitors of cholesterol synthesis (see below).

Sitosterol is a natural plant sterol that competes with cholesterol for uptake into the intestinal cell by endocytosis, but is then ejected back into the gut (see previous slide). Ezetimibe is an inhibitor of NPC1L1. Lomitapide and CP-113,818 are experimental inhibitors of MTTP and of ACAT, respectively.

10.6.6 Inhibition of HMG-CoA reductase with statins



The key regulated step in cholesterol biosynthesis is catalyzed by hydroxymethylglutaryl-CoA (HMG-CoA) reductase, which converts its substrate to mevalonate. This enzyme is targeted by the so-called "statin" drugs. Mevastatin, a natural antibiotic, was the first such drug to be isolated. Atorvastatin is a synthetic inhibitor; it is shown on the right within the active site of HMG-CoA reductase. Both inhibitors contain a moiety that resembles mevalonate.



10.6.7 Overview of blood coagulation

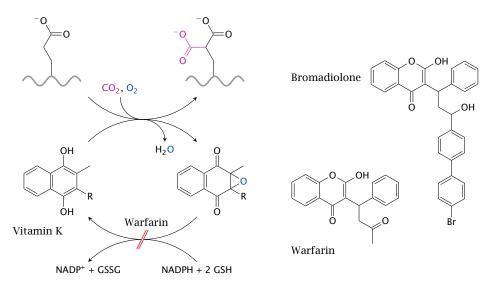
While reduction of cholesterol and blood pressure help to prevent the progress of atherosclerosis, they cannot do much to revert manifest vascular lesions. In patients with advanced atherosclerosis, it therefore becomes important to lower the risk of thrombus formation atop those lesions. This is done by partial inhibition of blood coagulation. Such treatment must be used with care; if coagulation is inhibited too strongly, the increase in the risk of hemorrhage may exceed the reduction in the risk of thrombotic infarction.

Blood coagulation is initiated by defects in the vascular endothelium, which expose collagen fibers. Platelets (thrombocytes) bind to collagen via their collagen receptors (GP1a-IIa, GPVI) and begin to secrete ADP and thromboxanes. These mediators activate further platelets through purine (P2Y) and thromboxane (TP) receptors, respectively. Plasmatic coagulation factors sequentially activate one another by proteolysis at the site of the lesion and on the surface of activated platelets; this culminates in the activation of thrombin (factor II). Thrombin then cleaves fibrinogen to fibrin, which forms the clot. It also cleaves protease-activated receptors (PAR) on platelets and so further amplifies platelet activation. Platelets adhere to fibrinogen or fibrin via GPIIA/IIIA receptors.

Both plasmatic coagulation and platelets can be inhibited with drug therapy. On platelets, all of the receptors discussed above can be targeted. We have already seen ticlopidine, which inhibits P2Y receptors (slide 6.16.1), as well as ramatroban, which blocks both the synthesis and receptors of thromboxane A₂ (slide 9.6). Low-dose aspirin therapy has been discussed as well (slide 9.5.1).

The fibrin clot is ultimately dissolved by plasmin, which is proteolytically released from plasminogen by tissue plasminogen activator (tPA). The latter enzyme is used to dissolve blood clots and so restore organ perfusion in acute cases of myocardial infarction and stroke. The related protease urokinase (see slide 1.1.2) can be used for the same purpose.

10.6.8 Inhibition of plasmatic blood coagulation with warfarin



Plasmatic coagulation can be inhibited by antimetabolites of vitamin K. Several coagulation factors (II, VII, IX, and X) require posttranslational modification of glutamate residues to γ -carboxyglutamate. The modified residue enables these proteins to chelate calcium, which in turn lets them bind to the surfaces of activated thrombocytes. The enzyme that carries out the modification, γ -glutamyl carboxylase, requires reduced vitamin K as a cosubstrate and converts it to an epoxide. The latter is reduced again by vitamin K epoxide reductase, which is inhibited by the drug warfarin. (In the structures of vitamin K and its epoxide, R represents a side chain containing several isoprenoid residues.)

Complete inhibition of vitamin K reductase is, of course, deleterious, and indeed is used to kill rats with poisons such as bromadiolone. Therefore, this type of drug has a very low therapeutic index and requires careful monitoring. Thrombocyte aggregation inhibitors are less precarious and are usually preferable in clinical practice.

Chapter 11

Chemotherapy of infectious diseases

11.1 Introduction

In the treatment of infections, the goal is to kill the microbes, or at least to disrupt their ability to propagate, so that the immune system can get the better of them. While this is simple in principle, the difficulties in practice are twofold. Firstly, the drugs must kill the microbes but spare the host; this is the principle of selective toxicity. Secondly, microbes tend to develop resistance to drugs that are initially effective. The problem of resistance is both grave and unavoidable, since microbes are subject to genetic variation, and each drug use, medically justified or not, will select for more resistant variants. Therefore, antimicrobial drug discovery may have to continue in perpetuity.

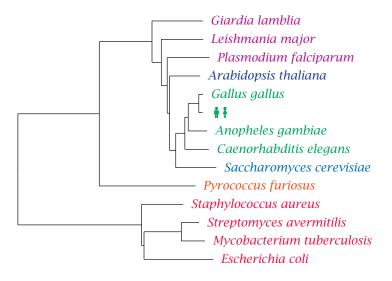
11.2 Diversity of infectious pathogens

- Bacteria
- Fungi
- Parasites—eukaryotes other than fungi
 - Protozoa—unicellular
 - Metazoa—multicellular
- Viruses

Among the parasites, the category "protozoa" is a bit of a historical relic. When infectious agents were first identified, the genetic and biochemical relationships between them were not understood, and newly discovered pathogens were classified somewhat haphazardly and mostly according to appearance and shape. The term "protozoa" means "primitive animals". However, as shown in the next slide, the protozoa are rather distantly related to humans and other real animals, and even to one another.

The large evolutionary distance between protozoa and humans corresponds to significant differences in cellular biochemistry. Some of these biochemical differences can be exploited to achieve selective toxicity in chemotherapy.

11.2.1 The tree of life, slightly pruned



This phylogenetic tree shows *Homo sapiens* in the company of some pathogens and reference organisms. *Arabidopsis* is a plant, and *Saccharomyces* is a yeast; both are more closely related to humans than are *Plasmodium*, *Leishmania* and other single-celled eukaryotic parasites that have been lumped in with the protozoa. The nematode *Caenorhabditis elegans* is not a pathogen itself but is included as a proxy for human worm pathogens such as *Wuchereria bancrofti* or *Ascaris lumbricoides*.

*Pyrococcus furiosus*¹ belongs to the Archaebacteria. These organisms have a prokaryotic cell structure but are phylogenetically closer to us than to the Eubacteria, to which all pathogenic bacteria belong. Among these, *Staphylococcus aureus* represents the Gram-positives, whereas *Escherichia coli* represents the Gram-negatives. These two major groups have different cell wall structures (see slide 11.4.1) that differ in their permissiveness both for the dyes used in the Gram stain procedure and for antibiotics. Mycobacteria such as *Mycobacterium tuberculosis*, which are genetically almost equidistant from both, have another distinct cell wall architecture that is particularly hard to penetrate for antibiotics.

The cell wall structure of the streptomycetes resembles that of the Grampositives, but they are genetically closer to the mycobacteria. They occasionally occur as pathogens but are most notable as purveyors of antibiotics; the majority of all known natural antibiotics is produced by member species of *Streptomyces* or related genera.

¹Literally translated: the "raging fireball"

11.2.2 Drug targets for antimicrobial therapy

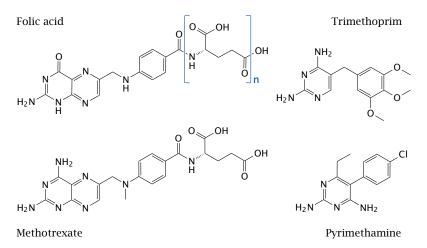
- Macromolecules that occur in the cells of the pathogen but not within the human host. Examples:
 - the bacterial cell wall (penicillin)
 - *de novo* synthesis of folic acid (sulfonamides)
- Macromolecules that occur in both humans and the pathogen but are structurally divergent. Examples:
 - ribosomes (chloramphenicol)
 - dihydrofolate reductase (trimethoprim)
 - DNA topoisomerase (ciprofloxacin)

This list of examples shows that both pathogen-specific drug targets and those that are shared but divergent are important and viable in clinical practice.

In recent years, the genomes of many pathogens have been sequenced and searched for genes that are essential for the life of the pathogens, and at the same time do not have counterparts in the human genome. The products of such genes should indeed make good candidate drug targets. However, considering the proven value of many drugs that act on gene products that *do* have homologues in host cells, we should not exclude the latter from our search for novel targets.

Both types of drug targets—those with and those without homologues in the host cell—tend to be more abundant in pathogens with greater evolutionary distance from humans. Thus, the number of suitable targets is smaller in protozoal parasites than in bacteria, and yet smaller in pathogenic fungi.

11.2.3 Structures of folic acid and of three inhibitors of dihydrofolate reductase



Dihydrofolate reductase is a good example of a drug target that is found in both host and pathogen. It is essential in all organisms for sustaining the folic acidmediated transfer of single-carbon groups in biosynthetic reactions. Nevertheless, the enzyme molecules found in various organisms are sufficiently different to allow their selective inhibition with various chemotherapeutic drugs.

Trimethoprim inhibits the bacterial enzyme. It acts synergistically with sulfonamides, which inhibit the bacterial *de novo* synthesis of folic acid (see slide 1.3.3). Trimethoprim is typically used in combination with a sulfonamide.

Pyrimethamine inhibits the enzymes of protozoans such as *Toxoplasma gondii* and *Plasmodium falciparum*. The human enzyme is not significantly inhibited by either but is inhibited by methotrexate, which is used in anticancer and immunosuppressive therapy.

11.2.4 Microbial resistance mechanisms

- Mechanisms affecting the target:
 - Structural alteration / mutation
 - Compensatory overexpression
- Mechanisms affecting the drug:
 - Reduced uptake
 - Active extrusion
 - Enzymatic inactivation

Resistance to chemotherapy occurs with all kinds of pathogenic microbes. While it poses obvious and significant practical problems, it is also a fascinating microcosm of Darwinian evolution through mutation and selection. Larger organisms mostly evolve too slowly for us to observe; with drug resistance in microbes, we get a timelapse view of evolutionary adaptation. This is due to both the short generation time of microbes and the rigorous nature of the selection.

This slide lists the principal mechanisms of resistance that apply to all classes of pathogenic microbes. We will see specific examples for most of these mechanisms in the following sections.

11.3 Overview of antibacterial chemotherapy

- Targets
 - Cell wall
 - Ribosomes
 - Enzymes related to cell division
 - Intermediate metabolism
- Antibiotic resistance
 - Bacteria have short generation times—fast *de novo* evolution of resistance
 - Resistance genes exist in producers of antibiotics—can spread to pathogenic bacteria by gene transfer

We will start our exploration of chemotherapeutic agents with those that act on bacteria, which are the most common class of pathogens.

Most antibacterial drugs are *antibiotics*, that is, natural compounds isolated from other microbes, or derivatives thereof. While penicillin was famously isolated from a mold, most antibiotics—for example, tetracyclins, aminoglycosides like streptomycin, and macrolides like erythromycin—are actually produced by *Streptomyces* species or related soil bacteria. Since these producer bacteria must be resistant to their own poisons, it follows that mechanisms and genes for bacterial resistance must exist for any of these natural antibiotics. Such genes may migrate to clinical pathogens and spread among them if we apply the proper selection pressure through the medical use and misuse of those antibiotics.²

Some antibacterial compounds are indeed fully synthetic; we have already seen sulfonamides and trimethoprim as examples. With these agents, resistance genes may not exist *a priori*; however, resistance often emerges through spontaneous mutations that sharpen the target enzymes' ability to discriminate between the drugs and the proper substrates.

With both natural and synthetic antibiotics, an important strategy to prevent, or at least delay, the emergence of resistance is combination therapy. When several drugs are combined that each alone are able to kill the pathogen, and each of which addresses a different target, the pathogen would have to simultaneously modify all targets in order to survive. With increasing number of agents simultaneously applied, this rapidly becomes unlikely.³

11.3.1 Gene transfer mechanisms in bacteria

- Transformation: cellular uptake of naked DNA
- · Conjugation: plasmid-encoded active transfer between bacterial cells
- Transduction: gene transfer mediated by bacteriophages
- Transposons: transfer of genes between carrier DNA molecules (chromosomes, plasmids)

Transformation, conjugation and transduction all work within and also *between* bacterial species.⁴ Transfer between species is important in the migration of resistance genes from producer strains, or other soil bacteria that are naturally exposed and have developed resistance to a given antibiotic, to pathogenic bacteria.

²This problem seems to have been overlooked in the recent stampede towards 'mining the gut microbiome' for new antibiotics. Should they indeed exist, such antibiotics should also be accompanied by resistance genes; and these genes, since they are already present in humans, would have a particularly easy time of hopping over to some proper pathogens.

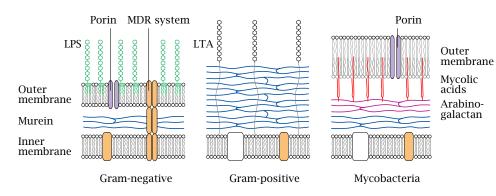
³Combination therapy is absolutely essential in the treatment of tuberculosis and leprosy. The hardy mycobacteria that cause these diseases succumb to antibiotics only slowly, and their prolonged survival increases their chances of acquiring mutations that confer resistance.

⁴For an individual carrier plasmid or bacteriophage, the range of host species will often be quite narrow. However, transposons help resistance genes to move from one carrier molecule to the next, and thus to cross multiple species barriers successively.

Resistance genes are often carried by mobile genetic elements called *transposons*, which encode enzymes with endonuclease and ligase activity that allow them to excise themselves from one host DNA molecule and then join another. Given the right selection conditions, multiple resistance transposons may wind up on a single plasmid molecule. Such a plasmid will then confer resistance to several unrelated antibiotics all at once, and the use of any single one of these drugs will cause this multiple resistance to spread further. The first such multiresistance plasmids were observed in the late 1950s, only about ten years after the start of the antibiotic era.

11.4 Antibiotics and the bacterial cell wall

In contrast to human cells, which are simply delimited by their cytoplasmic membranes, most bacteria have cell walls that consist of one or more protective layers stacked on top of their cytoplasmic membranes. While these cell walls may protect the bacteria from antibiotics, they also provide targets for chemotherapy. Note that some bacteria—mycoplasmas, and the vegetative forms of rickettsias and chlamydias—have no cell wall at all and therefore are not susceptible to the agents discussed in this section.



11.4.1 Bacterial cell wall structure

The innermost layer of a bacterial cell wall consists of murein or peptidoglycan, a meshwork of polysaccharide strands crosslinked by oligopeptides. Gram-negative bacteria have a comparatively thin peptidoglycan layer (blue) that is surrounded and protected by an outer membrane. The outer leaflet of this lipid membrane consists mostly of lipopolysaccharide (LPS, green), which is also known as *endotoxin.*⁵ Porins in the outer membrane facilitate diffusion of small polar solutes. Multidrug resistance (MDR) proteins that extrude antibiotics from the cell may be located in the cytoplasmic membrane alone or span both membranes.⁶

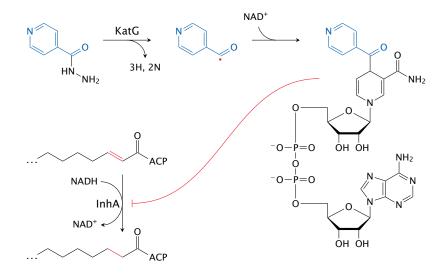
⁵The "toxic" effect of endotoxin results not from any intrinsic biochemical activity of the LPS molecule but instead from a violent response of the non-specific immune system to it.

⁶The two types of pumps cooperate synergistically in extruding drugs from the cell—see [95] and references therein.

Gram-positive bacteria lack an outer membrane but have a much thicker murein layer, which is decorated with lipoteichoic acids (LTA). The lack of an outer membrane makes them more amenable to penicillin (see slide 1.3.10) and many other antibiotics.⁷

In mycobacteria, the murein layer is surrounded by arabinogalactan polysaccharide, to which branched, long-chain fatty acids (mycolic acids, red) are attached. The mycolic acids act as anchors for a particularly thick, wax-like and impenetrable outer membrane. Because of their sturdy cell wall,⁸ mycobacteria have always been among the most difficult microbes to treat—although decades of selection have bred some real champions of resistance among the Gram-positives and Gram-negatives also.

11.4.2 Action mechanism of isoniazid (INH)



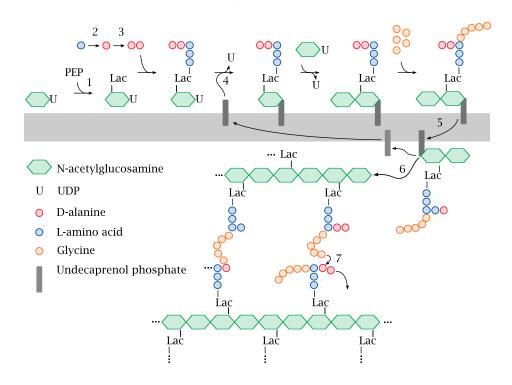
Isoniazid is effective against the pathogen *Mycobacterium tuberculosis*. It inhibits the synthesis of mycolic acids, the long-chain fatty acids that are characteristic and essential components of the mycobacterial cell wall. This inhibition arises in a rather unique manner.

Inside the mycobacterial cell, INH is activated by the oxidative enzyme KatG⁹ to a radical form. This radical then reacts with NAD⁺. The adduct inhibits InhA, an enoyl-CoA reductase involved in mycolic acid synthesis.

⁷The outer membrane in Gram-negatives excludes crystal violet, and therefore prevents the bacteria from staining dark blue in the Gram stain procedure. In contrast, Gram-positives absorb and retain the dark-blue dye. The cell wall of mycobacteria excludes both, and therefore these cells don't show up in a Gram stain at all.

⁸The thick cell wall enables the mycobacteria to persist inside host phagocytes, which further impedes access of drugs to bacterial targets.

⁹KatG has both superoxide dismutase and catalase activity, that is, it catalyzes both of the reactions $2O_2^- \rightarrow O_2 + H_2O_2$ and $2H_2O_2 \rightarrow H_2O+O_2$. I have not found a precise equation for the KatGmediated conversion of INH to the radical intermediate; the one given in the slide is not to be taken literally.



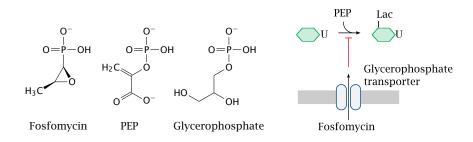
11.4.3 Outline of bacterial murein synthesis

Murein, or peptidoglycan, is found in the cell walls of Gram-positives, Gramnegatives, and mycobacteria. Inhibitors of various enzymes in the murein synthesis pathway are active against member species of all three classes.

The synthetic pathway involves the following stages: Phosphoenolpyruvate (PEP) supplies a lactate residue (Lac) that is attached to N-acetylglucosamine, which yields N-acetyl-muramic acid (1). Onto the latter, a pentapeptide is built in a series of ATP-activated reactions. The free end of this peptide contains two D-alanine residues that are supplied by alanine racemase (2) and D-alanine ligase (3). This nascent building block is transferred to the lipid carrier undecaprenol phosphate (4) and subsequently extended by another molecule of N-acetylglucosamine and five glycine residues.

The completed precursor molecule, named lipid II, is flipped across the cytoplasmic membrane (5). The glycopeptide moiety is transferred from lipid II to a growing murein strand in the transglycosylase reaction (6). The final transpeptidase reaction (7) cross-links the new subunit to an adjacent murein strand, releasing the terminal D-alanine residue.

The transglycosylase and transpeptidase activities are both located on the same enzyme protein, variously referred to as muramyl-transpeptidase or penicillin-binding protein (PBP). Most bacterial species have several PBP subtypes that may differ in susceptibility to penicillins and related antibiotics.

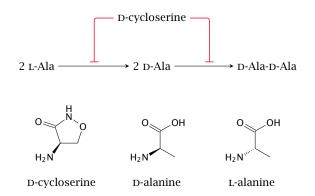


11.4.4 Fosfomycin mimics both phosphoenolpyruvate and glycerophosphate

Fosfomycin is an antimetabolite of phosphoenolpyruvate (PEP) in the first step of murein precursor synthesis. Considering its structure, it may not surprise you to learn that the reaction with the target enzyme is covalent and involves the thiol group of a cysteine residue [96].

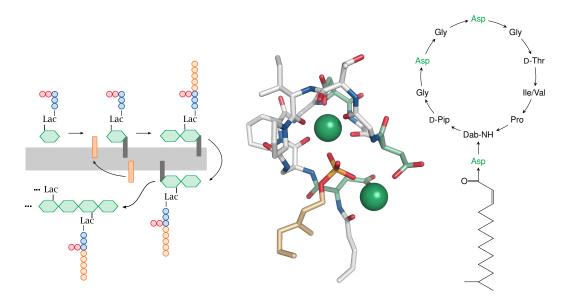
For uptake across the cytoplasmic membrane, fosfomycin piggybacks on a transport protein that mediates the uptake of glycerophosphate. This transporter is not essential for the bacterial cell; therefore, mutations that inactivate the transporter tend to cause rapid development of resistance under therapy. Fosfomycin can therefore only be used in combination with other antibiotics that are less prone to rapid evolution of resistance.

11.4.5 Cycloserine inhibits alanine racemase and D-alanine ligase



D-cycloserine is an antimetabolite of D-alanine in both the alanine racemase and the D-alanine ligase reactions. It is used mostly against mycobacterial infections, though active in principle against other types of bacteria also.

Interestingly, D-cycloserine is also a partial agonist at the sole glycine-specific subunit of the NMDA-type glutamate receptor (see section 6.11). As such, it has been tried therapeutically in various neurological and psychiatric conditions [97].

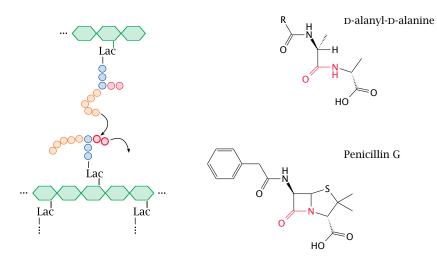


11.4.6 The lipopeptide laspartomycin sequesters undecaprenol phosphate

The acidic aspartate side chains participate in the binding of two calcium ions (green balls in the structure), which mediate binding to the likewise acidic phosphate headgroup of the undecaprenol carrier. Structure rendered from 500Z.pdb.

While mechanistically interesting, this antibiotic cannot be used in humans, at least not systemically, since it also interferes with the dolichol phosphate-dependent posttranslational glycosylation of proteins and therefore is toxic.

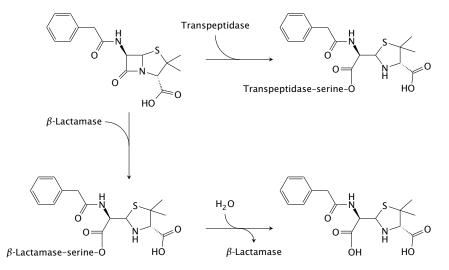
11.4.7 β -Lactam antibiotics resemble the substrate of the transpeptidase reaction



We now skip ahead to the last step of murein synthesis, that is, the transpeptidase reaction that crosslinks the assembled linear strands of murein and thereby confers mechanical stability to the cell wall.¹⁰

 β -Lactam antibiotics, which inhibit the transpeptidase reaction, are the most widely used class of antibiotics. The class comprises penicillins, cephalosporins and a few other variants. Penicillin G, which is shown here, was the first β -lactam antibiotic to be introduced into clinical practice.

11.4.8 Reactions of penicillin G with transpeptidase and β -lactamase



The shared structural feature that gave the class its name is also at the heart of its antibacterial action. The sterically strained four-membered β -lactam ring is spring-loaded like a mousetrap and readily reacts with the catalytic serine residue of the transpeptidase enzyme. The enzyme is unable to free itself from the covalent modification and remains irreversibly inactivated.

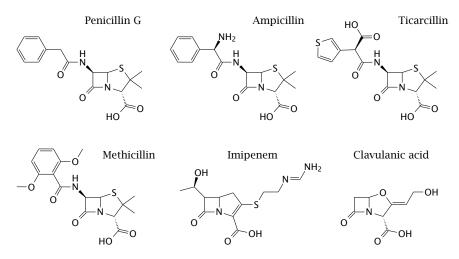
The most prevalent mechanism of bacterial resistance to β -lactams is enzymatic inactivation by β -lactamases. These enzymes fall into two major functional classes. Like transpeptidase, the first type of β -lactamase also contains a catalytic serine. However, in contrast to transpeptidase, the β -lactamase regenerates its free serine residue through hydrolysis, and it thus can successively degrade a large number of antibiotic molecules. Some enzymes in this class can be inhibited with β -lactamase inhibitors.

The second class of β -lactamases comprises metalloenzymes. In contrast to the serine β -lactamases, these enzymes do not form covalent intermediates, and no clinically useful inhibitors are currently available. Gram-negative organisms with metallo- β -lactamases have become a major problem worldwide. The Dmitrienko

¹⁰A crosslinked, stable cell wall is necessary to contain the high osmotic pressure of the bacterial cytoplasm. If crosslinking is inhibited with vancomycin or β -lactam antibiotics, the cells will suffer osmotic lysis at physiological extracellular salt concentrations. Osmotic lysis can be prevented by adding 600 mM sucrose to the medium. In this way, cell wall-less *protoplasts* (with Gram-positive bacteria) or *spheroplasts* (with Gram-negatives) can be generated.

group in the UW chemistry department is working on the synthesis and characterization of such inhibitors [98].

11.4.9 Structures of β -lactam antibiotics (1)



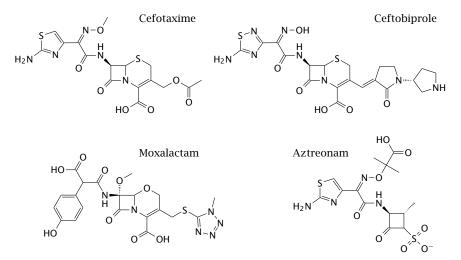
The first β -lactam to be used clinically, penicillin G, is active almost exclusively against Gram-positive bacteria, since it fails to penetrate the outer membrane of most Gram-negatives (see slide 1.3.10). Other β -lactams that were developed later have progressively extended the spectrum to include most Gram-negatives and even some mycobacteria. Some of these derivatives are also, to varying degrees, protected from inactivation by bacterial β -lactamases.

Penicillin G itself is readily cleaved by β -lactamases, and while it was active on almost all strains of *Staphylococcus aureus* when first introduced, more than 90% of all clinical isolates of this pathogen now possess β -lactamases that render them resistant.

To counter this resistance mechanism, semisynthetic drugs such as methicillin were developed, which escape cleavage by the enzyme through steric obstruction. This obstruction also renders such drugs about ten times less active against the muramyl-transpeptidase target, but because of the generally very high therapeutic index of the penicillins, this does not compromise their clinical utility. However, methicillin-resistant *S. aureus* strains (MRSA) have emerged and become increasingly widespread. Through horizontal gene transfer from another staphylococcal species, these strains have acquired a peculiar variant of the transpeptidase that no longer binds methicillin and related compounds. MRSA are also resistant to most other β -lactams; they are, however, susceptible to some new cephalosporins such as ceftobiprole (see next slide).

Ampicillin was the first penicillin derivative with useful activity against Gramnegatives such as *Escherichia coli*, and ticarcillin the first one to be active against *Pseudomonas*. Both are susceptible to now widespread β -lactamases, but they can still be used when combined with β -lactamase inhibitors such as tazobactam or clavulanic acid (see below). Imipenem is an erstwhile "wonder drug" that killed just about anything when it first appeared in the market. It is intrinsically quite resistant to cleavage by serine-type enzymes but is cleaved by the increasingly common metalloenzyme β -lactamases. Imipenem lacks the sulfur atom in the ring and is therefore referred to as a *carbapenem*. Such structures were first observed among the thienamycins, a class of β -lactams produced by certain *Streptomyces* strains.

11.4.10 Structures of β -lactam antibiotics (2)

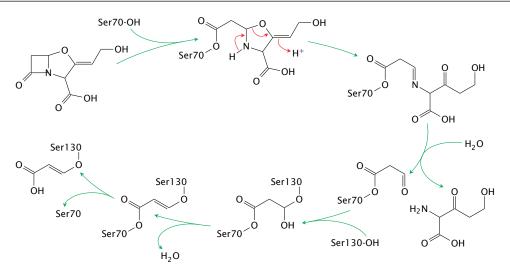


Cefotaxime and ceftobiprole are semisynthetic cephalosporins. Natural cephalosporins are produced by fungal species that belong to the genus *Cephalosporium*. Cephalosporins differ from the penicillins in the structure of the central nucleus. The cephalosporin nucleus is amenable to semisynthetic derivatization and variation in two positions. Like imipenem, many cephalosporins are quite resistant to serine β -lactamases but are susceptible to metalloenzymes. Cefotaxime has broad activity against both Gram-negative and Gram-positive bacteria; the more recently introduced ceftobiprole has the added bonus of being active against MRSA.

Moxalactam is an interesting cephalosporin analog with very good activity against some difficult Gram-negative pathogens, but it was retired due to interference with the plasmatic blood coagulation cascade. Aztreonam is a monobactam, an unusual, fully synthetic β -lactam antibiotic with a single ring structure. It has strong activity against *Pseudomonas* species.

11.4.11 Inactivation of SHV-1 β -lactamase by clavulanic acid

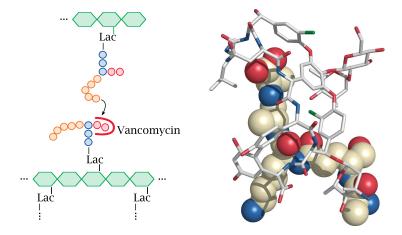
Clavulanic acid is a natural β -lactam antibiotic. Its intrinsic antibacterial activity is weak, but it is a suicide inhibitor of serine-type β -lactamases and as such can be used in combination with other, more active but β -lactamase-susceptible compounds such as ampicillin or ticarcillin.



The initial acylation of the catalytic serine 70 residue resembles the reaction of the enzyme with other β -lactam antibiotics. However, with clavulanic acid, this initial reaction is followed by a sequence of reactions that traps a second serine residue in the active site. The modification of serine 130 means that the active site remains blocked even after serine 70 is freed by hydrolysis.

Resistance to clavulanic acid can arise through mutation of serine 130 to glycine, which does not compromise the catalytic activity of the β -lactamase on penicillin derivatives. Reaction scheme simplified after [99].

11.4.12 Vancomycin sequesters the substrate of the transpeptidase reaction

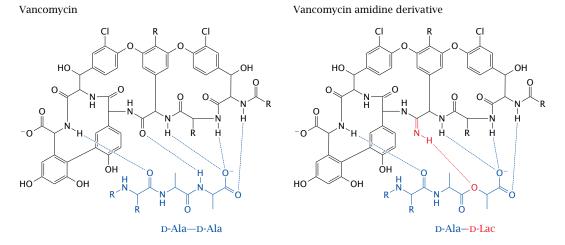


Vancomycin inhibits the same reaction as the β -lactams. However, it does so in a very different and rather unusual manner: Instead of binding to the enzyme, the antibiotic binds to the terminal D-alanine dipeptide on one of the two substrates. Vancomycin is active on Gram-positives but not Gram-negatives.

The structure (rendered from lgac.pdb) shows not vancomycin itself but the closely related glycopeptide antibiotic A82846B, bound to its substrate. The antibiotic is shown in stick representation, while the peptide moiety of lipid II is shown

as spheres. The uppermost part of the peptide consists of the two linked D-alanine residues.

11.4.13 Vancomycin can be modified to overcome bacterial resistance



Vancomycin has been widely used in the treatment of serious infections with Grampositive bacteria, and in particular it has been invaluable in the treatment of MRSA infections. Compared to most other antibiotics, resistance took a long time to develop, which may be related to its unusual mode of action that precludes simple point mutations as a resistance mechanism. However, vancomycin-resistant staphylococci and enterococci have emerged and are spreading. Again through horizontal gene transfer, these resistant bacteria have acquired an enzyme that replaces the terminal D-alanine residue with D-lactate. This removes one hydrogen atom and breaks one of the hydrogen bonds between vancomycin and the substrate, which substantially lowers affinity.

Interestingly, high-affinity binding to the D-alanine–D-lactate substrate can be restored by introducing an amidine group into the vancomycin molecule opposite the ester oxygen in the substrate [100]. Reportedly, the amidine derivative inhibits both vancomycin-sensitive and -resistant bacteria. If a practical and cost-effective method can be found to produce this derivative, it should be of great clinical value.¹¹

Another interesting approach for restoring the activity of vancomycin against resistant bacteria —and one that lends itself more readily to synthesis on a pharmaceutical scale—is the semisynthetic attachment of lipophilic groups to the molecule, whose affinity for the bacterial membrane offsets the decreased affinity for the altered substrate peptide. Several such derivatives are discussed in [101].

11.5 Antibiotics that inhibit ribosomal protein synthesis

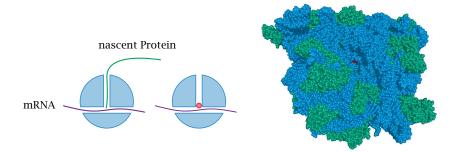
· Aminoglycosides

¹¹Schemes redrawn after reference [100]. The letter R denotes several different substituents that have been omitted here for simplicity.

- Tetracyclines
- Macrolides
- Chloramphenicol
- Puromycin
- ...

Bacterial ribosomes are substantially smaller and structurally different from those found in eukaryotic cells. Many, but not all antibiotics that inhibit ribosomal protein synthesis selectively inhibit the bacterial ones, and can therefore be used for antibacterial chemotherapy in humans. Note, however, that the ribosomes in mitochondria resemble those in bacteria. Inhibition of mitochondrial ribosomes may cause toxicity, and drugs in this class tend to have smaller therapeutic ranges than β -lactams.

11.5.1 Chloramphenicol lodges into the peptidyl-transfer site of the ribosome



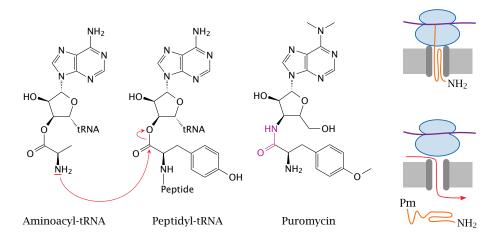
Chloramphenicol binds right within the center of the ribosome and blocks the transfer of the peptide from the peptidyl-tRNA to the next aminoacyl-tRNA. In the ribosome structure on the right-hand side, we are peeping down the peptide exit tunnel to catch a glimpse of the chloramphenicol molecule, which is rendered in red. Ribosomal RNA is shown in blue, and ribosomal proteins are shown in green.

This structure was rendered from 1ko1.pdb. Crystal structures of macromolecules in general are amazing, but the structure of the whole ribosome is a truly mind-boggling accomplishment!

11.5.2 Structure and action mechanism of puromycin

Puromycin disrupts protein synthesis in both prokaryotic and eukaryotic ribosomes, and therefore cannot be used for chemotherapy in humans. However, it has an intriguing structure and mode of action, which has been instrumental in working out the mechanics of protein synthesis itself.

The peptidyl transfer step of protein synthesis involves a single amino acid and the nascent peptide, each of which is bound via an ester bond to a tRNA molecule. In the reaction, the amino group of the incoming amino acid attacks the ester between the growing peptide chain and the corresponding tRNA molecule. The new amino acid residue thereby appends itself to the C-terminus and becomes the anchor of the entire peptide chain. The now empty previous tRNA gets released, and the new peptidyl-tRNA is translocated and awaits attack by the next incoming aminoacyl-tRNA.



Puromycin resembles an aminoacyl-tRNA molecule, and it can therefore attach itself to the C-terminus of a growing peptide chain. However, instead of an ester bond, it contains an amide bond (highlighted) which does not yield to the nucleophilic attack by the next amino acid. Peptide transfer fails, synthesis of the peptide is aborted, and the incomplete peptide is prematurely released.

The disruption of protein synthesis as such is bad enough, but there is more. Proteins that are destined for incorporation into membranes, or for secretion across them, are synthesized at the ER membrane,¹² where the ribosome attaches to a so-called translocon pore that guides the nascent peptide into or across the bilayer. Premature peptide release induced by puromycin leaves the translocon in a leaky state and thus renders the membrane permeable towards small solutes.

11.6 Diverse inhibitors of bacterial macromolecular synthesis

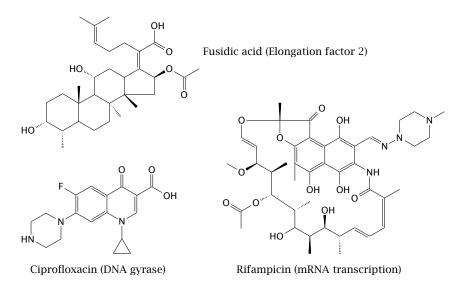
Ciprofloxacin is a synthetic inhibitor of bacterial DNA topoisomerase, an enzyme which reversibly uncoils the DNA in the tightly packed bacterial chromosome. Inhibition of this enzyme prevents DNA transcription and replication, which is obviously incompatible with life. DNA topoisomerase, or gyrase, inhibitors typically have a broad antibacterial spectrum. Resistance arises through point mutations in the enzyme.¹³

Rifampicin inhibits RNA polymerase 2, which performs mRNA synthesis. The drug is active against various bacterial species but clinically most valuable in the

¹²Bacterial cells have no ER, and synthesis of membrane or secreted proteins occurs directly at the cytoplasmic membrane.

¹³Interestingly, overexpression of DNA topoisomerase *increases* susceptibility to ciprofloxacin [102]. The cited paper gives a convincing explanation.

treatment of mycobacterial infections (tuberculosis and leprosy). Resistance tends to develop rapidly, and combination with other drugs is therefore mandatory.

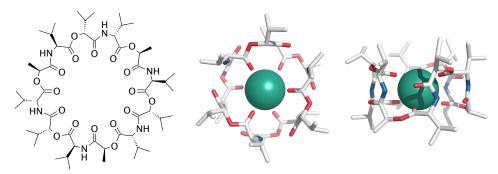


Fusidic acid inhibits ribosomal protein synthesis by binding to elongation factor 2. It has a sterol ring structure, but bacteria (including *Streptomyces* species) don't have sterols, from which one might guess that it is not produced by a bacterium. This is indeed the case; the producer organism is a fungus named *Fusidium coccineum*.

11.7 Antibiotics that act on bacterial cell membranes

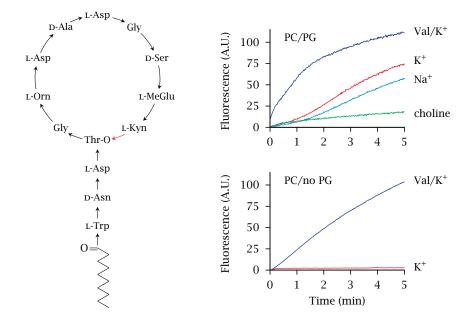
Bacterial cell membranes are attractive targets in principle, because they are more exposed and accessible than intracellular targets. However, only few antibiotics that target cell membranes are sufficiently selective for bacterial ones to be of use for antibacterial therapy.

11.7.1 Structure of the potassium ionophore valinomycin



Valinomycin permeabilizes both prokaryotic and eukaryotic cells for potassium and therefore cannot be used for antimicrobial chemotherapy. However, it is useful in biophysical and cell-biological experiments. Left: The circular molecule contains alternating ester and peptide bonds and exclusively hydrophobic side chains. Right: Valinomycin tightly wraps around a potassium ion, coordinating it with the double-bonded oxygen atoms of the ester bonds. The hydrophobic exterior of the complex enables it the cross cell membranes easily.

11.7.2 Daptomycin permeabilizes membranes containing phosphatidylglycerol

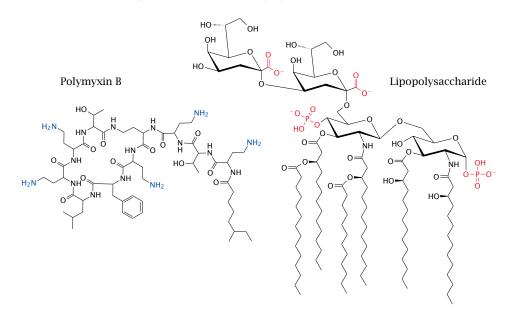


Daptomycin is a lipopeptide antibiotic produced by the soil bacterium *Streptomyces roseosporus*. It is only active on Gram-positive bacteria, because it fails to penetrate the outer membrane of Gram-negatives. It binds to the cytoplasmic membrane and then forms oligomeric pores [103, 104].

The experiment shown here is a fluorescence assay that measures the permeabilization of liposomes by daptomycin. Daptomycin permeabilizes liposomes only when they contain phosphatidylglycerol (PG). This lipid is abundant in the cytoplasmic membranes of bacteria but not in human cell membranes, which is most likely the basis for its selective toxicity. ¹⁴ Permeabilization is similarly efficient for K⁺ and Na⁺, but considerably less so for choline, which is likely due to the greater size of the choline molecule.

In contrast to daptomycin, valinomycin (Val) permeabilizes both types of membranes, which fits with its known toxicity for both prokaryotic and eukaryotic cells. Figure prepared from original data in [106].

¹⁴It has recently been shown that daptomycin binds PG and undecaprenol intermediates simultaneously [105]. Undecaprenol intermediates alone would not confer selective toxicity (see laspartomycin, above); however, their presence will likely render bacterial membranes more susceptible to daptomycin, and daptomycin may in this way also inhibit murein synthesis.



11.7.3 Structures of polymyxin B and lipopolysaccharide

Lipopolysaccharide (LPS) is the major component of the outer leaflet of the outer membrane in Gram-negative bacteria (see slide 11.4.1). The structure shown here depicts the minimal size of the molecule compatible with bacterial life, which has been characterized in biosynthetic mutants; wild type LPS species contain much longer polysaccharide chains that vary in sugar sequence depending on the bacterial species and strain.

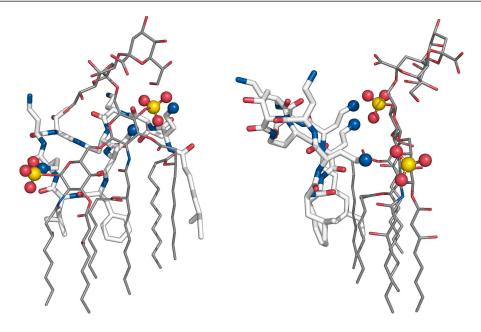
Polymyxin B, a lipopeptide antibiotic produced by the Gram-positive bacterium *Paenobacillus polymyxa*, binds specifically to LPS and disrupts the Gram-negative outer membrane. LPS contains several negative charges, which interact with the positive charges on polymyxin. In addition, there are hydrophobic interactions between the two molecules (see next slide).

11.7.4 A model of the polymyxin B-LPS complex

This structure of polymyxin bound to a LPS molecule was not obtained experimentally but through molecular simulation.¹⁵

Amino groups in polymyxin B that form ion pairs with the phosphates of lipid A are shown as blue balls. Several hydrophobic side chains of polymyxin B insert between the acyl chains of adjacent lipid A molecules and disrupt the membrane. The lower ends of the acyl chains in LPS, which don't engage in any contacts with polymyxin, were truncated in this figure.

¹⁵Rendered from coordinates kindly provided by P. Pristovsek.



11.8 Antifungal chemotherapy

Drug targets:

- ergosterol in cell membranes (amphotericin B)
- ergosterol synthesis (ketoconazole; terbinafine)
- thymidylate synthase (5-fluorocytosine)
- 1,3-β-glucan synthesis (echinocandins)

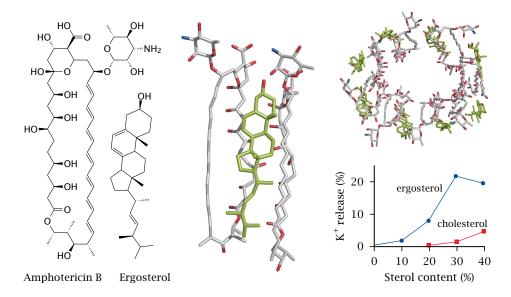
Fungi are eukaryotic, and therefore macromolecules like ribosomes and DNA topoisomerase are more similar to those in human cells and not suitable as drug targets for antimicrobial chemotherapy.

Like most bacteria, fungi have cell walls, which contain chitin and $1,3-\beta$ -glucan polysaccharides. In addition, fungal cell membranes contain ergosterol instead of cholesterol. The synthesis of cell wall polysaccharide and of ergosterol, as well as the ergosterol-containing bilayer itself, are targeted by antifungal agents.

11.8.1 Structure and action mode of amphotericin B

Amphotericin B is a polyene antibiotic produced by a *Streptomyces* species. It interacts specifically with ergosterol in fungal membranes, and the oligomeric complexes form cation-selective pores in the fungal cell membranes. The top right panel in this slide shows a hypothetical molecular structure of these complexes, as determined by molecular simulation.¹⁶ The panel in the middle shows a blow-up from that structure, with one molecule of ergosterol sandwiched between two molecules of amphotericin B.

¹⁶Coordinates kindly provided by Dr. Maciej Baginski.



The plot at the bottom right (redrawn from [107]) shows the release of potassium ions from liposomes varying in sterol content. The extent of release increases with the ergosterol concentration. Liposomes containing high (but physiological) concentrations of cholesterol are also permeabilized.¹⁷

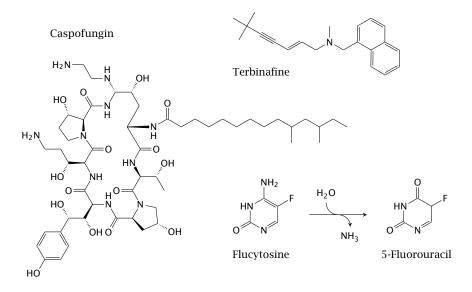
The activity of amphotericin B on cholesterol-containing membranes accounts for its toxicity, which can be quite severe. Cell damage is most pronounced in the distal tubules of the kidney, particularly when the pH of the nascent urine is low [108]. Amphotericin toxicity is mitigated when the drug is applied bound to liposomes; presumably, this avoids noxious spikes in the free drug concentration immediately after intravenous application (see slide 14.4.6).

11.8.2 Other antifungal drugs

Terbinafine inhibits squalene epoxidase, which catalyzes the cyclization step in the sterol synthesis pathway. This step is common to the synthetic pathways for both cholesterol and ergosterol; therefore, any selective toxicity is not due to absence of this enzyme activity from humans, but rather to molecular differences between the human and the fungal enzymes. Also note that, in contrast to the other drugs listed here, terbinafine is only used externally, so that the question of selective toxicity is not very pressing.

The biosynthesis of ergosterol is also inhibited by ketoconazole and related imidazole derivatives. These drugs inhibit 14α -demethylase, a cytochrome P450 enzyme that catalyzes a step in the conversion of the initial sterol intermediate, lanosterol, to ergosterol. As we had seen earlier, a side effect of ketoconazole is the inhibition of cytochrome P450 enzymes involved in human drug metabolism (see slide 4.2.4); therefore, the modes of action and of toxicity are related.

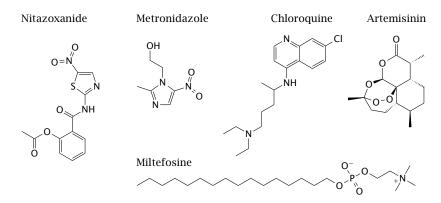
¹⁷The antibiotic used in this experiment is not amphotericin B itself but the related polyene antibiotic pimaricin.



Flucytosine is a prodrug that in fungal cells undergoes deamination to fluorouracil, which inhibits thymidylate synthase (see slide 12.6.2). Human cells do not deaminate the flucytosine, which is the basis for selective toxicity. Note, however, that the deaminase enzyme is not essential for fungal cells either, and mutational inactivation of the enzyme enables rapid development of resistance. Therefore, flucytosine is only useful in combination with other antifungal drugs.

Caspofungin is a lipopeptide antibiotic of the echinocandin type that inhibits an enzyme involved in the biosynthesis of $1,3-\beta$ -glucans, an essential component of the fungal cell wall.

11.9 Antiprotozoal drugs



As noted earlier, the term "protozoa" covers a pretty diverse lot of single-celled pathogens, and so it is not surprising that there is no such thing as a broad-spectrum antiprotozoal drug. We will here look at a few example drugs with different and interesting modes of action.

The plant alkaloid artemisinin inhibits a sarco-endoplasmatic Ca^{++} -ATPase (SERCA) in malaria parasites; this pump is essential for transporting Ca^{++} from

the cytosol into the ER and thereby terminating calcium signals. Its recently increasing use has already given rise to resistance that has been traced to point mutations in SERCA [109]; this association has been disputed, however [110], which suggests the existence of alternate action mechanisms.

Miltefosine is a lipid analogue that inhibits cytochrome C oxidase [111]. Metronidazole and nitazoxanide are reduced to toxic nitro radicals by anaerobic metabolism; this is explained below.

$\begin{cases} 80 \\ 60 \\ 40 \\ 20 \\ 0 \\ 0 \\ 100 \\ 200 \\ 300 \\ 400 \\ Time (min) \end{cases}$

11.9.1 Chloroquine inhibits formation of β -hematin

Chloroquine was developed in the 1930s and came to be widely used during World War II. For several decades, chloroquine remained the standard drug for prevention and treatment of malaria. Its mechanism of action is quite unusual and indirect.

Malaria plasmodia that live and grow in erythrocytes feed on hemoglobin, or rather only its protein component (globin). Free heme is left over and is toxic to the parasites, because it catalyzes the formation of reactive oxygen species from molecular oxygen.¹⁸ To combat heme toxicity, malaria parasites induce its crystal-lization (right); these β -hematin or hemozoin crystals are visible in blood smears as the so-called malaria pigment.¹⁹ Chloroquine interacts with the surfaces of growing β -hematin crystals, inhibits deposition of further heme molecules (left), and it thereby indirectly promotes formation of toxic reactive oxygen species.²⁰

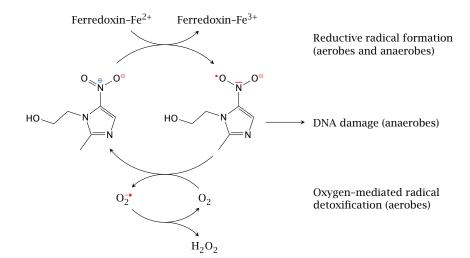
The accumulation of chloroquine inside the parasite's vacuole is driven by a pH gradient—the interior of the vacuole is acidic, which retains the basic drug molecule by way of non-ionic diffusion (see slide 3.4.7).

Considering the immutable nature of its target, it is clear that resistance to chloroquine must be mediated by removal or inactivation of the drug. Indeed, the most important mechanism of resistance consists in extrusion from the plasmodial cells by ABC type transporters. Resistance is now widespread among the parasites.

¹⁸This ROS toxicity may account for the benefit of glucose-6-phosphate dehydrogenase deficiency in malaria—lacking regeneration of reduced glutathione prevents scavenging of ROS, which then inflict damage on the parasites.

¹⁹The crystallization seems to be initiated by lipids in the parasite's vacuolar membranes, but the exact role of the lipids is still a bit contentious [112, 113].

²⁰Plot prepared from original data in [114]. β -Hematin structure rendered from coordinates kindly provided by Katherine de Villiers.



11.9.2 Action mechanism and selective toxicity of nitroimidazoles

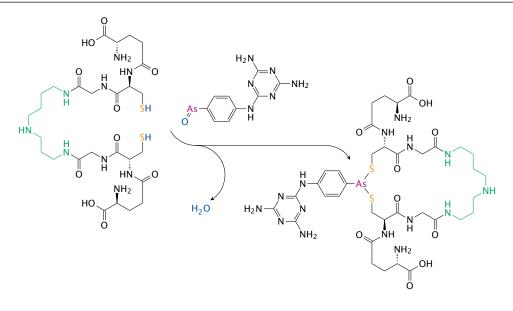
Nitroimidazoles are active on protozoa whose energy metabolism is anaerobic and which contain *hydrogenosomes*. These organelles produce ATP from the conversion of pyruvate to acetate, CO₂, and H₂. Hydrogenosomes contain high activities of the redox-active protein ferredoxin [115]. Reduction of nitroimidazoles by ferredoxin produces nitro radicals which then attack the parasites' DNA; intriguingly, the extent of damage to the DNA is strongly correlated with A/T content [116]. Interestingly, nitroimidazoles are also active and clinically used against anaerobic bacteria such as *Bacteroides fragilis*.

Mammalian cells don't produce large amounts of nitroimidazole radicals, and in addition they are able to detoxify them through reaction with molecular oxygen. The superoxide formed in the latter reaction is then scavenged by superoxide dismutase.

11.9.3 Melarsene oxide binds trypanothione

Organic arsenic compounds such as melarsene oxide are used in the treatment of infections caused by *Trypanosoma* species that are transmitted by tsetse flies and cause sleeping sickness.

The major intracellular thiol in *Trypanosoma* cells is trypanothione, which consists of two molecules of glutathione that are connected by a spermidine linker, which is shown here in green. Trypanothione and melarsene oxide form a chelate [117], which then acts as an antimetabolite to the free thiol itself in trypanothione-dependent reductive enzyme reactions. This causes oxidative damage to the cell. Resistance of the parasites to melarsene oxide is due to increased production of trypanothione and to active extrusion of the complex [118].



11.10 Antiviral chemotherapy

Viruses are heterogeneous in more than one way. They differ greatly in size; small viruses measure less than 30 nm across and contain only a handful of genes, whereas large ones approach bacteria in size and genomic complexity. The genome may be single- or double-stranded, consist of RNA, of DNA, or of both. It may be packaged as a single molecule or in multiple segments; if the former, the viral proteins may be translated separately or in the form of single precursor polyprotein. The virus particle may be coated with a protein capsid only, or the capsid may be surrounded by a lipid membrane. Finally, replication may occur entirely the cytoplasm or in part in the nucleus.

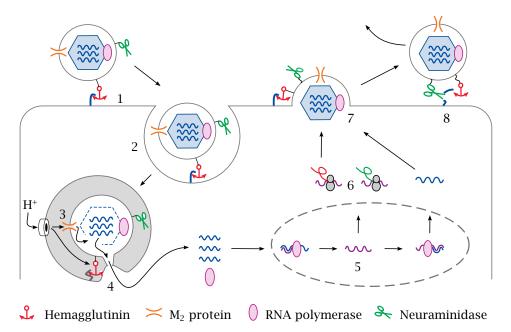
Like the viruses themselves, strategies for chemotherapy are diverse. Here, we will not consider them systematically but only by example.

11.10.1 The life cycle of influenza virus

Influenza virus contains a segmented minus-strand RNA genome and is enveloped by a lipid membrane. Embedded in the lipid membrane are several proteins that fulfill their respective functions in different stages of the viral life cycle.

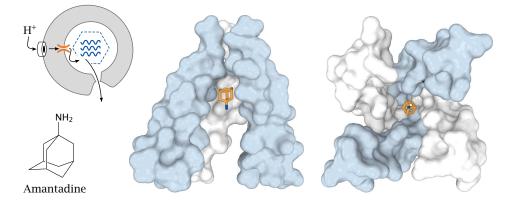
In the first stage of infection, the virion attaches to neuraminic acid residues on the target cell via its hemagglutinin surface protein (1). It is then taken up by endocytosis (2), and the cell begins to pump protons into the endocytotic vesicle. Protons enter the virus particle through the M₂ channel (3), which induces the viral protein capsid to disintegrate and release the viral RNAs.

Acidification of the vesicle also triggers the membrane fusion activity of the viral hemagglutinin (4), which creates a passageway through which the uncoated viral RNAs and the RNA polymerase enter the cytosol. Both translocate to the nucleus (5), where the viral RNA is transcribed and replicated. Plus strand RNA transcripts are translated by ribosomes in the cytosol (6). Progeny virions assemble and bud at the cytoplasmic membrane (7), from which they are released by neuraminidase (8).



The drugs shown in the next two slides interfere with step (3) and step (8) of this life cycle, respectively. Nucleoside analogues that inhibit the RNA polymerase (step 5) are in clinical use as well but are not shown here.

11.10.2 Amantadine blocks the M₂ proton channel

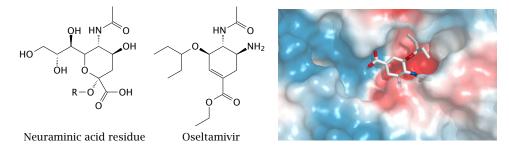


As we have just seen, uncoating and release of the viral RNA requires that protons reach the interior of the virion through the M₂ proton channel. The drugs amantadine and rimantadine block this channel and therefore disrupt this step of the viral life cycle.

Center: Side view of amantadine bound in the cavity of the channel. One of the four subunits has been removed to provide a view into the channel interior. Right:

View into the blocked channel cavity from inside the virus particle. Structures rendered from 3c9j.pdb.

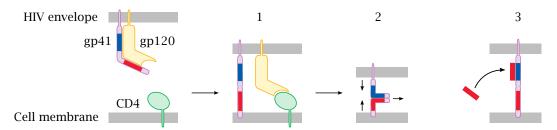
11.10.3 Oseltamivir inhibits influenzavirus neuraminidase



Neuraminidase is important in the final step of viral life cycle, that is, in detaching the particle from the expiring host cell. The enzyme cleaves neuraminic acid from the free end of a cell surface oligosaccharide, which is represented by R in the formula of neuraminic acid.

Oseltamivir mimics the transition state of neuraminic acid that occurs in the cleavage reaction. The drug is shown here bound within the active site of neuraminidase (rendered from 2hu4.pdb). To the left of the cavity occupied by oseltamivir, another, unoccupied groove is visible that presumably accommodates the adjacent sugar moiety of the substrate polysaccharide.

11.10.4 Inhibition of HIV fusion with target cells by the peptide enfuvirtide

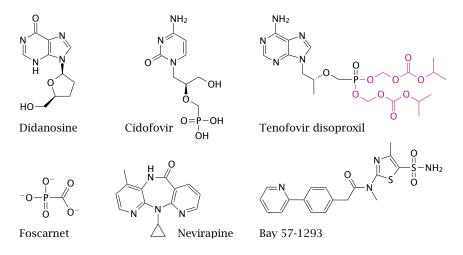


While HIV (human immunodeficiency virus) is not related to influenza virus, it also contains a lipid membrane, which must fuse with the host cell membrane to initiate the infection process. Fusion is controlled by two HIV surface glycoproteins, gp41 and gp120, which initially are associated with each other. Binding of gp120 to its cellular receptor, CD4, and to a co-receptor (not shown) releases the fusion protein gp41, the tip of which inserts into the target cell membrane (1). Two complementary heptad repeat motifs in gp41 (red and blue) then zip up against one another, causing the two membranes to converge (2) and eventually fuse. The drug enfuvirtide, a synthetic peptide, resembles one of these repeats and accordingly associates with the other, which disrupts gp41 activity (3).

While—ostensibly for the sake of simplicity, but really due to a limited supply of artistic talent—gp41 was rendered here as a monomer, it is actually a trimer.

Accordingly, polymeric analogues of enfuvirtide have shown superior affinity and inhibition of cell infection [119].

11.10.5 Inhibitors of virus genome replication



The most general approach to antiviral chemotherapy remains the inhibition of the viral DNA or RNA polymerases that replicate the viral genomes.

Didanosine and tenofovir disoproxil are chain terminators, and nevirapine is an allosteric inhibitor of HIV reverse transcriptase. Didanosine is phosphorylated by cellular kinases.²¹ Tenofovir disoproxil is a resorption ester of a phosphonate analogue of adenosine. Cidofovir, which is active against many viruses, likewise carries a phosphonate group and therefore bypasses the first phosphorylation step.

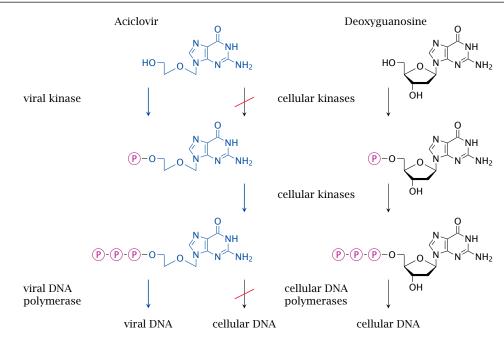
While most inhibitors resemble substrates of nucleic acid synthesis, foscarnet resembles a product. Pyrophosphate is produced in each addition of a nucleotide to a growing nucleic acid strand and then rapidly hydrolyzed by pyrophosphatase. On several viral polymerases, the hydrolysis-resistant analogue foscarnet blocks the binding site for pyrophosphate, at concentrations below those that would be inhibitory for human polymerases.

Bay 57-1293 is an experimental inhibitor of Herpesvirus helicase/primase, an enzyme that unwinds DNA and synthesizes a short oligonucleotide primer for the DNA polymerase.

11.10.6 Activation of acyclovir

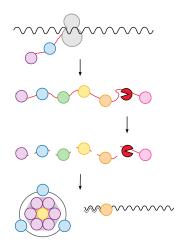
Acyclovir is a guanosine analogue that has been mutilated to a considerable extent, and accordingly is rejected as a substrate by cellular nucleoside salvage kinases. However, it is phosphorylated by a thymidine kinase encoded by herpes virus. Cellular nucleotide kinases then convert the monophosphate to the triphosphate, which is a substrate for the viral DNA polymerase, but not for the cellular enzyme.

²¹In HIV patients, long-term use of didanosine can give rise to gout (see slide 10.3.6).



In contrast to most antimicrobial drugs, which bind to a single microbial target, acyclovir selectively interacts with two viral enzymes in series. Because of this, acyclovir has a particularly high degree of selective toxicity, and serious side effects are less common with acyclovir than with most other nucleoside analogues used in antiviral chemotherapy.

11.10.7 Function of virus proteases



Many viruses, including hepatitis C virus and HIV, translate their genome into *polyproteins*, that is, large precursor proteins which contain the final, functional proteins as sub-domains like pearls on a string.

One of these sub-domains is a protease that cleaves first itself and then the other components. After cleavage, the released proteins travel to different destinations to serve in their respective roles during virus replication and assembly.

If the viral protease inhibited, most viral proteins will not become functional; in particular, assembly of the virus particle will be prevented. Inhibition of this crucial step is therefore extremely disruptive to viral replication.

In the case of HIV, the advent of protease inhibitors marked a very major improvement in the effectiveness of treatment, causing the life expectancy of the patients to jump up from just a few years after diagnosis to an almost normal life span. The HIV protease inhibitor saquinavir is shown in slide 1.3.6.

Chapter 12

Cancer chemotherapy

12.1 Overview

The purpose of cancer chemotherapy is analogous to that of antimicrobial chemotherapy, namely, to selectively kill the pathogenic cells. Of the two, cancer chemotherapy tends to be more toxic, yet less reliably effective; or in other words, it fails to achieve the level of selective toxicity that is possible with antimicrobial chemotherapy. This is not surprising, since the cells that we aim to destroy originate in our own bodies, and therefore contain few or no drug targets that are truly distinct from those found in healthy cells. Considering this lack of molecular distinctiveness, it is rather remarkable that cancer chemotherapy even works as well as it does.

Like pathogenic microbes, cancer cells may develop resistance to anticancer drugs under therapy. To mitigate this problem, anticancer drugs are almost always used in combination.

12.1.1 Forms of cancer therapy

- Surgery
- Radiation
- Chemotherapy

Criteria for therapy selection

- Benign or malignant tumor
- Tissue of origin, histological variant of tumor
- Stage of tumor—early and localized vs. advanced and disseminated

Broadly speaking, surgery is applied locally, radiation regionally, and chemotherapy systemically.

Benign tumors don't infiltrate the surrounding tissue and don't give rise to metastases; therefore, they can usually be cured through surgery alone. In most solid malignant tumors, surgery is used to remove the main tumor mass as far as possible. Subsequently, radiation or chemotherapy are applied alone or together in order to extirpate any regionally or systemically disseminated tumor cells and thus prevent resurgence of the cancer. However, leukemias and lymphomas, as well as some specific solid tumors, are treated primarily with chemotherapy.

As we had seen in Chapter 7, the cells in some of our organs depend on growth stimulation by hormones. Tumor cells originating in these organs may retain this hormone dependency; important examples are breast and prostate cancers, which often remain dependent on growth stimulation by sexual steroid hormones. In such cases, withdrawal of the hormonal stimulus, using receptor antagonists or inhibitors of hormone synthesis, can be an effective part of chemotherapy. Where such specific hormone dependency does not exist, broadly cytotoxic drugs that interfere with mitosis, DNA synthesis, or other fundamental aspects of cell biology are often the only option. In any case, such cytotoxic drugs are part of most combination regimens.¹

It is important to understand that cytotoxic drugs don't simply disrupt and destroy the cancer cells with brute force. Instead, they trigger regulatory pathways within the cell that initiate programmed cell death, or *apoptosis*. Accordingly, mutations that inactivate apoptotic pathways are often responsible for resistance of tumor cells to chemotherapy.

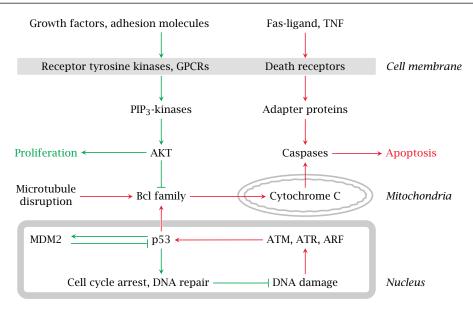
12.2 Cellular pathways that control proliferation and apoptosis

The lifetimes and rates of proliferation of the cells in our bodies are highly variable. Most nerve cells in the brain don't ever divide but just stay put throughout life,² while those in the bone marrow and the intestinal epithelia proliferate rapidly; lymphocytes often end their days through apoptosis, which is necessary to avoid damage to the body through autoimmune reactivity. The pathways that determine whether a cell should persist, proliferate, or commit apoptosis are subject to both intra- and extracellular signals.

Growth factors and adhesion molecules stimulate the PIP₃ kinase pathway and thereby stimulate proliferation and inhibit apoptosis. Conversely, mediator proteins such as Fas-Ligand and tumor necrosis factor (TNF) act on so-called "death receptor" proteins in the cell membrane. This leads to the activation of *caspases*, a family of cysteine proteases that cleave their numerous substrates at aspartate residues, and thereby destroy the cell.

¹Radiation therapy is similar in its mode of action to DNA-modifying cytotoxic drugs. "Ionizing" radiation creates not only ions but also radicals, which are the actual cytotoxic agents that react with DNA to induce strand breaks and other kinds of modifications.

²Some of them, of course, don't stay. From first-hand experience, I would recommend securing tenure before too many of them are gone.



In the intrinsic pathway of apoptosis, DNA damage leads to the activation of protein kinases such as ATM and ATR, which in turn activate the transcriptional regulator p53. Subsequently, increased expression of certain regulatory proteins in the Bcl family leads to the permeabilization of mitochondria by Bax, another Bcl family member. Cytochrome C released from the mitochondria then again activates caspases.

Mutations in many of the proteins shown here have been implicated in the induction or progression of cancer. One such protein, p53, is disabled by mutation in as many as 50% of all malignant tumors, which highlights its central role in tumor suppression.

12.2.1 Dysregulation of growth in tumor cells

Normal body cells

- grow or persist only when stimulated by growth factors
- undergo apoptosis when deprived of growth factor stimulation

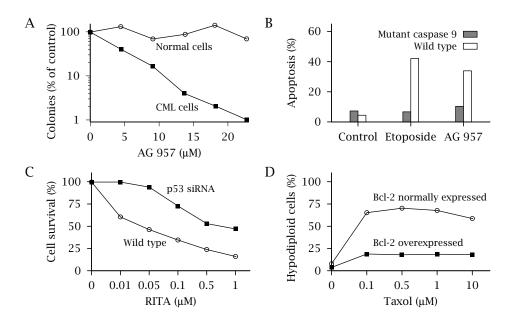
Tumor cells contain mutations that

- create surrogate growth stimuli
 - constitutively active growth factor receptors
 - autocrine secretion of growth factors
- disrupt activation of apoptosis downstream of growth factor deprivation
- *but also* make tumor cells *more* susceptible to *some* apoptotic stimuli than normal cells

It was noted above that p53 is very frequently inactivated in malignant tumors. This protein has an ambivalent role: while it promotes apoptosis when maximally activated, at lower degrees of activation it can actually promote cell survival. It

does this by initiating a pause in cell division and activating DNA repair systems. Failure to pause division and perform DNA repair may be one of the reasons why tumor cells actually tend to be *more* susceptible to certain apoptotic stimuli than normal cells. On the other hand, failure of DNA repair also increases the mutation rate, which in turn accelerates the progression of the tumor to more malignant and invasive behavior, as well as the development of resistance to chemotherapy.

The readiness to plunge into apoptosis varies not only between normal cells on one hand and tumor cells on the other, but also between normal cells from different tissues. For example, lymphocytes and their precursors are driven into apoptosis extremely easily (cf. slide 10.3.2). Tumors derived from lymphocytes—lymphomas and lymphatic leukemias—often retain this trait, and therefore are much more amenable to chemotherapy than most other tumors. On the other hand, tumors derived from normal cells that have little inclination to commit apoptosis may inherit this trait also, and therefore tend to be quite impervious to chemotherapy. Examples are cancers originating in the brain and the kidneys.



12.2.2 Cellular models of dysregulated apoptosis

This slide shows several experiments that illustrate some of the points made above on the dysregulation of apoptosis in tumors.

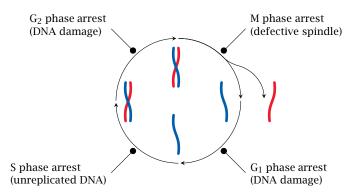
- (A) Apoptotic response triggered by the tyrosine kinase inhibitor AG 957 in white blood cells isolated from a patient with chronic myeloic leukemia (CML) and in normal control cells. This drug inhibits receptor tyrosine kinases, including a mutant one that is constitutively active and sustains the proliferation of the malignant cells. Inhibition of this type of protein kinase causes apoptosis more readily in the malignant cells than in comparable normal ones.
- (B) Expression of a mutant of caspase 9 in a leukemic cell line (K562) inhibits apoptosis in response to two anticancer drugs, namely the topoisomerase II

inhibitor etoposide as well as AG-957. Caspase 9 is part of the destructive machinery that is activated downstream of different proapoptotic stimuli (see slide 12.2). In some tumors, mutants of the enzyme may be expressed that not only are inactive themselves but even suppress the activity of simultaneously expressed active protease; that is, they are *dominant negative*. As expected, such mutants inhibit the induction of apoptosis by anticancer drugs.

- (C) The experimental compound RITA³ inhibits the binding of the inhibitory protein MDM2 to p53 (see slide 12.2), thus increasing the proapoptotic activity of p53. Inhibiting the expression of p53 with a specific siRNA in a model cell line reduces apoptosis in response to this drug.
- (D) The drug taxol disrupts microtubules, which indirectly leads to the activation of pro-apoptotic proteins in the Bcl family. Overexpression of the inhibitory regulator protein Bcl-2 reduces apoptosis, quantified here by FACS counting of "hypodiploid cells," which are not intact cells but rather fragments of nuclei released from apoptotic cells.

The plots in this slide were redrawn from data found in various sources [120-122].

12.3 The cell cycle and its checkpoints



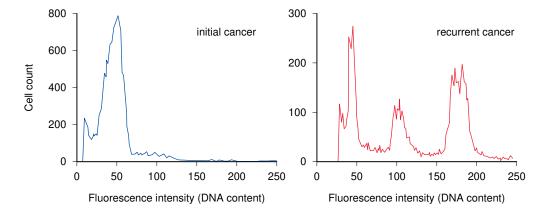
Somatic cell proliferation goes through successive coordinated phases, which together constitute the cell cycle. Daughter cells arise by mitosis, which within the cell cycle is referred to as the M phase. After the G_1 phase,⁴ a cell prepares for the next round of division by entering the S phase, in which the DNA is replicated, that is, a second double-stranded DNA copy is synthesized for each chromosome. Following another short intermission, the G_2 phase, mitosis repeats.

In each of these phases, corresponding checkpoint proteins are activated that will arrest the cell cycle if they detect phase-specific forms of genetic damage. Depending on the extent of damage, the arrest may be followed by DNA repair and resumption of the cell cycle, or apoptosis may be triggered.

³Chemical name: 2,5-bis-(5-hydroxymethyl-2-thienyl)-furan

⁴The "G" in G_1 and G_2 denotes "gap". While these phases are gap phases with respect to change in cellular DNA content, the cell is not idle in these stages. The G_1 phase, in particular, can be very long and comprises the time during which a cell performs its specific physiological role.

In the M phase, cell cycle arrest may be induced if defects are found in the *mitotic spindle*, that is, the cytoskeletal apparatus that distributes the chromosomes evenly between the two daughter cells. Defects in the M phase checkpoint proteins will permit the survival of cells that have gained or lost chromosome fragments, entire chromosomes, or even acquired extra copies of the whole genome. Cells whose chromosome complement deviates from the regular diploid one are called *aneuploid*. The occurrence of aneuploid cells is a hallmark of cancer and contributes greatly to genetic variability and development of chemoresistance.



12.3.1 Progressive cell aneuploidy in a recurring tumor

In the experiment shown, the nuclei of cells obtained from a *sarcoma*, that is, a malignant tumor derived from a non-epithelial tissue, were isolated and incubated with a fluorescent dye such as propidium iodide, which emits fluorescence only after intercalation into DNA. The stained nuclei were then passed through a flow cytometer. As the nuclei pass the instrument's laser beam in single file, each nucleus causes a fluorescence pulse, the intensity of which is proportional to its DNA content.

In the original tumor (A), the DNA content per cell shows one major peak, corresponding to the diploid chromosome complement. In the recurring tumor (B), there are multiple peaks, indicating aneuploidy and clonal divergence.

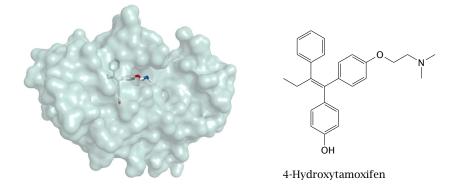
Aneuploidy is very common with malignant tumors. The additional or missing gene copies will change the expression levels of individual proteins, including ones that influence tumor proliferation, apoptosis, and susceptibility or resistance to anticancer drugs. From the genetically heterogeneous cell population, particularly resistant cell clones may be selected under drug therapy. Figure prepared from original data in [123].

12.4 Cell type-specific anticancer drugs

- Hormones and growth factors: interferon- α in hairy cell leukemia
- · Hormone antagonists: most significant with breast and prostate cancer
- Tissue-specific prodrug activation: mitotane in adrenal gland tumors
- Tissue-specific accumulation of radioactive iodine: thyroid cancer

Among the numerous anticancer drugs, we can broadly distinguish those that act only on specific cell types from those that are generally cytotoxic. We will now consider some examples of drugs in the first category.

12.4.1 Sexual hormones and receptor antagonists



Estrogens like estradiol or progestins like progesteron are required by many breast or uterine cancers, which can therefore be treated with estrogen receptor antagonists like tamoxifen or progestin receptor antagonists like mifepristone. Similarly, androgen-dependent prostate cancers can be targeted with inhibitors testosterone- 5α -reductase or androgen receptor antagonists (see slide 7.4.9).

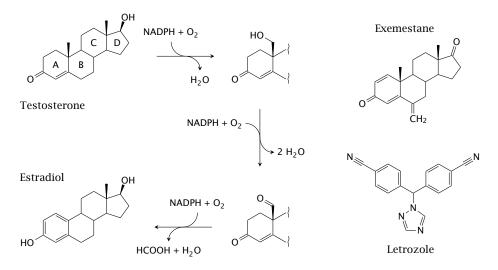
The structure (rendered from 2p7z.pdb) in this slide shows 4-hydroxy-tamoxifen, an active metabolite of tamoxifen, bound to the ligand-binding domain of the estrogen receptor; the formula of the free compound is rendered in a similar orientation. Mifepristone is shown in slide 7.4.5.

12.4.2 Aromatase and two of its inhibitors

Aromatase is a cytochrome P450 enzyme that converts androgens to estrogens (see slide 7.4.8). The left part of this slide shows details of the aromatase reaction. The methyl group between rings A and B is converted in two steps to an aldehyde. The keto group in ring A then becomes its enol tautomer, which creates a second double bond in the ring. The third double bond is introduced into ring A concomitantly with oxidative cleavage of the exocyclic aldehyde as formic acid.

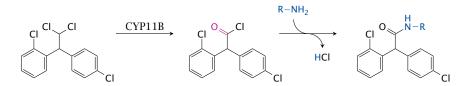
The slide also shows the structures of the clinically used aromatase inhibitors exemestane and letrozole. Exemestane is a steroidal, covalent aromatase inhibitor. The exocyclic C=C double bond likely reacts with a nucleophile in the active site

[124]. Letrozole is a nonsteroidal, noncovalent aromatase inhibitor. It is somewhat similar in structure to the antifungal drug miconazole, an inhibitor of $14-\alpha$ -sterol demethylase (see section 11.8.2) that, like aromatase, belongs to the cytochrome P450 family.



12.4.3 The anticancer prodrug mitotane is selectively activated in the adrenal cortex

Mitotane is converted to a reactive acyl chloride by $11-\beta$ -hydroxylase, a cytochrome P450 variant (CYP11B). The acyl chloride then reacts with amino groups or other nucleophiles in proteins or nucleic acids.



11- β -Hydroxylase participates in the synthesis of cortisol from progesterone in the cortex of the adrenal glands (see slide 7.4.3). It is highly expressed only in this tissue, which is therefore selectively susceptible to mitotane. Adrenal gland tumors that retain the expression of this enzyme can be treated with mitotane.

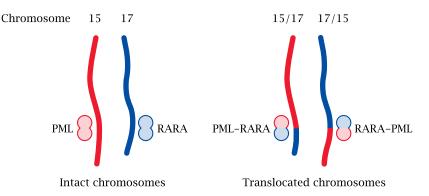
12.5 Anticancer drugs that target specific oncoproteins

- α -Retinoic acid in promyelocyte leukemia
- Imatinib in chronic myeloic leukemia; other tyrosine kinase inhibitors in various tumors
- Monoclonal antibodies against growth factor receptors on the cell surface, for example Her2/neu ("herceptin") in breast cancer

Oncoproteins are mutant proteins that drive the proliferation of aberrant cells in tumors. Key examples are the oncoproteins involved in promyelocyte leukemia and in chronic myeloic leukemia, which both arise from chromosome translocations; this is discussed in more detail below.

The growth factor receptor Her2/neu is an oncoprotein found in a large number of breast cancers and also in some other carcinomas. Mutations affecting Her2/neu function found in tumors typically do not change the structure of the protein as such, but instead cause it to be overexpressed or excessively activated by phosphorylation.

12.5.1 A chromosomal translocation causes promyelocytic leukemia



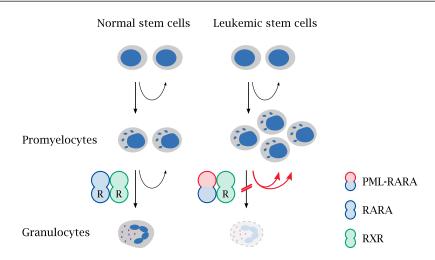
Bone marrow stem cells give rise to mature blood cells in several successive stages of proliferation and differentiation. In all stages except the final ones, individual cells can either divide and produce like daughter cells, or advance to the next stage of differentiation.

One of the intermediate stages in granulocyte differentiation is the *promyelocyte*. In promyelocytic leukemia (PML), this cell type proliferates excessively and displaces the regular bone marrow, which causes shortage of all sorts of normal blood cells. PML is caused by a specific chromosomal *translocation*, that is, the reciprocal exchange of chromosome fragments between chromosomes 15 and 17.

Two genes, respectively named PML and RARA, span the fault lines of the translocation on chromosome 15 and 17, respectively. Translocation gives rise to two chimeric genes, which are referred to as RARA-PML and PML-RARA. Of the two gene products, PML-RARA is the one that functions as an oncoprotein.

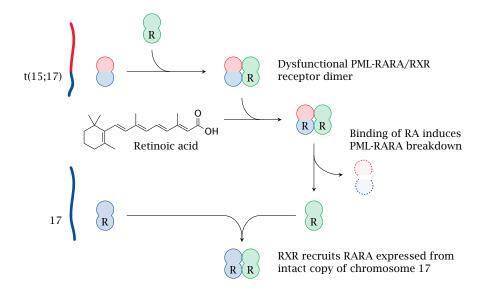
12.5.2 The mutant PML-RARA receptor blocks promyelocyte differentiation

PML-RARA is an in-frame fusion between the N-terminal portion of the PML gene product and the C-terminal part of the RARA gene product, which is the retinoic acid receptor α . White blood precursor cells that express this protein can proliferate and progress through the early stages of differentiation seemingly without disturbance.



Differentiation stalls, however, at the *promyelocyte* stage. The progression to the next (the *myelocyte*) stage requires the α -retinoic acid receptor to form a functional heterodimer with another retinoid acid-binding receptor, the retinoid X receptor (RXR).⁵ PML-RARA still forms heterodimers with RXR. However, at normal levels of retinoic acid, PML-RARA fails to bind its ligand, and in this form the receptor dimer engages in aberrant transcriptional regulation that causes cell differentiation to stall. Dysfunctional promyelocytes accumulate and proliferate, thereby displacing normal blood cell formation.

12.5.3 Retinoic acid therapy restores promyelocyte differentiation



⁵RXR cooperates also with other nuclear hormone receptors besides RARA, such as thyroid hormone receptor (slide 7.3.1) and the pregnane X receptor (slide 4.3).

Fortunately, in most patients,⁶ this condition can be treated with high dosages of retinoic acid. While PML-RARA has a reduced affinity for retinoic acid, this can be overcome by applying it as a drug at high concentrations. This changes the regulatory activity of the receptor dimer and also drives the drug-bound PML-RARA protein into accelerated degradation. With the aberrant protein gone, RXR can combine with intact RARA that continues to be expressed from the intact copy of chromosome 17; cell differentiation resumes, and excessive promyelocyte proliferation ceases.⁷

12.5.4 Chronic myeloid leukemia

- Caused by reciprocal translocation between chromosomes 9 and 22 ("Philadelphia chromosome")
- Translocation produces a chimeric, constitutively active protein tyrosine kinase (*Bcr-Abl*) that drives the proliferation of myeloid precursor cells
- Treatment with imatinib or other tyrosine kinase inhibitors controls, but usually does not eradicate leukemic cells
- After several years, CML typically ends in a "blast crisis", which resembles an acute myeloid leukemia

The two stages of chronic myeloid leukemia (CML) are somewhat resemblant of a solid tumor transitioning from benign to malignant growth. In contrast to such a benign tumor, CML grows diffusely and in multiple locations right away; however, this may under certain circumstances be observed with normal bone marrow tissue also. As in a benign tumor, the growth-dysregulated cells in early-stage CML remain genetically homogeneous, and their growth can be suppressed by inhibiting a single oncoprotein.

Like the acute myeloic leukemia it resembles, the terminal blast crisis is treated with combinations of multiple cytotoxic drugs. We will see examples of such drugs a little further on this chapter.

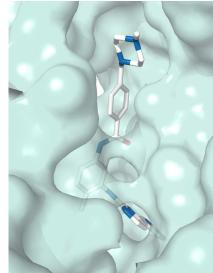
12.5.5 Imatinib bound to its target enzyme c-Abl kinase

The mutant Bcr-Abl kinase that induces CML inherits the tyrosine kinase domain from the normal cellular kinase c-Abl, which is the protein that was used in this crystallographic experiment.

The structural formula of imatinib is oriented similarly to the conformation of the drug molecule inside the enzyme. Structure rendered from 1m52.pdb.

⁶The translocation is somewhat molecularly heterogeneous, and not all variants or the resulting protein respond to the treatment equally well.

⁷Sounds complicated? It is actually simplified—see reference [125] for more detail.



12.6 Cytotoxic anticancer drugs

- Antimetabolites
- Inhibitors of DNA topoisomerase
- Proteasome inhibitors
- Inhibitors of mitosis
- DNA-alkylating and other DNA-damaging agents

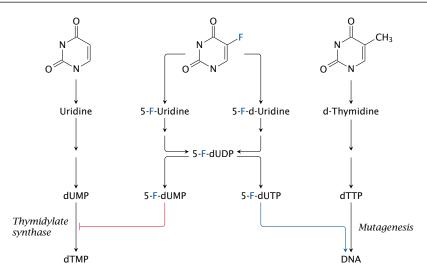
Cytotoxic anticancer drugs have multiple different targets and modes of action, but a common motif is that they inflict damage on the cells in order to activate the intrinsic pathway of apoptosis. While different cell types may respond more or less readily to such treatment, the apoptotic pathway itself is functional in all cells, and it is usually not possible to limit the cytotoxic effect to specific cell types.

We will now look at selected examples for the different types of cytotoxic anticancer drugs, starting with the antimetabolite 5-fluorouracil.

12.6.1 Dual action mode of 5-fluorouracil

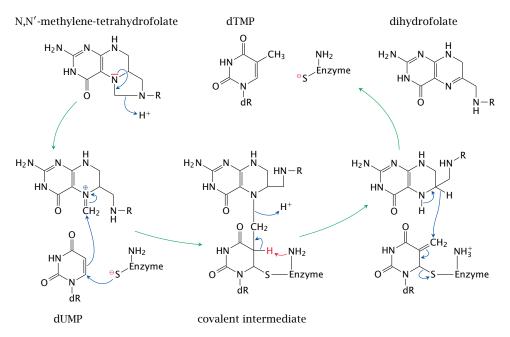
5-fluorouracil (5-FU) is a base analog that mimics both uracil and thymine; the dual resemblance gives rise to a twofold mode of metabolic activation and of action. One of the initial activation products, 5-fluorodeoxyuridine, is also used as a drug itself.

The metabolic activation of 5-FU occurs along the so-called salvage pathways that recycle bases and nucleosides released by nucleic acid degradation. 5-FU is activated by the same enzymes that salvage uracil or thymine and is thereby converted to 5-FdUMP, which is a suicide substrate for thymidylate synthase (see next slide). This is its major mode of action and the one that makes it an antimetabolite.



The precursor of 5-FdUMP is the diphosphate (5-Fluoro-dUDP). Due to the resemblance between 5-FU and thymine, 5-Fluoro-dUDP may also be further phosphory-lated to to 5-Fluoro-dUTP, which is incorporated into DNA; this will cause point mutations (see slide 12.6.3).

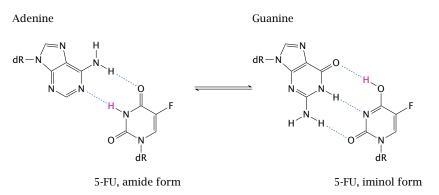
12.6.2 Catalytic mechanism of thymidylate synthase



Thymidylate synthase attaches a methyl group onto uracil to form thymine, at the level of the deoxyriboside monophosphate. It acquires the methyl group from the cosubstrate N,N'-methylene-tetrahydrofolate, which is thereby converted to dihydrofolate.

Halfway through the reaction, the enzyme, the substrate and the cosubstrate are all covalently connected. Resolution of this covalent intermediate depends on the abstraction of a hydrogen atom from position 5 of uracil (highlighted in red). In 5-FU, the place of this hydrogen is taken by fluorine. The fluorine will resist abstraction, the complex will fail to resolve, and the enzyme will remain covalently trapped and inactivated. The resulting lack of dTMP and dTTP inhibits DNA replication.

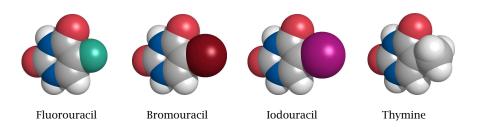
12.6.3 Mutagenesis through mispairing of the 5-FU iminol tautomer



5-F-dUTP can be incorporated into DNA and promote point mutations. This is due to the fluorine, which withdraws electrons from the ring; this, in turn, pulls electrons into the ring at other positions and encourages the molecule to assume the iminol configuration. In this configuration, the base no longer pairs with adenine but with guanine.

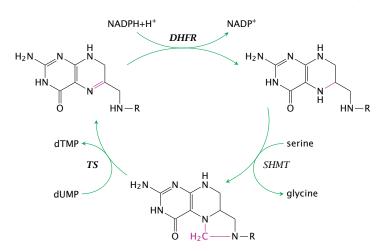
If the iminol configuration is present during DNA replication, guanine will be selected and incorporated into the opposite strand. This mutagenic effect of 5-FU augments its anticancer effect.

12.6.4 Thymine and various halogen analogues



The bromo- and iodo-analogs of deoxythymidine, 5-bromouracil-deoxyriboside (5-BUdR) and idoxuridine, are also incorporated into DNA. Bromine and iodine are larger than fluorine and similar to a methyl group in size. Therefore, the activated triphosphates more closely resemble dTTP and are more effectively incorporated into DNA than the 5-FU derivative.

Like fluorouracil, idoxuridine is used in tumor therapy. 5-BUdR is not used clinically but has been widely used for shotgun mutagenesis experiments in genetic research.



12.6.5 Blockade of dihydrofolate reductase also inhibits thymidylate synthesis

As we have seen in slide 12.6.2, thymidylate acquires a methyl group from N,N'tetrahydrofolate, which is thereby turned into dihydrofolate. Recycling dihydrofolate involves two successive enzyme reactions, namely the reduction to tetrahydrofolate, followed by the acquisition of a methylene group from serine hydroxymethyl transferase (SHMT) or from the glycine cleavage system. If dihydrofolate reductase (DHFR) is inhibited, this will indirectly also inhibit the synthesis of dTMP by thymidylate synthase (TS).

In contrast to 5-FU, inhibitors of dihydrofolate reductase do not resemble nucleotides and will not directly interact with DNA to cause mutations. Therefore, they are less toxic and are used not only in cancer but also for long-term treatment in autoimmune diseases such as lupus erythematosus or myasthenia gravis.⁸

12.6.6 Inhibitors of dihydrofolate reductase

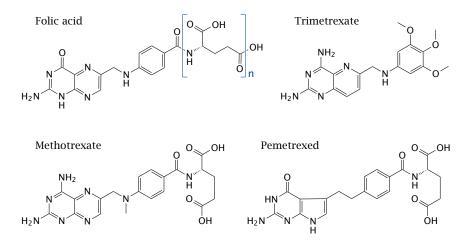
Methotrexate and pemetrexed inhibit the human dihydrofolate reductase enzyme (DHFR). Pemetrexed also inhibits thymidylate synthase directly, as well as glycinamide ribotide formyltransferase, a THF-dependent enzyme from the purine nucleotide biosynthesis pathway. Since it acts on multiple targets, it is less prone to resistance through DHFR gene amplification and overexpression, which is a common resistance mechanism with drugs that inhibit this enzyme exclusively.

Trimetrexate inhibits DHFR not only in humans but also in some microbes, and it can be used for example in the treatment of infections with the fungus *Pneumocystis carinii*.

In this slide, the brackets in the structure of folic acid represent a polyglutamate moiety that is attached to folic acid in human metabolism; it is involved in active transport of folate in the human body. Methotrexate and pemetrexed retain a single glutamate residue that is extended into a polyglutamate tail inside the body

⁸The cells that one aims to destroy in immunosuppressive therapy are lymphocytes, which mediate the autoimmune reactions. The principle is similar as in anticancer treatment, in that the cells are driven into apoptosis through interference with DNA replication.

also. In contrast, trimetrexate does away with this polyglutamate and crosses cell membranes on its own by passive diffusion. This feature likely is important for its activity against not only human cells but also microbes.



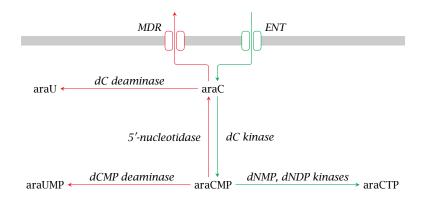
12.6.7 Structure of cytosine arabinoside (araC)

In the last chapter, we had seen some examples of nucleotide analogues that inhibit virus replication through interfering with nucleic acid polymerization (see slide 11.10.5). The same approach is used with human DNA polymerases for cancer chemotherapy.⁹



An example of a base analogue that interferes with human DNA polymerases is cytosine arabinoside (araC). This molecule contains arabinose, an epimer of ribose, instead of ribose or deoxyribose itself. The hydroxyl group in position 2 of this sugar points "up" rather than "down" as it does in ribose. Interference with DNA synthesis is due to this aberrant hydroxyl group.

⁹Some of the analogues used in antiviral treatment, such as dideoxyadenosine for example, were indeed originally conceived as anticancer drugs.

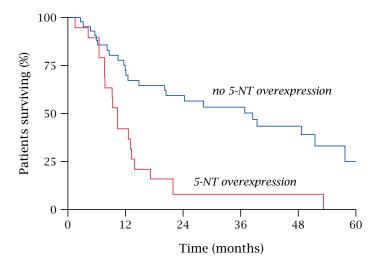


12.6.8 Metabolic activation and inactivation of araC

AraC enters the cell by facilitated diffusion through the equilibrative nucleoside transporter (ENT). In order to become a substrate for DNA polymerase, araC must be phosphorylated to the triphosphate (araCTP); this is accomplished by enzymes of the nucleoside salvage pathways.

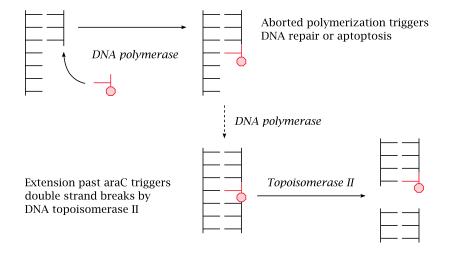
The activation of araC can be reversed at several stages. Extrusion of araC itself is mediated by multi-drug resistance transporters (MDR) such as P-glycoprotein (see slide 3.5.1). Like cytidine and deoxycytidine, araC can undergo deamination either as a free nucleoside or as a monophosphate, and the initial phosphorylation can be reversed by the enzyme 5'-nucleotidase. Increased expression of MDR or of enzymes that counteract the activation of araC to araCTP cause tumor cell resistance (see slide 12.6.9).

12.6.9 Overexpression of 5'-nucleotidase in leukemic cells correlates with reduced survival rates



AraC is an important drug in the treatment of acute myeloic leukemia (AML). Reduced susceptibility to araC due to increased expression of 5'-nucleotidase correlates with a significant shortening of relapse-free survival in AML patients. Figure prepared from original data in [126].

12.6.10 Action mode of araCTP



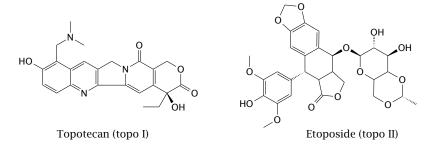
AraCTP is accepted as a substrate by DNA polymerase and becomes incorporated into a growing strand of DNA. The polymerase may or may not manage to continue past an incorporated araC molecule. If not, DNA repair will be activated, and the base will be excised and replaced; this amounts to a delay of DNA synthesis and constitutes a proapoptotic signal.

If DNA polymerase manages to continue past an incorporated araC molecule, the latter becomes part of a continuous DNA double strand. Such interposed araC residues then become preferred substrate sites for DNA topoisomerase II, which cleaves the DNA double strand [127]. Resealing of the double strand may fail and lead to chromosome breaks.

12.6.11 Function of DNA topoisomerases

DNA topoisomerases introduce coils and super-coils into DNA molecules or remove them. They do so by breaking and rejoining the DNA molecules. By transiently "straightening out" the DNA molecules, they make them accessible to replication and transcription. DNA topoisomerase I breaks one strand of a DNA molecule, swivels the ends around the other strand and then re-ligates them. It thus changes the number of coils in an individual double-stranded DNA molecule. DNA topoisomerase II operates analogously on two stretches of double-stranded DNA that are twisted around one another, breaking both strands of one double helix and pushing the other through the gap before rejoining the first one.

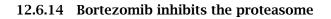
12.6.12 DNA topoisomerase inhibitors

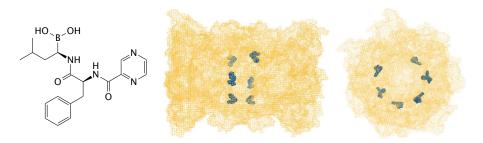


As these two examples show, inhibitors of both topoisomerase I and II are used in cancer chemotherapy. The effect of topotecan on its target is illustrated in the next slide.

12.6.13 Topotecan bound to topoisomerase I and DNA

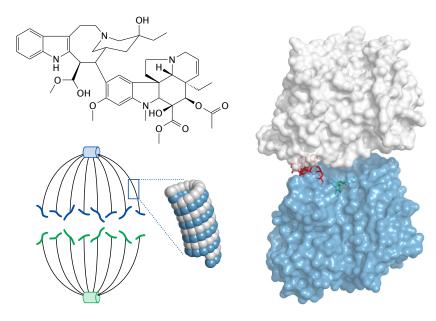
The drug (shown in red) binds to the enzyme such that it inserts itself between the free ends of the cleaved DNA strand and thereby prevents re-ligation. Structure rendered from 1k4t.pdb.





Proteasomes are drum-shaped, large protease complexes. They unfold and cleave intracellular proteins destined for degradation.¹⁰ Bortezomib binds and inhibits the protease active sites in proteasomes. This causes all manner of dysregulation [128] and again promotes apoptosis.

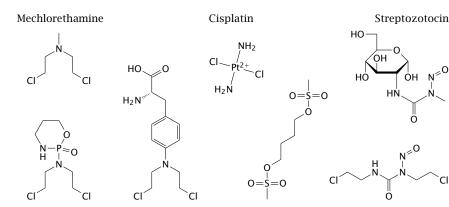
12.6.15 Vinblastine inhibits tubulin polymerization



The mitotic spindle is the cytoskeletal apparatus that separates the chromosomes during the metaphase of mitosis. The spindle consists of microtubules, which are helical assemblies of tubulin $\alpha\beta$ -heterodimers. Within the dimer, the drug vinblastine (red) binds between α -tubulin (white) and β -tubulin (blue). This distorts the geometry of the dimer and, by extension, that of the polymeric assembly. Tubulin structure rendered from 1z2b.pdb.

In addition to inhibiting mitosis, the disruption of tubulin polymerization also promotes apoptosis via activation of Bcl proteins. The molecular connection between these two events is only partially understood [129].

¹⁰Cellular proteins get tagged for degradation by ligation to the small protein ubiquitin. The protein MDM2 (slide 12.2) is a ubiquitin ligase that tags p53 for proteosomal degradation.



12.6.16 Alkylating anticancer drugs



Alkylating drugs introduce covalent modifications into DNA and thus cause mutations and interference with replication. In contrast to the other types of cytotoxic drugs that we have covered so far, their effect is not limited to actively dividing cells but also affects resting cells. Since tumors typically contain a significant fraction of cells that are resting, alkylating drugs are part of most therapeutic drug combinations.

Two functional groups found in many alkylating drugs are the bis-chloroethylamine (N-mustard) group and the nitrosurea group. Mechlorethamine, cyclophosphamide, and melphalan all contain the N-mustard moiety, with its chloride leaving groups.¹¹ Bis-chloroethyl-nitrosurea (BCNU, carmustine) and cisplatin have chloride leaving groups as well, whereas busulfan has methylsulfonate leaving groups. BCNU additionally contains the nitrosurea group, which is also found in the antibiotic streptozotocin.

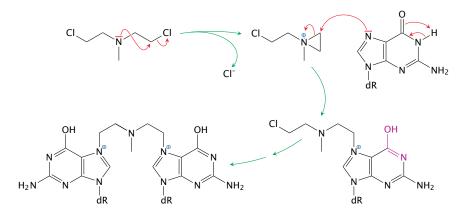
12.6.17 Reaction of mechlorethamine with DNA

Mechlorethamine provides a straightforward example of DNA modification by the N-mustard drugs. The drug is activated by conversion of one chloroethyl group to the corresponding aziridine. The site in the DNA most likely to react with this aziridine is the N7 position of guanine. In the adduct, the aziridinium ring has been opened, which allows the next chloroethyl group to react. Successive reaction of two bases with the same drug molecule may crosslink the two DNA strands. Since such crosslinks are not amenable to excision repair, they form very effective and highly mutagenic lesions.

In the guanine adduct, the iminol tautomer is favored (highlighted in the first reaction step). This changes the base-pairing preference from cytosine to thymine and

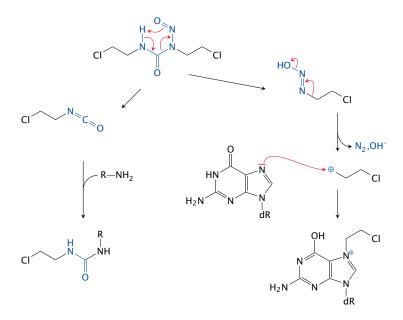
¹¹Melphalan also contains a phenylalanine moiety, which makes it a substrate for amino acid transporters. Cyclophosphamide undergoes metabolic conversion to another N-mustard derivative plus acrolein ($CH_2 = CH - CHO$), which is an alkylating agent in its own right.

may cause mutations during DNA replication. In this manner, even non-crosslinked modified bases may give rise to mutation.

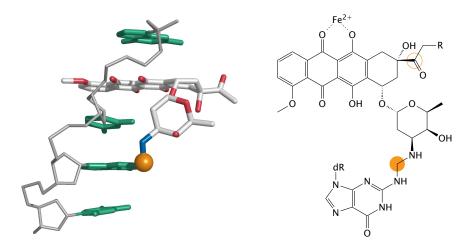


12.6.18 BCNU decay and adduct formation

The nitrosourea moiety in BCNU decomposes spontaneously, yielding an isocyanate compound that reacts with primary amines (left) and a diazo compound that decays further into a carbocation, which in turn reacts with many nucleophiles, including bases in DNA (right). Each of the two BCNU fragments retains one chloroethyl moiety, which can engage in further reactions.



The antibiotic streptozotocin (slide 12.6.16) consists of a nitrosourea group attached to glucosamine. The sugar moiety facilitates its uptake by β cells in the pancreatic islets, which it selectively destroys. Streptozotocin is used therapeutically in β cell carcinomas, and experimentally in animals to induce diabetes mellitus.



12.6.19 Reaction of daunorubicin with DNA

Daunorubicin (R=H) and doxorubicin (R=OH) are antibiotics produced by a soil bacterium (*Streptomyces peucetius*). Mediated by their planar polycyclic rings, these molecules intercalate between the stacked bases of DNA double strands (but only one DNA strand is shown here; structure rendered from 2d34.pdb.). The amino group on the daunosamine sugar moiety of the drug then becomes linked by a methylene bridge to the N2 amino group of a guanine base (highlighted).

The carbon that forms this bridge is derived from formaldehyde, which arises from cellular precursors or, with doxorubicin, from the hydroxyacetyl side chain on the drug itself (also highlighted) through a series of redox reactions that occur between glutathione, iron and the drug's quinoid rings.

Another antibiotic that has been (but no longer is) used in cancer chemotherapy is calicheamicin (see slide 14.5.2).

Chapter 13

Ribonucleic acids as drugs and drug targets

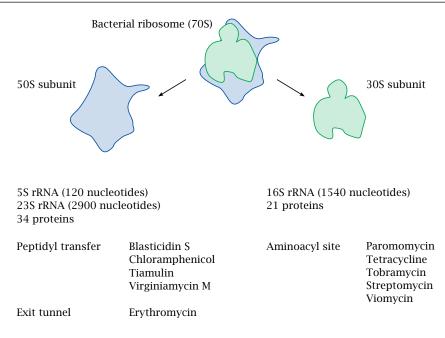
13.1 Introduction

As we have seen in the preceding chapters, most drugs target proteins, but some drugs act on ribonucleic acids (RNAs) instead. Among these, the currently most widely used ones are antibiotics that inhibit the bacterial ribosome (see slide 11.5). However, as more has been learned about the diverse and crucial roles of RNA in eukaryotic cell biology, novel RNA drug targets have emerged and become a focus of ongoing drug development efforts.

In this chapter, we will first have a closer look at some of the antibiotics that interact with the RNA of the bacterial ribosome, and then turn to some of the experimental strategies that target RNA molecules in human cells.

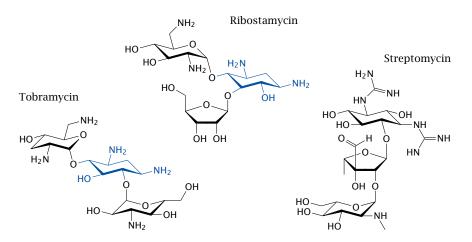
13.2 Antibiotics that inhibit the bacterial ribosome

The bacterial ribosome consists of two subunits that can be separated under mild conditions. Different classes of antibiotics bind to either of these, and in the case of the larger subunit to distinct functional sites within. While ribosomes consist of both RNA and protein molecules, structural studies have determined that most of the interactions with antibiotics involve the ribosomal RNA rather than the ribosomal proteins.



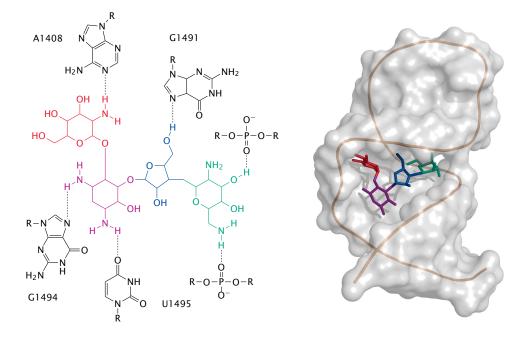
13.2.1 Some aminoglycoside antibiotics

Aminoglycoside antibiotics are produced by *Streptomyces* species and related soil bacteria. As the name suggests, they are oligosaccharides substituted with primary or secondary amines and related functional groups. These antibiotics bind to the aminoacyl acceptor site in the small subunit of the bacterial ribosome.



Among the antibiotics shown here, ribostamycin is a "typical" aminoglycoside that belongs to the 4,5-disubstituted deoxystreptamine group, and tobramycin a typical aminoglycoside with 4,6-disubstituted deoxystreptamine (the deoxystreptamine moiety is shown in blue). Streptomycin does not contain a deoxystreptamine group and thus is an "atypical" aminoglycoside.

Tobramycin is clinically important in the treatment of infections due to Gramnegative bacteria and in particular *Pseudomonas aeruginosa*, against which it counts among the most effective antibiotics. Streptomycin is used in combination with other antibiotics in treating tuberculosis.



13.2.2 Paromomycin in the ribosomal aminoacyl acceptor site

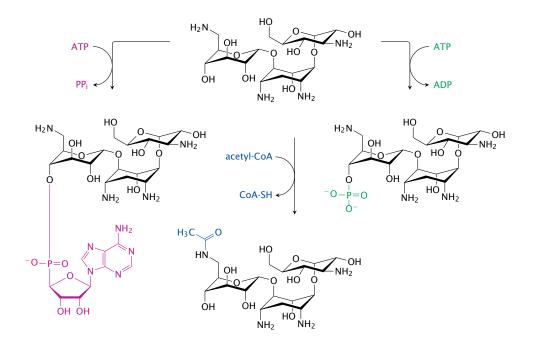
Paromomycin is another aminoglycoside antibiotic. This three-dimensional structure of the RNA-paromomycin complex was determined by NMR spectroscopy, using a short model RNA that mimics the crucial double-stranded section of the (much larger) ribosomal RNA [130]. Rendered from 1pbr.pdb.

The "flattened" structure on the left shows the network of hydrogen bonds between the drug and the nucleotides of the ribosomal RNA. It can be seen that bonds involve both the bases and the backbone phosphates of the RNA.

13.2.3 Inactivation of kanamycin by resistance enzymes

Aminoglycosides will be rendered inactive by covalent modification of the amino and hydroxyl groups that form hydrogen bonds with the bacterial RNA. Enzymes that perform such covalent modifications mediate bacterial resistance to these antibiotics.

This slide illustrates the reactions catalyzed by several resistance enzymes that inactivate kanamycin A, which is yet another aminoglycoside. Adenylylation (purple) is catalyzed by aminoglycoside O-adenylyltransferases, acetylation (blue) by aminoglycoside N-acetyltransferases, and phosphorylation (green) by aminoglycoside Ophosphotransferases. The semisynthetic kanamycin derivative amikacin has been functionalized on an amino group that is not essential for binding to the ribosome. This modification protects the drug from some resistance enzymes. Amikacin is valuable in infections with Gram-negative bacteria that are difficult to treat otherwise.

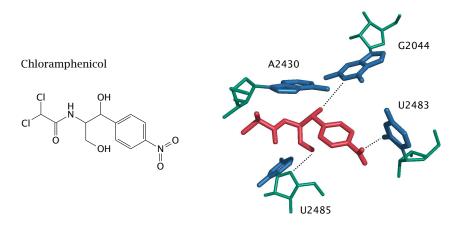


13.2.4 Interactions of chloramphenicol with RNA in the peptidyl transferase site of the ribosome

Chloramphenicol is a small, fairly lipophilic molecule with a broad spectrum of antibacterial activity and very favorable pharmacokinetic properties, but it is almost never used in developed countries because of allergic bone marrow toxicity.¹ The drug binds within the peptidyltransferase site of the ribosome (see slide 11.5.1), which is part of the large subunit of the ribosome.

Like paromomycin, chloramphenicol interacts exclusively with the ribosomal RNA; this interaction involves both of its hydroxyl groups. The acetylation of one hydroxyl group by chloramphenicol acetyltransferase is an important resistance mechanism.

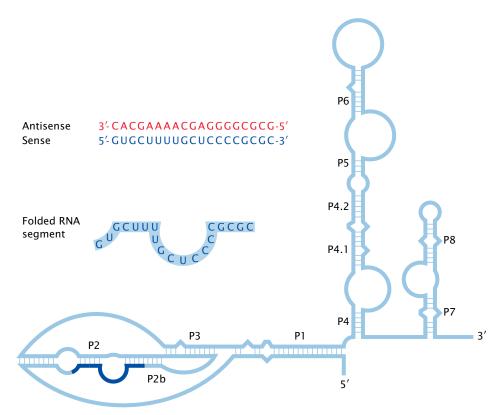
¹Fatalities due to chloramphenicol bone marrow toxicity are actually no more frequent than fatal outcomes of penicillin allergy. Nevertheless, chloramphenicol is shunned, while penicillin is accepted. I suspect that this is because penicillin allergy kills instantaneously, and the unfortunate victim will therefore rapidly leave the scene. In contrast, bone marrow failure due to chloramphenicol is a slow and drawn-out affair that will cause much dismay and anxiety among a larger number of attendant personnel.



13.3 Novel RNA drug targets

The antibiotics discussed above show that ribosomal RNA makes a perfectly fine drug target, which suggests that RNA targets other than ribosomes should also be amenable to small drug molecules. This paradigm is still at the experimental stage.

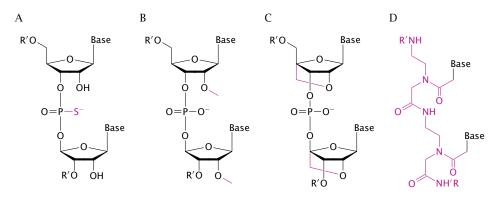
13.3.1 The RNA component of human telomerase



Telomerase is an enzyme that synthesizes repetitive DNA segments and attaches them to the *telomeres*, that is, the free ends of the chromosomes. Telomeres get shortened during each mitotic cell division, and if not renewed by telomerase, cumulative shortening during repeated cell divisions will ultimately wear them down entirely, putting an end to cell proliferation.

As one might guess from the foregoing, telomerase is often overexpressed in tumor cells, contributing to their ability to proliferate indefinitely. This has prompted research into telomerase inhibitors as anticancer agents. The enzyme consists of both protein and RNA moieties. The RNA component, which serves as a template for the polymerization of DNA, is essential for the enzyme's function, and therefore suitable in principle as a drug target. One class of inhibitory molecules are *antisense oligonucleotides*, which hybridize to some strategic part of the target RNA and thereby disrupt its function.

The picture shows the secondary structure of the human telomerase RNA component. A possible binding site for inhibitory antisense oligonucleotides is highlighted. The antisense nucleotide shown will base-pair with all bases in the target sequence, including those that are not base-paired within the native RNA molecule. This will maximize the affinity of the antisense molecule for the target.



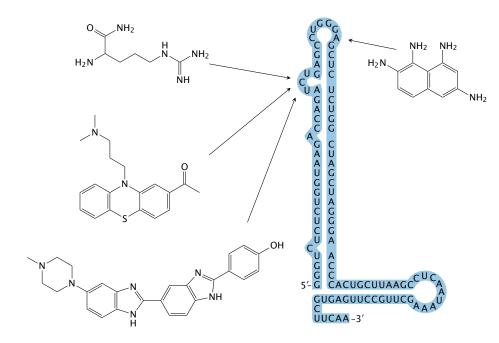
13.3.2 Unusual nucleotide linkages in synthetic oligonucleotides

The above example of telomerase illustrates the conceptual elegance and simplicity of the RNA antisense approach: Affinity and specificity of an oligonucleotide for a given target are much more predictable than is the case with small organic molecules that bind to proteins. Nevertheless, antisense oligonucleotides have found very few applications in clinical practice. Why is this?

Compared to conventional drug molecules, antisense oligonucleotides are rather large—they must contain approximately 15–20 bases to achieve sufficient specificity for a unique target sequence—as well as polar, which is due to the phosphodiester bonds in the backbone. These phosphodiester bonds are also subject to rapid degradation by RNAse or DNAse enzymes. Therefore, overall, oligonucleotides have very unfavorable pharmacokinetic properties.

To increase stability *in vivo*, synthetic oligonucleotide analogues with modified backbone structures have been developed. The picture shows some examples; the structural deviations from regular RNA backbone structure are highlighted.

Phosphorothioates (A), 2'-O-methyl RNA (B) and "locked nucleic acids" (LNA; C) have modified backbones that render them resistant to degradation by nucleases. In "peptide nucleic acids" (PNA, D), the sugar-phosphate backbone is replaced entirely with a polyamide structure that confers not only resistance to cleavage but also reduces polarity.



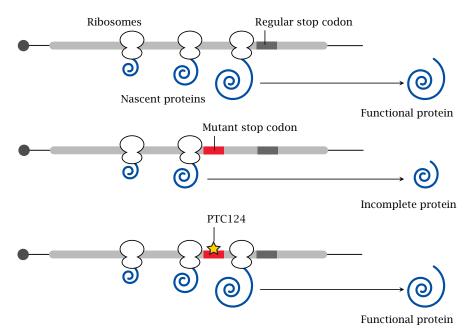
13.3.3 The HIV transactivation-responsive region (TAR)

The transactivation-responsive region (TAR) is an RNA motif that occurs at the 5'-end of human immunodeficiency virus (HIV) RNA transcripts. On a nascent transcript, this region forms a characteristic stem-loop structure that is recognized by the viral *tat* protein, which then recruits cellular proteins to assist with the completion of transcription. Disrupting the interaction between TAR and *tat* might be a viable strategy for inhibiting HIV multiplication.

The figure shows the secondary structure of TAR, as well as several small molecules that have been screened from a library for binding to this RNA [131]. The arrows indicate the approximate binding sites within the TAR.

13.3.4 Blocking premature translational termination with PTC124 (ataluren)

Many genetic diseases are caused by point mutations that inactivate some important enzyme or other proteins. Very often, such inactivating mutations are diverse, affecting different positions within the reading frame of the protein in question, and either causing the replacement of a functionally crucial amino acid residue, or creating a premature stop codon. In the latter case, it may be possible to restore expression of a functional protein by promoting translational *miscoding*, that is, incorporation of some amino acid or other vis-a-vis the mutant stop codon, which then enables the ribosome to keep translating right through to the regular stop $\operatorname{codon.}^2$



An experimental drug that promotes translational suppression of premature stop codons is PTC124 [132], also named ataluren. Its biochemical mode of action is not known exactly; however, it is noteworthy that several aminoglycoside antibiotics have similar effects, and some are also being investigated for similar therapeutic uses [133].

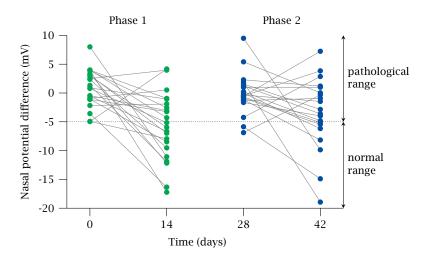
13.3.5 Ataluren in cystic fibrosis

The effectiveness of ataluren in various genetic diseases has been tested in clinical pilot studies. This slide illustrates one such study, which was performed on cystic fibrosis patients. This disease is caused by a deficiency in a membrane transport protein that exports chloride ions from cells, which impedes the secretion of fluid in all kinds of exocrine glands. Multiple organs are functionally impaired, but the most serious consequences arise from the build-up of viscous mucus in the lungs, which facilitates chronic bacterial infections and ultimately leads to organ destruction.

In a sizable minority of all CF patients, the gene defect consists in a premature stop codon, and in this group translational antitermination is a plausible therapeutic strategy. The data illustrate the response to the drug among such patients, during two successive phases of treatment. The effect of the drug is measured as the potential that exists across the nasal mucous membrane; this potential is affected by

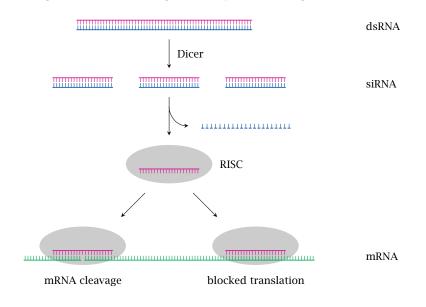
²If you are wondering now how this can be done without suppressing the orderly termination of translation at the regular stop codon, we have no answer for you, but nevertheless commend you for paying attention. If you were *not* wondering about this, you were probably not really studying but just cramming for an exam.

the capacity of the epithelial cells to export chloride. Lines connect measurements on individual patients before and after each course of treatment. Figure prepared from original data in [134].

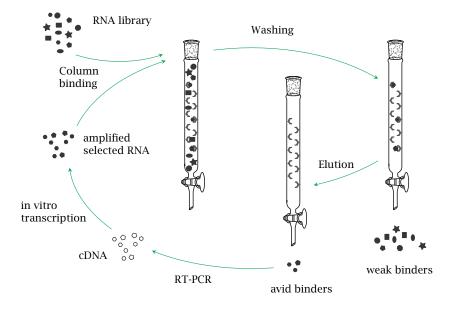


13.3.6 RNA interference

In RNA interference, the presence of double-stranded RNA (dsRNA) suppresses the translation of a corresponding single-stranded messenger-RNA. In this process, the dsRNA is cleaved into small fragments by an enzyme known as *dicer*. The double-stranded fragments—referred to as silencing RNA, or siRNA—are picked up by RNA-Induced Silencing Complex (RISC), which contains multiple protein subunits and catalytic activities. This complex degrades one strand of the siRNA and uses the other to bind to the complementary mRNA, which it inactivates either simply through binding as such, or through subsequent cleavage.



RNA interference is important as a non-specific immune mechanism against the proliferation of RNA viruses, which must go through a dsRNA stage during genome replication and transcription.³ However, RNA interference is not limited to viral RNA as a target, and synthetic siRNA is now often used in cell culture experiments to inhibit the expression of specific genes, for example to validate the corresponding protein as a drug target. Application of siRNA in pharmacotherapy is hampered by the same problems that also afflict antisense oligonucleotides.



13.3.7 The SELEX process for generating RNA aptamers

RNA aptamers are artificial RNA molecules with affinity for a given ligand of interest. They are of potential interest for therapeutic use in a manner similar to therapeutic antibodies, although pharmacokinetic limitations again restrict such uses in practice.

RNA (and similarly, DNA) aptamers can be obtained through an ingenious combination of molecular biology and conventional affinity chromatography that is referred to as *systematic evolution of ligands by exponential enrichment*, or SELEX for short. In such an experiment, the ligand of interest is bound to a solid phase within a chromatography column. A library of random RNA sequences is incubated with the immobilized ligand, whereafter RNAs that remained unbound are washed away. Ligand-bound RNAs are eluted and amplified through RT-PCR and in vitro transcription, and the process repeated using more stringent conditions for binding and washing. PCR conditions can be chosen so as to encourage mutations, which may further enhance the affinity of the selected aptamers.

³Some viruses, such as rotaviruses, possess a double-stranded genome to begin with. However, most RNA viruses have single-stranded genomes; one wonders how much RNA interference may have done to favor the emergence of single-strandedness during evolution.

Chapter 14

Drug delivery

14.1 Introduction

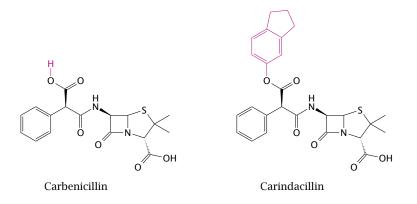
As we have seen in chapter 3, some drugs cannot get to their targets easily or even at all. In some cases, the best strategy is to use another drug altogether; however, in other cases, we can take measures to improve the uptake and retention of drugs, or to target them to specific sites of action in order to improve effectiveness and reduce toxicity. Such auxiliary techniques are collectively referred to as drug delivery. This chapter discusses a number of instructive examples.

14.2 Modifying drug molecules to improve intestinal uptake and distribution

We have already considered this topic earlier (see slide 3.4.5). Here, we will expand on it with several more examples of techniques and applications.

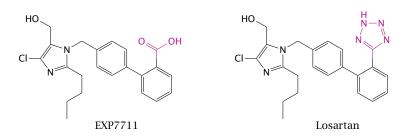
14.2.1 Protecting drugs from gastric acid through prodrug formation

Carindacillin is an orally applicable prodrug of carbenicillin, a penicillin derivative with good activity against various Gram-negative bacteria (but one which is no longer commonly used). Esterification with indanol (highlighted) improves acid resistance. In addition, it renders the prodrug more hydrophobic than the parent compound and promotes its uptake from the intestine.



14.2.2 Optimizing a drug structure for bilayer permeation

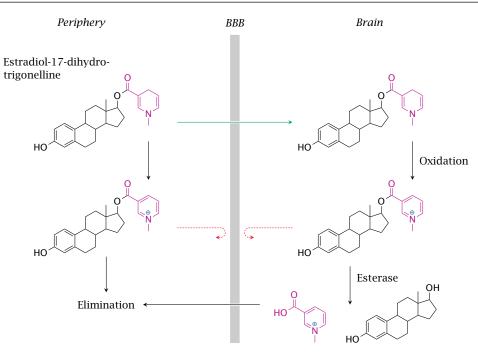
EXP7711 is an angiotensin receptor blocker with antihypertensive activity. The acidic carboxylate group in EXP7711 inhibits intestinal absorption, yet the negative charge in this position is necessary for tight receptor binding in order to achieve a high IC_{50} .



Like carboxylate, tetrazole is acidic and has a planar structure, but at the same time it is ten times more lipophilic. Replacement of the carboxylate in EXP7711 with tetrazole gives losartan, which retains inhibitory activity on the angiotensin receptor (see slide 1.2.5) yet is absorbed from the intestine much more readily.

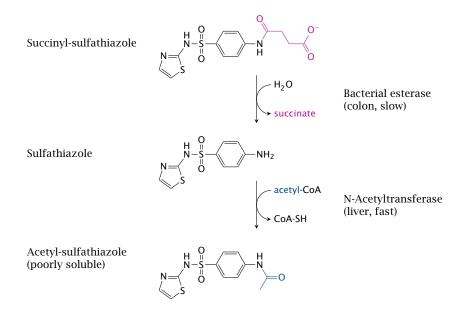
14.2.3 Trapping an estradiol prodrug inside the brain

The 1,4-dihydro-N-methylnicotinic acid (trigonelline) ester of estradiol can enter the brain by diffusion. Oxidation of the trigonelline moiety occurs both in the periphery and the brain. The charge introduced by oxidation will accelerate elimination in the periphery but inhibit elimination from the brain, since the charged molecule is no longer able to cross the BBB. Estradiol is slowly released from the trapped prodrug by esterases.



14.2.4 Succinylsulfathiazole, a prodrug designed for *reduced* absorption

Sulfathiazole is a sulfonamide like sulfamidochrysoidine (see slide 1.3.3) and shares its mode of action. Sulfathiazole is metabolized through N-acetylation. The acetylated compound is poorly soluble, which may cause it to precipitate inside the kidney tubules; this may be lethal.



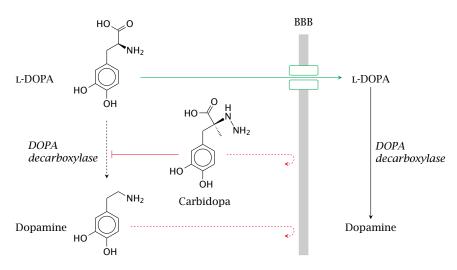
Succinylsulfathiazole is less toxic because it is ionized and thus not taken up efficiently. Once the drug reaches the large intestine, it is slowly hydrolyzed by bacterial

esterases. The low rate of hydrolysis means that its uptake will also be slow and protracted; systemic concentrations of sulfathiazole and the acetylated metabolite will remain low throughout, and renal complications will be avoided.

While succinylsulfathiazole illustrates an approach to mitigating drug toxicity that is interesting in principle, in practice we prefer to use other sulfonamides that are not encumbered with this kind of potential problems in the first place.

14.3 More on dopamine and its prodrugs

14.3.1 Inhibition of DOPA decarboxylase in the periphery improves L-DOPA uptake into the brain

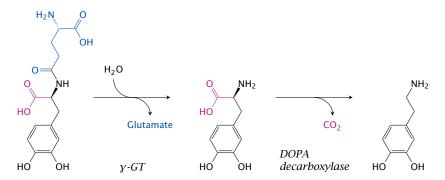


As discussed earlier (slide 3.5.5), dopamine does not cross the blood brain barrier (BBB); therefore, in the treatment of Parkinson's disease, its metabolic precursor L-DOPA is used as a prodrug. L-DOPA crosses the BBB by active transport and is converted to dopamine in the brain.

DOPA decarboxylase, the enzyme that converts L-DOPA to dopamine, is active both in the periphery and the CNS. Accordingly, if L-DOPA is applied alone, most of it undergoes decarboxylation in the periphery. The dopamine produced there cannot enter the brain but may instead be converted to norepinephrine or epinephrine and so cause side effects on blood pressure and glucose metabolism.

The premature conversion of L-DOPA can be prevented by combining L-DOPA with carbidopa or benserazide, which inhibit DOPA decarboxylase in the periphery. Like dopamine, these compounds do not cross the BBB and thus do not interfere with dopamine metabolism in the brain. Combination with such inhibitors permits the dosage of L-DOPA to be reduced by ~75% and very significantly reduces side effects.

14.3.2 Gludopa, a prodrug for selective release of dopamine in the kidneys



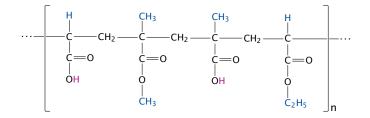
Dopamine receptors in the kidney vasculature improve kidney perfusion and function, and their selective stimulation may be clinically desirable. A prodrug to target dopamine preferentially to the kidneys is gludopa (γ -glutamyl-L-DOPA).

The kidneys (as well as the liver) contain L- γ -glutamyl-transpeptidase (γ GT) at high levels of activity. This enzyme transfers γ -glutamyl residues between different substrates, but can also simply hydrolyze them. Hydrolytic removal of γ -glutamate from gludopa in the kidneys releases L-DOPA, which is again converted to dopamine and stimulates the local dopamine receptors.

14.4 Using carriers and vehicles to improve drug delivery

Aside from modifying the drug molecules themselves, the distribution or organ targeting of drugs can sometimes be improved by suitably packaging them before application.

14.4.1 Protecting drugs from gastric acid through acrylate copolymer coating



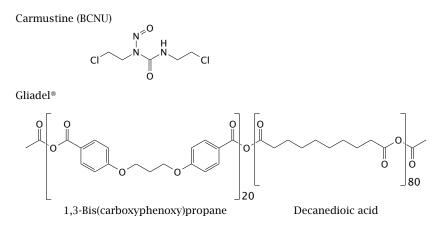
Some drug molecules contain acid-labile bonds such as carboxylic acid esters. These drugs must be protected from destruction by the fairly concentrated hydrochloric acid in the stomach. One way to achieve this protection is through micro-encapsulation in polymers that remain stable in an acidic milieu but dissolve once they reach the slightly alkaline environment within the small intestine. This technique is commonly referred to as *enteric coating*.

Widely used for this purpose are acrylate copolymers, often referred to using their trade name (Eudragit[®]). The acidic pH inside the stomach keeps the polymers' carboxylic acid groups protonated, and the whole particles insoluble. Upon entering

the small intestine, the carboxylic acid groups dissociate, and the ester bonds are cleaved. The polymers disperse and dissolve, releasing the cargo drugs enclosed within.

The slide shows one specific example, namely Eudragit[®] L 100-55; the substituents highlighted in blue differ in other commercial grades of this product (see [135] for details).

14.4.2 Site-selective delivery of BCNU



Bis-chloroethyl-nitrosurea is a bifunctional alkylating agent that is used in the treatment of cancer (see slide 12.6.18). The drug can cross the BBB in principle, but it is highly reactive and therefore consumed within a short range of penetration into the brain tissue, mostly before reaching the tumor. Therefore, systemic application for treating brain tumors is problematic.

For localized, sustained delivery in treating brain tumors, the drug is embedded in a polyanhydride polymer, and the product is shaped into wafers that go by the trade name Gliadel[®]. These are placed into the tumor resection cavity during tumor surgery.

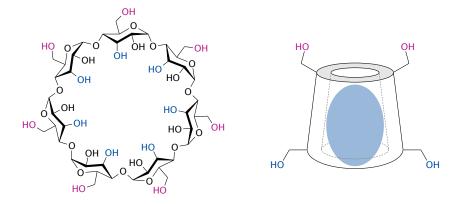
The ratio of carboxyphenoxypropane and sebacic acid building blocks in the polymer can be varied to control the rate of its hydrolysis and accordingly of drug release from the polymer matrix.

14.4.3 Cyclodextrins: Structure and use in drug delivery

Cyclodextrins are naturally occurring, circular glucose polymers. In aqueous solution, they adopt a conformation that encloses a central cavity, which can accommodate a hydrophobic guest molecule. The variants α -, β - and γ -cyclodextrin contain 6, 7 and 8 molecules of glucose, respectively. β -Cyclodextrin is most commonly used, but the others are of use with smaller or larger drug molecules.

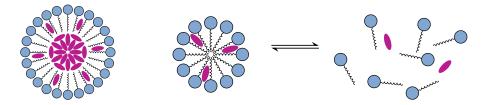
Cyclodextrins are small and polar enough to be renally eliminated, and their degradation will produce simply glucose; therefore, they are well tolerated and suitable for both oral and parenteral application. Piroxicam (see slide 9.4.2) and

prostaglandin E₁ are examples of drug molecules that are applied as cyclodextrin complexes.



Left: Structure of β -cyclodextrin. Right: Schematic of a cyclodextrin molecule encasing a hydrophobic drug molecule.

14.4.4 Solubilization of hydrophobic drugs with surfactants



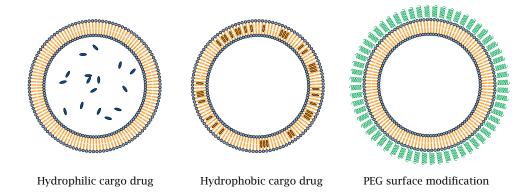
Surfactants, or detergents, are amphiphilic molecules that in aqueous solution aggregate into micelles. These micelles can accommodate hydrophobic drug molecules as aggregates (left) or as individual molecules (center).

Most surfactants rapidly equilibrate between the micellar and the monomeric state. When the surfactant is diluted to below its critical micellar concentration, the micelles will rapidly dissipate, and the drugs contained within will be released (right). The antifungal drug amphotericin B (see slide 11.8.1) used to be delivered in this manner, using deoxycholic acid as the detergent. However, as we will see below, liposomal delivery of this drug reduces toxicity and as now preferred.

14.4.5 Liposomes as drug delivery vehicles

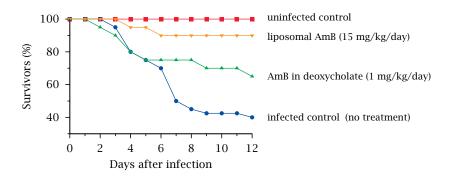
Liposomes are vesicles consisting of natural or synthetic phospholipids. They can vary widely in size, but the ones used for drug delivery are most often between 50 and 200 nm in diameter. Hydrophilic cargo drugs will be enclosed in the lumen (left), whereas hydrophobic ones will reside in the bilayer itself (center).

The phospholipids that constitute the liposomes have negligible water solubility; therefore, unlike detergent micelles, they will not simply disperse after intravenous application and dilution. However, their lifetime may still be cut short due to encounters with proteins of the complement system in the blood plasma, which may disrupt their membranes and mediate their removal from the circulation by phagocytosis. Disruption by complement proteins can be prevented through surface modification with polyethyleneglycol (PEG), which will greatly increase their stability and dwell time in the circulation (right).



14.4.6 Liposomal vs. deoxycholate-solubilized amphotericin B in a mouse infection model

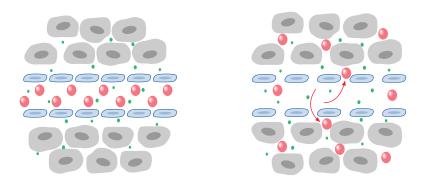
Amphotericin B is an antifungal antibiotic that binds to ergosterol in the fungal cell membrane, which it then renders permeable toward cations (see slide 11.8.1). The molecule is fairly hydrophobic and therefore must be solubilized in some way. One widely used preparation contains the surfactant deoxycholate, but liposomal preparations are now frequently used because of their greater therapeutic index.



The lower toxicity of liposomal amphotericin B (AmB) is illustrated here in a mouse model of a fungal infection. In preliminary experiments, the highest tolerable dosage for both the deoxycholate-based and the liposomal AmB preparations was established; a fifteen-fold higher dosage was tolerated when the drug was bound to liposomes. When these respective dosages were applied to mice that had been infected with the rather nasty fungal pathogen *Rhizopus oryzae*, the liposomal preparation came out ahead. Figure prepared from original data in [136].

Another interesting study explored the use of the related polyene antibiotic nystatin as a liposomal preparation. This drug had traditionally been considered too toxic for systemic application; however, when delivered using liposomes, the drug was tolerated by mice and had equal or better activity than amphotericin B against experimental infection with *Aspergillus fumigatus* [137].

14.4.7 Liposomes and the Enhanced Permeability and Retention (EPR) effect



Liposomes can also be used for targeted—or preferential—delivery of cytotoxic drugs to cancer tissue. In cancer, as well as in inflammation, the capillary barriers become leaky, and plasma proteins that are normally confined to the circulation can disperse in the interstitial space.¹

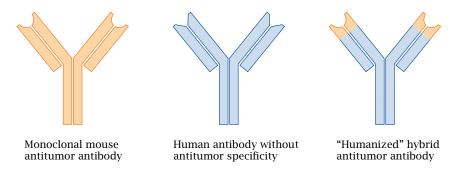
The leakiness also applies to liposomes up to approximately 200 nm in diameter, which will therefore preferentially accumulate in cancers and cancer metastases. PEG-modified liposomes loaded with the anticancer drug doxorubicin (see slide 12.6.19) have been FDA-approved for tumor therapy [138].

14.5 Antibodies as drug-targeting devices

Antibodies have exquisite affinity and selectivity for their targets (antigens) and can be used to direct drugs to specific tissues and cell. In some cases, the antibodies themselves can act as drugs; an example is "trastuzumab", the monoclonal antibody directed against the tumor-related growth factor receptor Her2/neu. Alternatively, the antibodies may be labeled with a radioactive isotope or coupled with a cytotoxic drug such as calicheamicin (see below), or they may be attached to the surface of drug-containing liposomes.

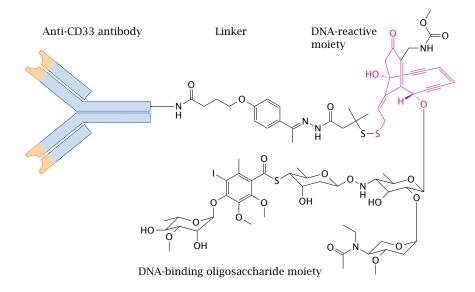
¹The physiological significance of this is that the most common cause of inflammation is infection, and many plasma proteins, such as antibodies and complement proteins, are involved in fighting infections.

14.5.1 Humanized antibodies



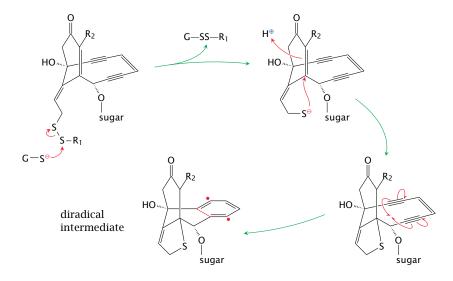
Monoclonal antibodies are conventionally derived from mice and thus immunogenic to humans, which means that patients receiving repeated applications may develop anti-antibodies that bind and neutralize them. The immunogenicity of mouse antibodies can be substantially reduced by creating hybrid molecules that retain only the variable, antigen-specific moieties from the mouse antibodies but derive the invariant remainder of the molecule from a human antibody.

14.5.2 A calicheamicin-antibody conjugate



This conjugate—poetically named "gemtuzumab ozogamicin"—contains a humanized IgG monoclonal antibody (hP 67.6), which binds to CD33, a surface antigen found on leukemic cells in acute myeloic leukemia (AML). A derivative of the cytotoxic antibiotic calicheamicin γ is linked to the antibody by (4-acetylphenoxy)butanoic acid. Two sugar residues within the oligosaccharide moiety of the antibiotic are responsible for binding of the minor groove of DNA. Several calicheamicin molecules may be coupled to one antibody. The covalent reaction of calicheamicin with DNA is explained below. The conjugate was FDA-approved for a brief period of time but has since been withdrawn due to unacceptable toxicity.

14.5.3 Activation of calicheamicins



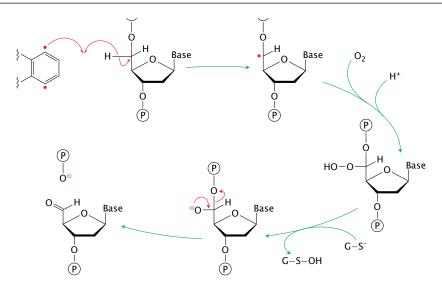
Calicheamicins are endiyne antibiotics, that is, they possess one alkene bond between two conjugated alkyne bonds. They also have a trisulfide group, which in the antibody conjugate discussed above is replaced by a disulfide.

Once the drug enters the cell, an intracellular nucleophile, usually glutathione, attacks the di- or trisulfide. Subsequent conjugate addition of the enone and Bergman rearrangement generate a reactive diradical intermediate, which reacts with DNA and induces DNA double strand breaks (see next slide).

14.5.4 DNA cleavage by activated calicheamicins

This slide shows the reaction of one of the two radicals in activated calicheamicin with a single DNA strand. Abstraction of a hydrogen atom from deoxyribose produces a carbon radical, which reacts with molecular oxygen to form a peroxide. The latter is reduced by glutathione to an alkoxide, which then cleaves the adjacent phosphoester bond.

Hydrogen abstraction and bond cleavage can occur at other positions of the deoxyribose moiety and result in other outcomes, including abasic sites. A common outcome seems to be cleavage of one DNA strand, combined with an abasic site on the other [139].

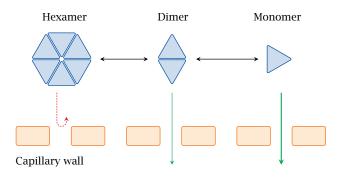


14.6 Delivery of insulin

Most body cells require insulin to facilitate uptake of glucose from the blood, and while the plasma insulin level rises and falls in keeping with blood glucose levels, the hormone must be continuously present in the blood in order to maintain metabolism in good working order. In diabetes mellitus, insulin is lacking or insufficient and must be substituted (see slide 10.5).

The plasma half-life of insulin molecules, once secreted, is only about ~15 minutes; the hormone is depleted both by peptidases and by receptor binding. Therefore, plasma levels have to be sustained by continued secretion from the pancreas. In insulin replacement treatment of diabetics, various techniques are used in order to achieve a sustained level of blood plasma insulin. A modern development are insulin pumps that deliver insulin continuously; still more commonly used, however, are techniques that achieve a protracted uptake of subcutaneously injected insulin into the circulation.

14.6.1 Aggregation of insulin delays its uptake into the circulation

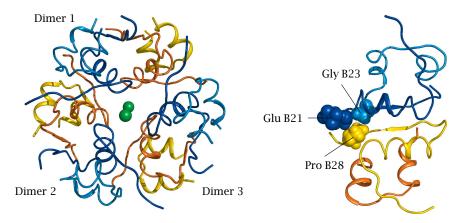


If insulin is injected intravenously, it will rapidly be inactivated through cleavage by peptidases. To achieve a more sustained activity, insulin is injected subcutaneously, that is, into the interstitial space.

Insulin exists in different states of association. Zinc ions and high insulin concentrations promote the formation of hexamers, which are too large to permeate across the capillary walls. The aggregation equilibrium can be shifted either way by point mutations or chemical modifications, which is exploited in the preparation of both fast- and slow-acting insulins.

Slow-acting insulins are optimized for subcutaneous application 2–3 times per day; each of the injected aliquots must then release insulin slowly over a time period of many hours. In contrast, fast-acting insulins are optimized for more frequent injections, or for use with insulin pumps, which infuse insulin continuously and at a rate that is adjusted according to frequent measurements of blood glucose.

14.6.2 Structure of the insulin hexamer



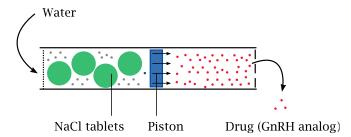
The insulin hexamer is composed of three dimers and stabilized by two centrally placed zinc ions. In each dimer, proline 28 of one B chain interacts with glutamate 21 and glycine 23 of the opposite B chain. In the recombinant fast-acting variant insulin lispro, proline B28 has been replaced with lysine, which destabilizes the interaction of the two monomers.

In another fast-acting variant, insulin aspart, proline 28 is replaced with aspartic acid, which also perturbs this interaction and additionally creates electrostatic repulsion with the opposite glutamate.

14.7 The Viadur[®] implant

The Viadur[®] implant is a device for the long-term delivery of leuprolide acetate, an analogue of gonadotropin-releasing hormone (GnRH), the hypothalamic hormone

that controls the release of FSH and LH from the anterior pituitary and, through these, of androgens.²



The device can sustain the release of the cargo peptide for many months; this is achieved with an "osmotic engine". A piston inside a cylinder separates two compartments, one of which contains salt, and the other the peptide. Both ends of the cylinder are capped with membranes.

The piston moves when water enters the compartment with the salt and dissolves it. This water uptake is osmotically driven, and its rate is controlled by the permeability of the membrane delimiting this compartment. Driven by the water, the piston compresses the opposite reservoir, from which it thus expels the peptide.

²Leuprolide acetate is a GnRH receptor agonist. However, GnRH receptor stimulation must occur in a pulsatile fashion in order to trigger release of LH and FSH from the pituitary. If present continuously, an agonist causes receptor desensitization and decreased LH and FSH secretion; this is the principle of long term leuprolide acetate application.

Chapter 15

Drug discovery

15.1 Stages of drug discovery

- Target molecule
 - selection
 - validation
- Candidate compounds
 - acquisition
 - screening

Biochemical and physiological discoveries suggest macromolecules that might make worthwhile targets for treating diseases. This is an important step toward new drugs, but only the first one. The subsequent steps that must be taken are the subject of this chapter.

Drug development is labor-intensive and costly, so it is important to experimentally ascertain whether a drug with the intended activity on our chosen target would indeed produce the expected physiological outcome. This stage of development is referred to as *target validation*.

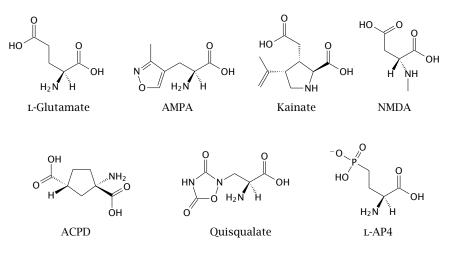
The choice of the experimental model for target validation depends on the intended physiological effect. Some effects, for example cytotoxicity, can readily be observed in cell cultures; on the other hand, for physiological functions such as pain or blood pressure, animal experiments will be required. If the drug under consideration is supposed to be an inhibitor, its effect can often be modeled by genetic knockout of the target or by RNA interference (see slide 13.3.6). On the other hand, if the intention is to activate the target, it should in principle often be possible to model this by overexpression (cf. slide 2.6.1); however, this approach does not seem to be commonly used in practice.

If the target has been experimentally validated, candidate compounds must be obtained and screened. Major pharmaceutical companies have large libraries (collections) of compounds that may be screened over and over against novel, unrelated targets. Alternatively, custom libraries may be synthesized and can be structurally focused around some existing agonist or antagonist of the target molecule.

The screening of large numbers of compounds—a recent study on a specific type of voltage-gated K⁺ channels tested as many as 650,000 compounds [140]— requires simple and robust high-throughput assays. If detailed structural information on the macromolecular target is available, it may be preferable to perform the initial screening *in silico*, that is, to use molecular docking software to examine the binding of real or virtual compounds to the target.

Once lead compounds have been identified that act on the target in the intended manner, they must also be tested with other macromolecules that are related to the target; the entirety of such data for a given compound is its *receptor profile*. Lead compounds must also be tested against *antitargets*, that is, macromolecules that are frequently involved in drug toxicity. One important antitarget are cardiac hERG potassium channels, whose inhibition by drugs gives rise to cardiac arrhythmias (see section 6.6).

15.1.1 Chemical structures of subtype-selective glutamate receptor ligands



Acronyms: ACPD, 1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; L-AP4, L-2-amino-4-phosphonobutanoic acid; NMDA, *N*-methyl-D-aspartate.

While a drug development project will usually start with a hypothesis that is based on the state of the art in biochemistry and physiology, one must nevertheless be ready to reevaluate this hypothesis as further experimental information accrues along the way. A common case in point is the discovery of pharmacologically distinct subtypes of receptors that at the outset were assumed to be homogeneous.

The molecules shown in this slide were prepared as candidate agonists for glutamate receptors in the central nervous system. As it turned out, these synthetic

ligands did not all act the same way, but instead activated different subsets of glutamate receptors, the existence of some of which was only thereby discovered.

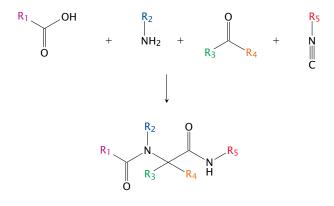
AMPA and quisqualate activate the same subtype of ionotropic glutamate receptors. Quisqualate also activates group I metabotropic glutamate receptors, which belong to the GPCR type. NMDA and kainate, respectively, activate two other ionotropic receptor subtypes. ACPD is an agonist of group I and II metabotropic glutamate receptors. L-AP4 is an agonist of group III metabotropic receptors (see section 6.11).

15.2 Sources of candidate compounds

- Synthetic libraries
- Natural compounds
- · Semisynthesis
- Gene technology

Candidate compounds can be obtained from various sources. Natural compounds or synthetic strategies can be used alone or in combination; the latter approach is termed *semisynthesis*. Gene technology is a promising development but not yet a major source for compounds in practice.

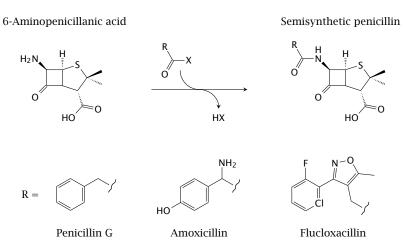
15.2.1 Combinatorial synthesis: the Ugi reaction



One important strategy for developing large compound libraries is referred to as *combinatorial synthesis*. In this approach, modular reactants that share similar reactive groups but differ in their side chains are combined in many permutations, all of which are processed in parallel, often with the help of robotic systems.

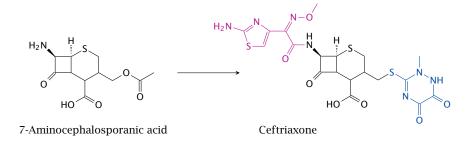
An example of a combinatorial synthetic strategy is the Ugi reaction. Here, a carboxylic acid, an amine, a ketone or aldehyde and an isonitrile condense to form a bis-amide. Through combinatorial variation of the functional groups R_1-R_5 , many different compounds can be prepared in parallel. An application of the Ugi reaction for the combinatorial synthesis of analogues of the antibiotic muraymycin is described in [141].

15.2.2 Semisynthesis of penicillins



The penicillins are good examples for the semisynthetic variation of natural compounds. The starting compound, 6-aminopenicillanic acid, is obtained from fermenter cultures of the fungus *Penicillium notatum*, and is chemically acylated at its unique amino group.

15.2.3 Semisynthesis of cephalosporins



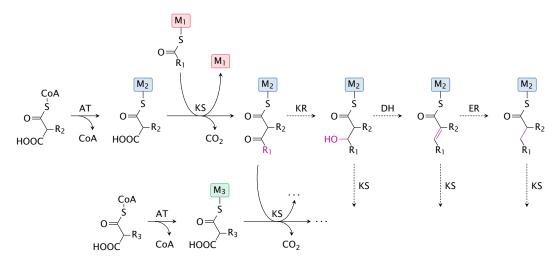
Ceftriaxone (shown here) and other cephalosporins (cf. slide 11.4.10) are derived through semisynthesis from 7-aminocephalosporanic acid. Here, two variable substituents are attached to the natural compound nucleus. More complex, multi-step chemistries are regularly utilized on a wide variety of natural products.

15.2.4 Biosynthesis of polyketides

Polyketides are natural compounds that are produced, in considerable variety, by *Streptomyces* species and other microorganisms. The biosynthesis of polyketides is chemically similar to that of fatty acids, with the following differences:

1. In contrast to fatty acids, which are assembled through stepwise attachment of a single precursor (malonyl-CoA), polyketides are contain various organic acids with different side chains. Each polyketide has its specific composition and sequence of side chains.

2. While the β -carbon of each successively added C₂ subunit is always completely reduced in fatty acid synthesis, in polyketides the reduction of β -carbons may be complete or partial.



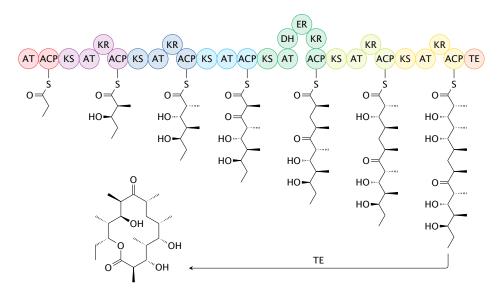
Enzyme activities: AT, acyltransferase; KS, ketosynthase; KR, ketoreductase; DH, dehydratase; ER, enoylreductase; M_1 - M_3 , enzyme modules for successively incorporating substituents with side chains R_1 - R_3 .

Polyketide synthases are large molecules, structured like assembly lines, in which a given nascent polyketide molecule travels from one module to the next. Each module selects and attaches a specific substrate acid, and then optionally reduces it; the level of reduction is determined by the presence or absence of ketoreductase, dehydratase, and enoylreductase activities in the module in question.

15.2.5 Structure of native 6-deoxyerythronolide B synthase

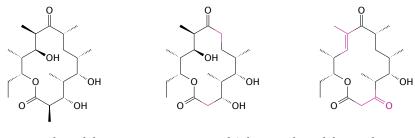
An example polyketide is 6-deoxyerythronolide B, which is a precursor of the antibiotic erythromycin. The synthase that makes this compound has six separate modules, five of which contain one or more reducing enzyme activities, which will cause partial or complete reduction of the corresponding β -carbon atom. The thioesterase (TE) that follows the last module cyclizes and releases the product.

The sequence of the various modules and the presence of reductase domains within these modules completely determine the structure of the product. Recombinant DNA technology can be used to replace individual domains and thereby create novel polyketide molecules.



15.2.6 Two compounds produced by engineered variants of 6-deoxyerythronolide B synthase

The two engineered variants of 6-deoxyerythronolide B were obtained by replacing several modules of the cognate synthase with corresponding ones from the rapamycin polyketide synthase. Data from [142].



6-Deoxyerythronolide B

Engineered 6-deoxyerythronolide B analogues

While this approach is intriguing and has the potential to provide novel compounds with intricate structures in a very scalable manner, the scope of structural variation that can be realized in this way will often be more limited than with organic synthesis or semisynthesis.

15.3 Compound screening

The best approach for testing the effect of candidate compounds varies with the nature of the target molecule. Enzymes are typically active in solution and can therefore be tested as purified molecules, which minimizes interference by other macromolecules. In contrast, the functions of GPCRs and ion channels can only be observed in cells or model membranes, and cell-based assays are most commonly

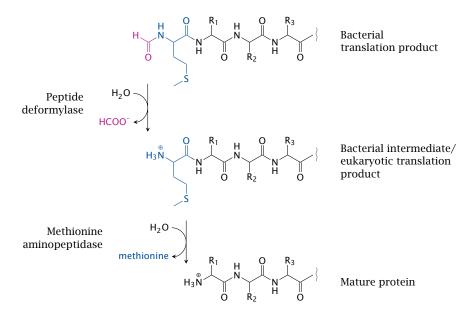
used. With both *in vitro* and cell-based assays, absorbance- or fluorescence-based readouts are preferable, since they lend themselves to automation and high throughputs.

Experimental approach	Typical applications
Activity assays on purified target proteins	Enzymes
Cell-based activity assays	GPCRs, ion channels
Computational screening	Targets with available 3D structure
Phenotypic screening	Cytotoxic activity

Computational screening is best used with target molecules for which detailed structural information is available. While it sometimes is involved and time-consuming, one great advantage of this approach is that the candidate compounds don't have to physically exist before the screen; actual synthetic effort can be focused on those candidates that score well *in silico*.

Phenotypic screening is different from the other entries here, since it is performed *without* any specific target molecule; instead, the desired physiological outcome, such as the killing of bacterial or cancer cells, is measured directly. Many anticancer and antimicrobial drugs have emerged from phenotypic screens.

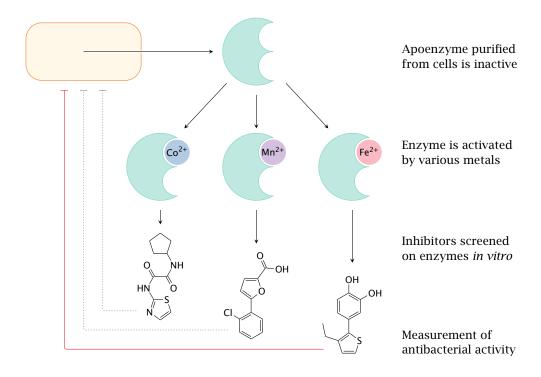
15.3.1 Peptide deformylase and Met aminopeptidase



Peptide deformylase and methionine aminopeptidase can serve as examples as drug targets amenable to *in vitro* screening. Both enzymes function in the posttranslational processing of protein N-termini. In bacterial proteins, but not in eukary-

otic cells, the N-terminal methionine residue is N-formylated. The formyl group is removed by peptide deformylase, which accordingly is a candidate target for antibacterial drugs.

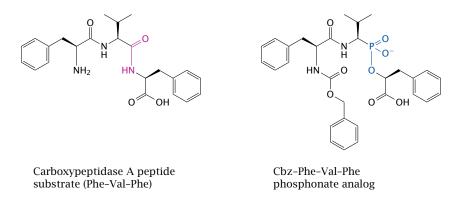
In both bacterial and eukaryotic cells, the N-terminal methionine residue is cleaved by methionine aminopeptidase; many proteins will not become functional when this methionine residue is not removed. Accordingly, inhibitors of the enzyme may be of use in both antibacterial and anticancer chemotherapy. The search for antibacterial inhibitors of methionine aminopeptidase brought some surprises that illustrate potential pitfalls of the seemingly straightforward approach of screening with purified enzymes *in vitro*.



15.3.2 In vitro screening of Met aminopeptidase inhibitors

Purification of the protein from *E. coli* yields the apoenzyme, which is inactive. It can be activated by saturating it with several different metal ions, which suggested the exact nature of the metal to not be critical. Accordingly, several differently metallated preparations of the enzyme were used, somewhat arbitrarily, to screen enzyme inhibitors.

As it turned out, however, the different screens produced different inhibitors, and only the ones screened with Fe⁺⁺-saturated enzyme turned out to have antibacterial activity. This finding suggests that only this form of the enzyme is relevant *in vivo* [143]. This example shows the importance of fully characterizing the target's behavior *in vivo*.

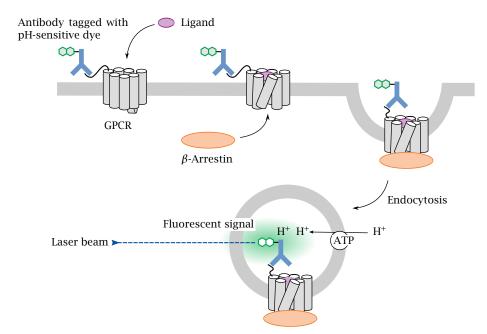


15.3.3 A non-covalent yet irreversible enzyme inhibitor

When screening drugs that bind to a specific target, how high should one aim in terms of affinity? The higher the affinity, the lower the required dosage of a drug; practical considerations indicate that a nanomolar affinity is desirable. While this may seem ambitious, even higher affinity can be achieved on occasion, as is the case with the inhibitor shown in this slide.

The target molecule in question is carboxypeptidase A. The structure on the left shows a tripeptide substrate of this enzyme; the one on the right is that of the inhibitor Cbz-Phe-Val-Phe phosphonate. The time constant for dissociation of this femtomolar inhibitor exceeds the lifetime of the enzyme, which means that inhibition is practically irreversible [144].

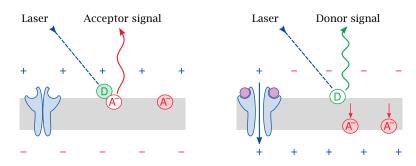
15.3.4 A generic assay for GPCR activation



GPCR function depends on several other cellular components, and therefore can only be screened with cell-based assays. Many screens rely on some specific signal that is triggered downstream of the activation of the specfic GPCR in question, or at least of a specific G protein. In contrast, the CypHer 5 assay illustrated here responds to endocytosis of the receptor itself, which in turn is triggered by receptor activation. The assay is therefore very general.

In preparation for the assay, the GPCR of interest is overexpressed in cells, with its N-terminus fused to an antigenic peptide that is then tagged with a cognate antibody carrying a pH-sensitive fluorescent dye. When a ligand activates the receptor, the receptor is phosphorylated and binds β -arrestin, which in turn triggers receptor endocytosis. When the endosome containing the labeled receptor is acidified by cellular proton pumps, the fluorescent dye is protonated, making it brightly fluorescent.

15.3.5 A cell-based fluorescence assay of membrane depolarization



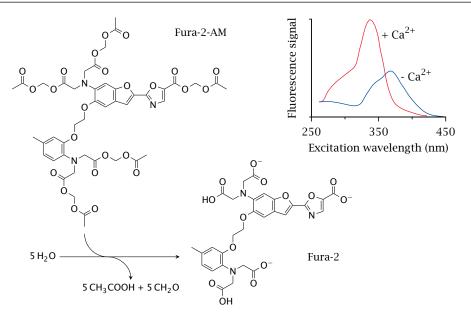
A technically challenging kind of drug target to screen in a high-throughput format are ion channels. The cell-based assay shown here uses fluorescence to detect membrane depolarization downstream of channel activation.

In preparation for the assay, the cell membranes are loaded with two lipophilic fluorescent dyes, which are spectrally matched for fluorescence resonance energy transfer (FRET) but differ in net charge and in transverse mobility.

At the resting membrane potential, the two dyes stay close to one another, and excitation of the donor dye causes FRET and long-wave emission from the acceptor dye. If, however, a ligand activates the channel and reverses the membrane potential, the dyes become separated; the donor will then emit directly within its own, characteristic wavelength range.

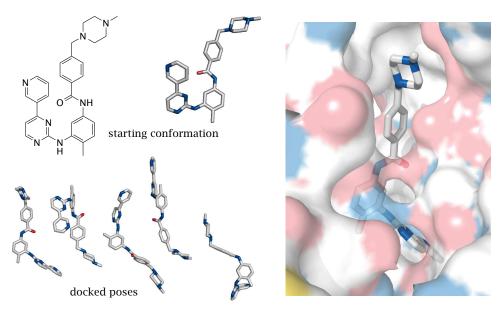
15.3.6 A fluorescence assay of Ca⁺⁺ influx

The cytosolic Ca⁺⁺ level is normally low, but it can rise due to influx of extracellular Ca⁺⁺ through Ca_V channels or to efflux from the ER downstream of GPCRs that couple to phospholipase C (see slide 5.3.2). To measure cytosolic calcium signals downstream of a drug target, cells can be loaded with a resorption ester of the fluorescent dye Fura 2. Cleavage by cellular esterases exposes the dye's two Ca⁺⁺ chelating functional groups.



When intracellular calcium increases, it binds to the dye, which leads to a strongly increased fluorescence signal upon excitation at 340 nm.

15.3.7 An in silico docking experiment



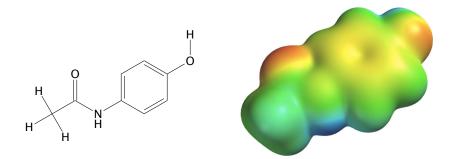
The model experiment illustrated here was carried out using the freely available program AutoDock Vina [145] and followed a detailed tutorial that can be found on that program's website.

The protein tyrosine kinase inhibitor imatinib was docked into the active site of the *abl* oncoprotein tyrosine kinase (cf. slide 12.5.4). The starting conformation of imatinib was given to the docking program, which was allowed to rotate the

single bonds of the drug molecule in order to optimize its fit to the enzyme (whose structure was treated as invariant).

Below the starting conformation, several docked *poses*, that is, energetically favorable ligand orientations, are shown. The most energetically favorable pose is almost indistinguishable from the one obtained by crystallography.

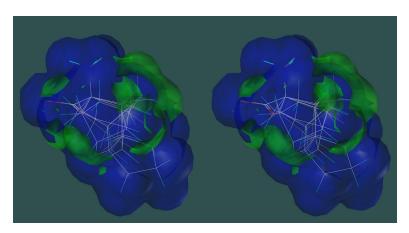
15.3.8 Electrostatic potential mapped onto the electronic density for acetaminophen



Docking programs such as AutoDock Vina that are designed for initial screens of large numbers of compounds treat molecules in a substantially simplified manner; among other things, all molecular charges are assigned to discrete point coordinates. For more accurate prediction of binding parameters, it is necessary to consider the electronic structure at higher resolution.

This slide illustrates the distribution of electrostatic charges across the van der Waals contour of the drug acetaminophen. The calculation was performed using the commercial software Spartan 08 (Wavefunction Inc, USA). The calculation was performed at the RHF/6-31G \star level of theory.

15.3.9 Hypothetical pharmacophore for inhibitors of ATP:L-Methionine S-Adenosyltransferase



While actual crystal structures of drug targets are highly desirable—and nowadays often available—for computational screening, another viable approach is based on the pharmacophore concept. In this approach, molecular features of the ligand that enhance or detract from the desirable effect on the target are determined by experimentally characterizing and comparing a set of reference compounds. The structures of the compounds are aligned in space, and each molecular feature that enhances or detracts from the desired effect is expressed by its magnitude, direction, and nature of binding force. The pharmacophore is the consensus set of favorable features. Once the pharmacophore has been established, further compounds can be compared to it by computational analysis.

The slide illustrates a pharmacophore for the enzyme ATP:L-Methionine Sadenosyltransferase, which regenerates the methyl group donor S-adenosylmethionine. In the construction of this pharmacophore, six different derivatives of 1aminocyclopentane-1-carboxylic acid were tested for their ability to displace methionine from the enzyme. White lines indicate the bonds of the aligned compounds. The volume that envelopes all active compounds is outlined in blue; the volumes of substituents that prevent binding extend out and are indicated in green. Based on data in [146].

Sybyl8.1 (Tripos, Inc, USA) software was used to produce this cross-eyed stereo figure.

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- Slide 6.10.1: Panels A-D from Nigel Unwin, with permission. Panels E-F by me.
- Slide 8.5.3: Adapted from a figure in [68].

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Bibliography

- [1] J.-M. Beaulieu et al.: A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell* 132 (2008), 125–136.
- [2] E. Stone: An Account of the Success of the Bark of the Willow in the Cure of Agues. *Phil. Trans. Roy. Soc. Lond.* 53 (1763), 195–200.
- [3] A. Fleming: On the antibacterial action of cultures of a Penicillium, with special reference to their use in isolation of B. influenzae. *Br. J. Exp. Pathol.* 10 (1929), 226–236.
- [4] J. Brunel: Antibiosis from Pasteur to Fleming. J. Hist. Med. Allied Sci. 6 (1951), 287– 301. PMID: 14873929.
- [5] P. Ehrlich: Partial cell functions. 1908. URL: http://nobelprize.org/nobel_ prizes/medicine/laureates/1908/ehrlich-lecture.html.
- [6] R. K. Bickerton: The response of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. *J. Pharmacol. Exp. Ther.* 142 (1963), 99–9110. PMID: 14076528.
- [7] D. A. Shapiro et al.: Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* 28 (2003), 1400–1411. PMID: 12784105.
- [8] K. A. Berg et al.: Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* 54 (1998), 94–9104. PMID: 9658194.
- [9] S. Strickland and J. N. Loeb: Obligatory separation of hormone binding and biological response curves in systems dependent upon secondary mediators of hormone action. *Proc. Natl. Acad. Sci. U. S. A.* 78 (1981), 1366–1370. PMID: 6262790.
- [10] L. Brown et al.: Spare receptors for β-adrenoceptor-mediated positive inotropic effects of catecholamines in the human heart. *J. Cardiovasc. Pharmacol.* 19 (1992), 222–232. PMID: 1376792.
- [11] B. G. Katzung: Basic and Clinical Pharmacology. Vol. -. McGraw-Hill, 2001, n/a.
- [12] S. G. Aller et al.: Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323 (2009), 1718–1722. PMID: 19325113.
- [13] R. J. P. Dawson and K. P. Locher: Structure of the multidrug ABC transporter Sav1866 from *Staphylococcus aureus* in complex with AMP-PNP. *FEBS Lett.* 581 (2007), 935– 938. PMID: 17303126.

- S. Bihorel et al.: Modulation of the brain distribution of imatinib and its metabolites in mice by valspodar, zosuquidar and elacridar. *Pharm. Res.* 24 (2007), 1720–1728.
 PMID: 17380257.
- [15] U. Tröger et al.: Tetraparesis associated with colchicine is probably due to inhibition by verapamil of the P-glycoprotein efflux pump in the blood-brain barrier. *BMJ* 331 (2005), 613. PMID: **16148013**.
- [16] D. R. Wada et al.: Computer simulation of the effects of alterations in blood flows and body composition on thiopental pharmacokinetics in humans. *Anesthesiology* 87 (1997), 884–899. PMID: 9357892.
- [17] M. L. Frenia et al.: Multiple-dose activated charcoal compared to urinary alkalinization for the enhancement of phenobarbital elimination. *J. Toxicol. Clin. Toxicol.* 34 (1996), 169–175. PMID: 8618250.
- [18] E. Zuccato et al.: Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environ. Health* 4 (2005), 14. PMID: 16083497.
- [19] L. L. Brunton et al.: *Goodman and Gilman's The pharmacological basis of therapeutics*. McGraw Hill, 2005.
- [20] K. He et al.: Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem. Res. Toxicol.* 11 (1998), 252–259. PMID: 9548795.
- [21] M. Ekroos and T. Sjögren: Structural basis for ligand promiscuity in cytochrome P450 3A4. *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006), 13682–13687. PMID: 16954191.
- [22] J. E. Chrencik et al.: Structural disorder in the complex of human pregnane X receptor and the macrolide antibiotic rifampicin. *Mol. Endocrinol.* 19 (2005), 1125–1134. PMID: 15705662.
- [23] J. Bauer et al.: A structural gap in Dpo4 supports mutagenic bypass of a major benzo[a]pyrene dG adduct in DNA through template misalignment. *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007), 14905–14910. PMID: 17848527.
- [24] M. R. McGill and H. Jaeschke: Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *Pharm. Res.* 30 (2013), 2174–87. PMID: 23462933.
- [25] H. W. Goedde et al.: Studies on pharmacogenetics. I. The enzymatic acetylation of isonicotinic acid hydrazide (INH). *Biochem. Pharmacol.* 13 (1964), 1671–1675. PMID: 14248395.
- [26] J. R. Rashid et al.: Acetylation status using hydralazine in African hypertensives at Kenyatta National Hospital. *East Afr. Med. J.* 69 (1992), 406–408. PMID: 1396201.
- [27] K. Golka et al.: The enhanced bladder cancer susceptibility of NAT2 slow acetylators towards aromatic amines: a review considering ethnic differences. *Toxicol. Lett.* 128 (2002), 229–241. PMID: 11869833.
- [28] S. S. Mattano et al.: Purification and biochemical characterization of hepatic arylamine N-acetyltransferase from rapid and slow acetylator mice: identity with arylhydroxamic acid N,O-acyltransferase and N-hydroxyarylamine O-acetyltransferase. *Mol. Pharmacol.* 35 (1989), 599–609. PMID: 2725469.
- [29] Y. Fu et al.: Crystal structure of quinone reductase 2 in complex with cancer prodrug CB1954. *Biochem. Biophys. Res. Commun.* 336 (2005), 332–338. PMID: 16129418.

- [30] P. Hein et al.: Gs activation is time-limiting in initiating receptor-mediated signaling. *J. Biol. Chem.* 281 (2006), 33345–33351. PMID: 16963443.
- [31] U. Gether et al.: Different binding epitopes on the NK1 receptor for substance P and non-peptide antagonist. *Nature* 362 (1993), 345–348. PMID: 7681152.
- [32] W. L. Hubbell et al.: Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. *Adv. Protein Chem.* 63 (2003), 243–290. PMID: 12629973.
- [33] S. Arnis et al.: A conserved carboxylic acid group mediates light-dependent proton uptake and signaling by rhodopsin. *J. Biol. Chem.* 269 (1994), 23879–23881. PMID: 7929034.
- [34] S. Chackalamannil et al.: Discovery of potent orally active thrombin receptor (protease activated receptor 1) antagonists as novel antithrombotic agents. *J. Med. Chem.* 48 (2005), 5884–5887. PMID: 16161991.
- [35] T. Galvez et al.: Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA_B receptor function. *EMBO J.* 20 (2001), 2152–2159. PMID: 11331581.
- [36] A. W. S. Ma et al.: Recovery of oligomers and cooperativity when monomers of the M₂ muscarinic cholinergic receptor are reconstituted into phospholipid vesicles. *Biochemistry* 46 (2007), 7907–7927. PMID: 17552496.
- [37] W. G. Rajeswaran et al.: Design, synthesis, and biological characterization of bivalent 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazole derivatives as selective muscarinic agonists. *J. Med. Chem.* 44 (2001), 4563–4576. PMID: 11741475.
- [38] M. Waldhoer et al.: A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers. *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005), 9050– 9055. PMID: 15932946.
- [39] A. Dalpiaz et al.: A novel conjugated agent between dopamine and an A2A adenosine receptor antagonist as a potential anti-Parkinson multitarget approach. *Mol. Pharm.* 9 (2012), 591–604. PMID: 22292533.
- [40] S. J. Matkovich et al.: Cardiac-specific ablation of G-protein receptor kinase 2 redefines its roles in heart development and β-adrenergic signaling. *Circ. Res.* 99 (2006), 996–991003. PMID: 17008600.
- [41] S. K. Shenoy et al.: β -Arrestin-dependent, G protein-independent ERK1/2 activation by the β_2 adrenergic receptor. *J. Biol. Chem.* 281 (2006), 1261–1273. PMID: 16280323.
- [42] G. A. Piersol: Human Anatomy. Vol. -. Lippincott, 1908, n/a.
- [43] A. J. dos Reis: Sigmund Freud (1856-1939) and Karl Köller (1857-1944) and the discovery of local anesthesia. *Rev. Bras. Anestesiol.* 59 (2009), 244–257. PMID: 19488537.
- [44] G. W. Zamponi and R. J. French: Dissecting lidocaine action: diethylamide and phenol mimic separate modes of lidocaine block of sodium channels from heart and skeletal muscle. *Biophys. J.* 65 (1993), 2335–2347. PMID: 8312473.
- [45] M. Roy et al.: HERG, a primary human ventricular target of the nonsedating antihistamine terfenadine. *Circulation* 94 (1996), 817–823. PMID: 8772706.
- [46] L. Huang et al.: NNC 55-0396 [(1S,2S)-2-(2-(N-[(3-benzimidazol-2-yl)propyl]-N-methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropane-

carboxylate dihydrochloride]: a new selective inhibitor of T-type calcium channels. *J. Pharmacol. Exp. Ther.* 309 (2004), 193–199. PMID: 14718587.

- [47] R. SoRelle: Withdrawal of Posicor from market. *Circulation* 98 (1998), 831–832. PMID: 9738634.
- [48] H. E. Hannon and W. D. Atchison: Omega-conotoxins as experimental tools and therapeutics in pain management. *Mar. Drugs* 11 (2013), 680–699. PMID: 23470283.
- [49] R. N. Willette et al.: Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J. Pharmacol. Exp. Ther.* 326 (2008), 443–452. PMID: 18499744.
- [50] J. C. Skou: The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* 23 (1957), 394–401. PMID: 13412736.
- [51] R. L. Post et al.: Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. *J. Biol. Chem.* 247 (1972), 6530–6540. PMID: 4263199.
- [52] B. Forbush: Rapid release of 42K and 86Rb from an occluded state of the Na,Kpump in the presence of ATP or ADP. *J. Biol. Chem.* 262 (1987), 11104–11115. PMID: 2440883.
- [53] D. C. Chiara et al.: Identification of amino acids in the nicotinic acetylcholine receptor agonist binding site and ion channel photolabeled by 4-[(3-trifluoromethyl)-3H-diazirin-3-yl]benzoylcholine, a novel photoaffinity antagonist. *Biochemistry* 42 (2003), 271–283. PMID: 12525154.
- [54] B. Katz and S. Thesleff: A study of the desensitization produced by acetylcholine at the motor end-plate. *J. Physiol.* 138 (1957), 63–80. PMID: 13463799.
- [55] A. C. Foster and J. A. Kemp: Glutamate- and GABA-based CNS therapeutics. *Curr. Opin. Pharmacol.* 6 (2006), 7–17. PMID: 16377242.
- [56] H. Stähle: A historical perspective: development of clonidine. *Best Pract. Res. Clin. Anaesthesiol.* 14 (2000), 237 –246. DOI: https://doi.org/10.1053/bean.2000. 0079.
- [57] D. Sulzer et al.: Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.* 75 (2005), 406–433. PMID: 15955613.
- [58] R. L. Robinson: Stimulation of the release of catecholamines from isolated adrenal glands by tyramine. *J. Pharmacol. Exp. Ther.* 151 (1966), 55–58. PMID: 5902179.
- [59] B.-O. Nilsson et al.: G protein-coupled oestrogen receptor 1 (GPER1/GPR30): a new player in cardiovascular and metabolic oestrogenic signalling. *Br. J. Pharmacol.* 163 (2011), 1131–1139. PMID: 21250980.
- [60] D. S. Reddy: Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Prog. Brain Res.* 186 (2010), 113–137. PMID: 21094889.
- [61] B. M. Vayssière et al.: Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol. Endocrinol.* 11 (1997), 1245–1255. PMID: 9259316.
- [62] M. Janka-Junttila et al.: The glucocorticoid RU24858 does not distinguish between transrepression and transactivation in primary human eosinophils. *J. Inflamm. Lond.* 3 (2006), 10. PMID: 16834783.

- [63] E. M. King et al.: Glucocorticoid repression of inflammatory gene expression shows differential responsiveness by transactivation- and transrepression-dependent mechanisms. *PLoS One* 8 (2013), e53936. PMID: 23349769.
- [64] R. Clapauch et al.: Total estradiol, rather than testosterone levels, predicts osteoporosis in aging men. *Arq. Bras. Endocrinol. Metabol.* 53 (2009), 1020–1025. PMID: 20126856.
- [65] C. E. Sanz-Rodríguez et al.: Bisphosphonates as inhibitors of Trypanosoma cruzi hexokinase: kinetic and metabolic studies. *J. Biol. Chem.* 282 (2007), 12377–12387. PMID: 17329254.
- [66] J. L. Pérez-Castrillón et al.: Effects of Atorvastatin on vitamin D levels in patients with acute ischemic heart disease. *Am. J. Cardiol.* 99 (2007), 903–905. PMID: 17398180.
- [67] T.-K. Lin et al.: Long-term effect of statins on the risk of new-onset osteoporosis: A nationwide population-based cohort study. *PLoS One* 13 (2018), e0196713. PMID: 29723231.
- [68] S. R. Jaffrey et al.: Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat. Cell Biol.* 3 (2001), 193–197. PMID: 11175752.
- [69] Z. Chen et al.: Identification of the enzymatic mechanism of nitroglycerin bioactivation. *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002), 8306–8311. PMID: 12048254.
- [70] Z. Chen et al.: An essential role for mitochondrial aldehyde dehydrogenase in nitroglycerin bioactivation. *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005), 12159–12164.
- [71] T. Ishibashi et al.: Effects of cyanamide, an aldehyde dehydrogenase type 2 inhibitor, on glyceryl trinitrate- and isosorbide dinitrate-induced vasodilation in rabbit excised aorta and in anesthetized whole animal. *Biol. Pharm. Bull.* 36 (2013), 556–563. PMID: 23546291.
- [72] R. Neubauer et al.: Aldehyde dehydrogenase-independent bioactivation of nitroglycerin in porcine and bovine blood vessels. *Biochem. Pharmacol.* 93 (2015), 440–448.
- [73] Y. Minamiyama et al.: Cytochrome P450 is responsible for nitric oxide generation from NO-aspirin and other organic nitrates. *Drug Metab. Pharmacokinet.* 22 (2007), 15-19. PMID: 17329906.
- [74] A. Schrammel et al.: Activation of soluble guanylyl cyclase by the nitrovasodilator 3morpholinosydnonimine involves formation of S-nitrosoglutathione. *Mol. Pharmacol.* 54 (1998), 207–212. PMID: 9658207.
- [75] S. Cuzzocrea et al.: Beneficial effects of GW274150, a novel, potent and selective inhibitor of iNOS activity, in a rodent model of collagen-induced arthritis. *Eur. J. Pharmacol.* 453 (2002), 119–129. PMID: 12393067.
- [76] R. Langenbach et al.: Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83 (1995), 483-492. PMID: 8521478.
- [77] S. G. Morham et al.: Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 83 (1995), 473–482. PMID: 8521477.
- [78] S. W. Rowlinson et al.: A novel mechanism of cyclooxygenase-2 inhibition involving interactions with Ser-530 and Tyr-385. *J. Biol. Chem.* 278 (2003), 45763–45769. PMID: 12925531.

- [79] C. N. Serhan: Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. *Prostaglandins Leukot. Essent. Fatty Acids* 73 (2005), 141–162. PMID: 16005201.
- [80] Y. H. Loo: Serotonin deficiency in experimental hyperphenylalaninemia. *J. Neurochem.* 23 (1974), 139–147. PMID: 4546911.
- [81] L. I. Woolf: The heterozygote advantage in phenylketonuria. *Am. J. Hum. Genet.* 38 (1986), 773–775. PMID: 3717163.
- [82] D. B. Kohn and H. B. Gaspar: How We Manage Adenosine Deaminase-Deficient Severe Combined Immune Deficiency (ADA SCID). *J. Clin. Immunol.* - (2017), n/a.
- [83] M. L. Joachims et al.: Inhibition of deoxynucleoside kinases in human thymocytes prevents dATP accumulation and induction of apoptosis. *Nucleosides Nucleotides Nucleic Acids* 27 (2008), 816–820. PMID: 18600545.
- [84] W. Löffler et al.: Influence of dietary purines on pool size, turnover, and excretion of uric acid during balance conditions. Isotope studies using ¹⁵N-uric acid. *Res. Exp. Med. Berl* 181 (1982), 113–123. PMID: 6294765.
- [85] D. L. Mehlhaff and D. S. Stein: Gout secondary to ritonavir and didanosine. *AIDS* 10 (1996), 1744. PMID: 8970701.
- [86] H. K. Choi et al.: Alcohol intake and risk of incident gout in men: a prospective study. *Lancet* 363 (2004), 1277–1281. PMID: 15094272.
- [87] M. Kishibe et al.: Chronic tophaceous gout secondary to self-induced vomiting in anorexia nervosa. *J. Dermatol.* 37 (2010), 578–580. PMID: 20536680.
- [88] P. U. Heuckenkamp and N. Zöllner: Fructose-induced hyperuricaemia. Lancet 1 (1971), 808–809. PMID: 4101315.
- [89] R. D. Oberhaensli et al.: Study of hereditary fructose intolerance by use of ³¹P magnetic resonance spectroscopy. *Lancet* 2 (1987), 931–934. PMID: 2889861.
- [90] H. K. Choi and G. Curhan: Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ* 336 (2008), 309–312. PMID: 18244959.
- [91] J. A. Barranger and E. O'Rourke: Lessons learned from the development of enzyme therapy for Gaucher disease. *J. Inherit. Metab. Dis.* 24 Suppl 2 (2001), 89–96. PMID: 11758684.
- [92] J. R. Colca et al.: Identification of a mitochondrial target of thiazolidinedione insulin sensitizers (mTOT) -relationship to newly identified mitochondrial pyruvate carrier proteins. *PLoS One* 8 (2013), e61551. PMID: 23690925.
- [93] A. S. Divakaruni et al.: Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier. *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013), 5422–5427.
 PMID: 23513224.
- [94] R. A. Miller et al.: Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature* 494 (2013), 256–260. PMID: 23292513.
- [95] M. Palmer: Efflux of cytoplasmically acting antibiotics from gram-negative bacteria: periplasmic substrate capture by multicomponent efflux pumps inferred from their cooperative action with single-component transporters. *J. Bacteriol.* 185 (2003), 5287–5289. PMID: 12923103.

- [96] J. L. Marquardt et al.: Kinetics, stoichiometry, and identification of the reactive thiolate in the inactivation of UDP-GlcNAc enolpyruvoyl transferase by the antibiotic fosfomycin. *Biochemistry* 33 (1994), 10646–10651. PMID: 8075065.
- [97] D. C. Goff: D-cycloserine: an evolving role in learning and neuroplasticity in schizophrenia. *Schizophr. Bull.* 38 (2012), 936–941. PMID: 22368237.
- [98] J. W. Johnson et al.: Cyclobutanone analogues of β-lactams revisited: insights into conformational requirements for inhibition of serine- and metallo-β-lactamases. J. Am. Chem. Soc. 132 (2010), 2558–2560. PMID: 20141132.
- [99] D. Sulton et al.: Clavulanic acid inactivation of SHV-1 and the inhibitor-resistant S130G SHV-1 β -lactamase. Insights into the mechanism of inhibition. *J. Biol. Chem.* 280 (2005), 35528–35536. PMID: 15987690.
- [100] J. Xie et al.: A redesigned vancomycin engineered for dual D-Ala-D-ala And D-Ala-D-Lac binding exhibits potent antimicrobial activity against vancomycin-resistant bacteria. J. Am. Chem. Soc. 133 (2011), 13946–13949. PMID: 21823662.
- [101] G. G. Zhanel et al.: New lipoglycopeptides: a comparative review of dalbavancin, oritavancin and telavancin. *Drugs* 70 (2010), 859–86. PMID: 20426497.
- [102] A. C. Palmer and R. Kishony: Opposing effects of target overexpression reveal drug mechanisms. *Nat. Commun.* 5 (2014), 4296. PMID: 24980690.
- [103] J. K. Muraih et al.: Oligomerization of daptomycin on membranes. *Biochim. Biophys. Acta* 1808 (2011), 1154–1160. РМІД: 21223947.
- [104] T. Zhang et al.: Mutual inhibition through hybrid oligomer formation of daptomycin and the semisynthetic lipopeptide antibiotic CB-182,462. *Biochim. Biophys. Acta* 1828 (2013), 302–308. PMID: 23084999.
- [105] F. Grein et al.: Ca²⁺-Daptomycin targets cell wall biosynthesis by forming a tripartite complex with undecaprenyl-coupled intermediates and membrane lipids. *Nat. Commun.* 11 (2020), 1455. PMID: 32193379.
- [106] T. Zhang et al.: Daptomycin forms cation- and size-selective pores in model membranes. *Biochim. Biophys. Acta* 1838 (2014), 2425–2430. PMID: 24857935.
- T. Teerlink et al.: The action of pimaricin, etruscomycin and amphotericin B on liposomes with varying sterol content. *Biochim. Biophys. Acta* 599 (1980), 484-492.
 PMID: 6967735.
- [108] I. Walev and S. Bhakdi: Possible reason for preferential damage to renal tubular epithelial cells evoked by amphotericin B. *Antimicrob. Agents Chemother.* 40 (1996), 1116–1120. PMID: 8723450.
- [109] A.-C. Uhlemann et al.: A single amino acid residue can determine the sensitivity of SERCAs to artemisinins. *Nat. Struct. Mol. Biol.* 12 (2005), 628–629. PMID: 15937493.
- [110] L. Cui et al.: Lack of association of the S769N mutation in *Plasmodium falciparum* SERCA (PfATP6) with resistance to artemisinins. *Antimicrob. Agents Chemother.* 56 (2012), 2546–52. PMID: 22354307.
- [111] J. R. Luque-Ortega and L. Rivas: Miltefosine (hexadecylphosphocholine) inhibits cytochrome c oxidase in *Leishmania donovani* promastigotes. *Antimicrob. Agents Chemother.* 51 (2007), 1327–1332. PMID: 17283192.
- [112] A. N. Hoang et al.: The neutral lipid composition present in the digestive vacuole of Plasmodium falciparum concentrates heme and mediates β -hematin formation

with an unusually low activation energy. *Biochemistry* 49 (2010), 10107–10116. PMID: 20979358.

- [113] S. Kapishnikov et al.: Oriented nucleation of hemozoin at the digestive vacuole membrane in Plasmodium falciparum. *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012), 11188-11193. PMID: 22745164.
- [114] J. Gildenhuys et al.: The Single Crystal X-ray Structure of β-Hematin DMSO Solvate Grown in the Presence of Chloroquine, a β-Hematin Growth-Rate Inhibitor. J. Am. Chem. Soc. 135 (2013), 1037–1047. PMID: 23253048.
- [115] M. Müller: The hydrogenosome. *J. Gen. Microbiol.* 139 (1993), 2879–2889. РМІD: 8126416.
- [116] D. I. Edwards: Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs. *Br. J. Vener. Dis.* 56 (1980), 285–290. PMID: 7000306.
- [117] A. H. Fairlamb et al.: Trypanothione is the primary target for arsenical drugs against African trypanosomes. *Proc. Natl. Acad. Sci. U. S. A.* 86 (1989), 2607–2611. PMID: 2704738.
- [118] R. Mukhopadhyay et al.: Trypanothione overproduction and resistance to antimonials and arsenicals in *Leishmania. Proc. Natl. Acad. Sci. U. S. A.* 93 (1996), 10383–10387.
 PMID: 8816809.
- [119] J. J. Dwyer et al.: Design of helical, oligomeric HIV-1 fusion inhibitor peptides with potent activity against enfuvirtide-resistant virus. *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007), 12772–12777. PMID: 17640899.
- [120] N. Issaeva et al.: Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nat. Med.* 10 (2004), 1321–1328. PMID: 15558054.
- [121] K. Janssen et al.: Apaf-1 and caspase-9 deficiency prevents apoptosis in a Baxcontrolled pathway and promotes clonogenic survival during paclitaxel treatment. *Blood* 110 (2007), 3662–3672. PMID: 17652622.
- P. A. Svingen et al.: Effects of the bcr/abl kinase inhibitors AG957 and NSC 680410 on chronic myelogenous leukemia cells in vitro. *Clin. Cancer Res.* 6 (2000), 237–249.
 PMID: 10656455.
- [123] M. L. Ostrowski et al.: Malignant chondroblastoma presenting as a recurrent pelvic tumor with DNA aneuploidy and p53 mutation as supportive evidence of malignancy. *Skeletal Radiol.* 28 (1999), 644–650. PMID: 10591928.
- [124] D. Giudici et al.: 6-Methylenandrosta-1,4-diene-3,17-dione (FCE 24304): a new irreversible aromatase inhibitor. *J. Steroid Biochem.* 30 (1988), 391–394. PMID: 3386266.
- [125] R. Nasr et al.: Eradication of acute promyelocytic leukemia-initiating cells through PML-RARA degradation. *Nat. Med.* 14 (2008), 1333–1342. PMID: **19029980**.
- [126] C. M. Galmarini et al.: In vivo mechanisms of resistance to cytarabine in acute myeloid leukaemia. *Br. J. Haematol.* 117 (2002), 860–868. PMID: 12060121.
- [127] S. D. Cline and N. Osheroff: Cytosine arabinoside lesions are position-specific topoisomerase II poisons and stimulate DNA cleavage mediated by the human type II enzymes. *J. Biol. Chem.* 274 (1999), 29740–29743. PMID: 10514448.
- [128] S. Frankland-Searby and S. R. Bhaumik: The 26S proteasome complex: an attractive target for cancer therapy. *Biochim. Biophys. Acta* 1825 (2012), 64–76. PMID: 22037302.

- [129] D. T. Terrano et al.: Cyclin-dependent kinase 1-mediated Bcl-xL/Bcl-2 phosphorylation acts as a functional link coupling mitotic arrest and apoptosis. *Mol. Cell. Biol.* 30 (2010), 640–656. PMID: 19917720.
- [130] D. Fourmy et al.: Structure of the A site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* 274 (1996), 1367–1371. PMID: 8910275.
- [131] A. Krebs et al.: Targeting the HIV trans-activation responsive region-approaches towards RNA-binding drugs. *Chembiochem* 4 (2003), 972–978. PMID: 14523913.
- [132] E. M. Welch et al.: PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 447 (2007), 87–91. PMID: 17450125.
- [133] T. Goldmann et al.: A comparative evaluation of NB30, NB54 and PTC124 in translational read-through efficacy for treatment of an USH1C nonsense mutation. *EMBO Mol. Med.* 4 (2012), 1186–1199. PMID: 23027640.
- [134] E. Kerem et al.: Effectiveness of PTC124 treatment of cystic fibrosis caused by non-sense mutations: a prospective phase II trial. *Lancet* 372 (2008), 719–727. PMID: 18722008.
- [135] S. Thakral et al.: Eudragit: a technology evaluation. *Expert Opin. Drug Deliv.* 10 (2013), 131–149. PMID: 23102011.
- [136] A. S. Ibrahim et al.: Liposomal amphotericin B, and not amphotericin B deoxycholate, improves survival of diabetic mice infected with *Rhizopus oryzae*. *Antimicrob. Agents Chemother.* 47 (2003), 3343–3344. PMID: 14506054.
- [137] D. W. Denning and P. Warn: Dose range evaluation of liposomal nystatin and comparisons with amphotericin B and amphotericin B lipid complex in temporarily neutropenic mice infected with an isolate of Aspergillus fumigatus with reduced susceptibility to amphotericin B. *Antimicrob. Agents Chemother.* 43 (1999), 2592–2599. PMID: 10543734.
- [138] Y. Barenholz: Doxil[®]—the first FDA-approved nano-drug: lessons learned. J. Control. Release 160 (2012), 117–134. PMID: 22484195.
- [139] P. C. Dedon et al.: Exclusive production of bistranded DNA damage by calicheamicin. *Biochemistry* 32 (1993), 3617–3622. PMID: 8466904.
- K. Liu et al.: High-throughput screening for Kv1.3 channel blockers using an improved FLIPR-based membrane-potential assay. *J. Biomol. Screen.* 15 (2010), 185–195.
 PMID: 20044579.
- [141] T. Tanino et al.: Mechanistic analysis of muraymycin analogues: a guide to the design of MraY inhibitors. *J. Med. Chem.* 54 (2011), 8421–8439. PMID: 22085339.
- [142] R. McDaniel et al.: Multiple genetic modifications of the erythromycin polyketide synthase to produce a library of novel "unnatural" natural products. *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999), 1846–1851. PMID: 10051557.
- [143] D.-Z. Wang: Neurotoxins from marine dinoflagellates: a brief review. *Mar. Drugs* 6 (2008), 349–371. PMID: 18728731.
- [144] A. P. Kaplan and P. A. Bartlett: Synthesis and evaluation of an inhibitor of carboxypeptidase A with a Ki value in the femtomolar range. *Biochemistry* 30 (1991), 8165–8170.
 PMID: 1868091.

- [145] O. Trott and A. J. Olson: AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31 (2010), 455-461. PMID: 19499576.
- [146] J. R. Sufrin et al.: Steric mapping of the L-methionine binding site of ATP:Lmethionine S-adenosyltransferase. *Mol. Pharmacol.* 19 (1981), 307–313. PMID: 7231391.
- [147] J. Mazzone: Copyfraud. N. Y. Univ. Law Rev. 81 (2006), 1026–1098. URL: https: //ssrn.com/abstract=787244.
- [148] R. P. Rubin: A brief history of great discoveries in pharmacology: in celebration of the centennial anniversary of the founding of the American Society of Pharmacology and Experimental Therapeutics. *Pharmacol. Rev.* 59 (2007), 289–359. PMID: 18160700.

Glossary

ABC transporter membrane protein that uses ATP for active transport of out of or, in some cases, into the cell. ABC transporters occur in human and microbial cells and may cause resistance to chemotherapy in both 332*i*

ACTH see adrenocorticotropic hormone

- **action potential** Transient depolarization (inversion) of the normally negative-inside electrical potential across the cytoplasmic membrane, mediated by voltage-gated ion channels. Occurs only on excitable cells 332*i*
- **active metabolite** metabolic conversion product of a drug that retains pharmacological activity, or acquires a novel one
- **adenylate cyclase** membrane-associated enzyme that converts ATP to the second messenger cAMP
- adenylate kinase enzyme that catalyzes the reversible reaction ATP+AMP \leftrightarrow 2 ADP 332*i*
- **adrenal gland** endocrine gland that sits atop the kidney (which means there are two glands). The outer layer, or cortex, produces various steroid hormones; the inner layer, or medulla, produces epinephrine (adrenaline) and norepinephrine (noradrenaline) 332*i*
- **adrenergic** literally: using adrenaline. The term is applied both to synapses that release adrenaline (epinephrine) or noradrenaline (norepinephrine) and to receptors that are activated by these transmitters. The terms 'dopaminergic', 'cholinergic' and so forth are used analogously 332*i*
- **adrenergic receptor** G protein-coupled receptor activated by adrenaline (epinephrine) or noradrenaline (norepinephrine) 332*i*
- adrenocorticotropic hormone peptide hormone secreted by the hypophyseal gland. Activates proliferation and hormone production in the cortex of the adrenal glands 332*i*agonist drug that activates its receptor 332*i*
- **agonist-specific coupling** response of a receptor to an agonist that leads to preferential activation of one of its downstream signaling cascades over the others
- **albumin** most abundant plasma protein. Produced in the liver; contributes prominently to the osmotic activity of blood plasma and to the protein binding of drugs
- **alkaloid** secondary (that is, non-essential) metabolite of plant or microorganism, usually rich in nitrogen, often poisonous 332*i*
- **alkylating anticancer drugs** drugs that covalently react with DNA to introduce alkyl moieties into it. The term is sometimes also loosely applied to drugs that cause other types of DNA modification. Active on both dividing and resting cancer cells 332*i*
- **allergy** Reaction of the specific immune system against drugs or other allergens such as pollen, foodstuffs or microbial macromolecules leading to formation of allergen-specific antibodies or lymphocytes and clinical symptoms upon repeated allergen exposure
- **allosteric** action mode of a drug that binds to a receptor outside of the regular ligand's binding site. The receptor can bind both the drug and the native ligand 332i

- α -motoneuron a nerve cell that resides in the brain stem or spinal cord and controls, via its long axon, a group of skeletal muscle cells
- $5-\alpha$ -reductase enzyme that reduces testosterone to the more potent androgen dihydrotestosterone. Inhibitors are used therapeutically in prostate cancer
- aminoglycosides class of antibiotics that inhibit bacterial protein synthesis 332*i*
- analgesic pain killer, pain-killing 332*i*
- **androgens** steroid hormones that induce development and sustain function of male sexual organs and body attributes. Testosterone and dihydrotestosterone are the most prominent androgens; they are produced in the Leydig cells of the testicles 332*i*
- **angina pectoris** intermittent of hypoxia and pain in the heart, usually caused by a combination of atherosclerotic constrictions and vasospasms. Classical indication for nitroglycerin treatment 332*i*
- antagonist drug that inhibits its receptor 332*i*
- **antidepressant** drug that counteracts the symptoms of depression, such as dark mood and lack of energy. Typically used in depressive episodes of bipolar disease and in monopolar depression 332*i*
- antimetabolite inhibitor of an enzyme that functions in a metabolic pathway
- **antimicrobial resistance** primary or secondary (acquired) insensitivity of pathogenic microbes to antimicrobial chemotherapy 332*i*
- **antisense oligonucleotide** a stretch of single-stranded DNA or RNA, typically 15–25 nucleotides in length, that is complementary to some cellular or viral RNA molecule, and selectively interferes with its function through base-pairing 332*i*
- **antitarget** a macromolecule that is frequently involved in drug toxicity and therefore must be specifically examined for susceptibility to novel compounds during drug development 332*i*
- anucleate not possessing a cell nucleus
- **aorta** main artery of the systemic circulation. Emerges from the left heart; initially points upward but then bends around to run downward along the spine 332i
- **apoptosis** programmed cell death. Occurs during embryonic development, in pruning autoreactive lymphocyte clones, and other physiological processes; can be triggered by radiation and other causes of DNA damage 332*i*
- **aptamer** DNA or RNA molecule that has been selected for affinity to a target molecule, which usually is not a nuceic acid 332*i*
- **aromatase** Enzyme of the cytochrome P450 family that introduces an aromatic ring into various androgenic steroids and thereby converts them to estrogens. Inhibitors are used therapeutically in gynecological tumors 332i
- **L-aromatic amino acid transporter** transports aromatic amino acids—phenylalanine, tyrosine, tryptophan, but also the prodrug L-DOPA—across cell membranes, including those at the blood brain barrier 332*i*
- **aromatic hydrocarbon receptor** nuclear receptor that, when bound to aromatic hydrocarbons, induces cytochrome P450 1A1 and other enzymes
- arteriole small artery 332*i*
- **atherosclerosis** inflammatory and degenerative disease of the arteries. Promoted by high cholesterol and blood pressure. Most common underlying cause of stroke and myocardial infarction, which occur when blood clots form on top of atherosclerotic lesions 332*i*

- **autoimmune disease** disease caused by formation of antibodies and/or T-lymphocytes that react with proteins or other macromolecules of the body
- autonomic ganglia see autonomic nervous system
- **autonomic nervous system** functional part of the nervous system that is not under voluntary, conscious control. Mainly concerned with regulation of circulation and interior organ function 333*i*
- **axon** branched structure of nerve cell that conducts action potentials generated in this cell to the synapses it forms with other nerve cells 333*i*
- **basal membrane** thin layer composed of proteins and proteoglycans to which endothelial or epithelial cells adhere. In capillaries and glomerular arterioles, the basal membrane functions as a molecular sieve that restricts the movement of macromolecules
- **benign tumor** disinhibited growth of clonal cells that remains local and confined within a clear anatomical boundary (often a connective tissue capsule). Most benign tumors are not treated with drugs but are surgically removed. May progress to malignant tumor when left untreated 333*i*
- β -arrestin protein that binds to phosphorylated GPCRs. This inactivates the receptors and tags them for endocytosis
- β -lactam antibiotics class of antibiotics that contain a reactive β -lactam ring and covalently inhibit muramyl-transpeptidase. Comprises penicillins, cephalosporins, and carbapenems
- β -lactamase bacterial enzyme that cleaves the β -lactam ring of β -lactam antibiotics and in this way inactivates them
- bile bladder see bile duct
- **bile duct** conduit that drains the bile produced in the liver lobuli toward the small intestine. Also connected to the bile bladder, which concentrates and stores surplus bile
- **biliary** involving or belonging to the bile or bile duct and bile bladder 333*i*
- **blood brain barrier** functional and anatomical barrier that restricts permeation of many small solutes, including drugs, from the circulation into the brain and spinal cord 333*i*
- **blood coagulation** formation of blood clots. Involves activation both of the plasmatic cascade of coagulation factors and of thrombocytes. Activated by contact of blood with matrix proteins or other surfaces different from vascular endothelium, as induced by cuts or other lesions to the blood vessels 333*i*
- **blood plasma** The acellular fluid fraction of the blood, which makes up \sim 55% of the total blood volume. Plasma still contains the proteins for clot formation (coagulation); in serum, these have been removed 333*i*

blood platelets see thrombocytes

bone matrix the acellular component of bone tissue. Composite material containing protein fibrils, mostly collagen, and bone mineral, mostly hydroxyapatite. Produced by osteoblast cells, dissolved by osteoclast cells 333*i*

bone mineral see **bone matrix**

- **calcitonin** peptide hormone produced in the C-cells of the thyroid gland in response to high blood calcium levels. Promotes mineral deposition in the bone matrix 333*i*
- **calcitriol** major form of vitamin D. Precursor can be photochemically synthesized in the skin from 7-dehydrocholesterol. Increases intestinal uptake of calcium and phosphate 333i
- calcium channel ion channel that selectively transports calcium 333*i*

- **calmodulin** small regulatory protein that binds calcium and then associates with multiple intracellular proteins to either activate or inactivate them 333i
- **capillary** tiny blood vessels between arteries and veins. Substrate and gas exchange between circulating blood and tissues occurs across their thin and porous walls 333*i*
- **capsid** protein shell, usually of icosahedral symmetry, that encases the nucleic acids of a viral genome. In naked viruses such as enteroviruses, the capsid forms the outermost layer; in enveloped viruses, it is contained within a lipid membrane 333*i*

carbapenems see beta lactam antibiotics

carcinoma malignant tumor derived from epithelial tissue 333i

cardiac arrhythmia Disturbance of heart rhythm, due to excessive activity, or lacking activity, of the heart's excitation-conduction system. Causes are diverse; often treated with drugs that modulate ion channel function

catecholamine this term comprises dopamine, norepinephrine, and epinephrine 333*i*

cell excitation see action potential

cephalosporins see beta lactam antibiotics

channel block blockade of an ion channel by a drug. Can take the shape of fast block, in wich a drug binds reversibly within the conducting pathway of the channel, or as a slow block, in which a drug binds and stabilizes the inactive channel conformation 333*i*

cholinergic see adrenergic

cholinesterase hydrolase that cleaves acetylcholine; found in cholinergic synapses 333*i* **collecting duct** part of the **nephron**

- **competitive inhibition** inhibition by a drug that reversibly binds to a receptor or enzyme and displaces the physiological ligand or substrate
- **complement system** a system of plasma proteins that participates in immune defense by facilitating phagocytosis or by directly attacking the cell membranes of pathogenic microbes. Activated by antibodies as well as by particles with non-physiological surface properties 333*i*
- **cooperativity** synchronized ligand binding and conformational transition by the multiple subunits of an oligomeric receptor or enzyme 333i
- **coronary artery** artery that supplies the heart itself with blood. There are three major coronary arteries. Occlusion of a coronary artery causes myocardial infarction
- **corticotropin releasing hormone** peptide hormone produced by the hypothalamus that activates the secretion of adrenocorticotropic hormone (ACTH) from the hypophyseal gland 333*i*
- **CRH** see corticotropin releasing hormone

cyan-fluorescent protein see green fluorescent protein

cyclic nucleotide-gated channel see ligand-gated ion channel

- **cyclodextrins** cyclical glucose polymers with 6–8 subunits. Useful for drug solubilization and delivery 333*i*
- **cyclooxygenase** enzyme that converts arachidonic acid to prostaglandin H_2 , a key intermediate in the synthesis of other prostaglandins and of thromboxanes. Important drug target for inhibiting inflammation, pain, and thrombocyte aggregation 333i
- **cys-loop receptor family** homologous family of pentameric ligand-gated ion channels. Contains the GABA_A receptor and the nicotinic acetylcholine receptor as major drug targets 334i

- **cystic fibrosis** genetic disease caused by a homozygous deficiency of a chloride transport protein 334*i*
- **cytochrome P450** class of enzymes that perform a wide spectrum on oxidative reactions on both endogenous metabolites and xenobiotics 334*i*
- **dendrite** branched structure of a nerve cell that forms synapses with upstream nerve cells 334*i*

depolarization see action potential

dextran metabolically inert glucose polymer produced by Leuconostoc bacteria 334i

- **diabetes mellitus** endocrine and metabolic disease caused by lack of insulin activity, either due to lack of hormone, lack of tissue sensitivity to insulin, or excess activity of hormones antagonistic to insulin 334*i*
- diaphorase NADH-dependent redox enzyme that participates in reductive drug metabolism. Also referred to as quinone reductase 334i
- **differentiation** the acquisition of cell type-specific morphological and biochemical traits by cells forming through division of undifferentiated stem cells. Often occurs successively during multiple cell generations 334*i*
- **diffusion potential** electrochemical that forms across membranes if these are selectively permeable toward an ion species for which there is a concentration gradient across the membrane 334*i*

distal tubule part of the nephron

distribution see drug distribution

dopaminergic see adrenergic

- **drug absorption** uptake of a drug from the compartment of application (for example the digestive tract) into the blood plasma 334*i*
- **drug conjugation** Coupling of drug molecules with functional groups derived from cosubstrates. Usually renders the drug less active, more polar, and more suitable for excretion
- **drug distribution** migration of the drug from the blood plasma to the rest of the organism 334i
- **drug elimination** removal of the drug from the body through excretion with the urine or bile, often subsequent to enzymatic modification 334*i*
- EC_{50} the concentration of a drug at which it exhibits 50% of its maximal effect 334i
- **Edman degradation** experimental technique for protein sequence determination through successive removal of single amino acids from the N-terminus 334*i*
- efficacy strength of a drug's functional effect at saturating concentrations 334*i*
- **eicosanoids** class of mediators biosynthetically derived from arachidonic acid. Comprises prostaglandins, leukotrienes, thromboxanes, lipoxins, and endocannabinoids 334*i*
- **electrical synapse** a connection between two neighboring excitable cells, mostly in heart and smooth muscle, that conducts ions and thereby allows action potentials to spread between the cells

elimination see drug elimination

- **endocannabinoids** class of eicosanoids that are involved in synaptic negative feedback regulation 334*i*
- endocrine hormone-secreting (gland) 334i
- **endothelium** innermost cellular layer in blood (and lymph) vessels. In a capillary, the endothelium forms the *only* cellular layer 334i
- endotoxin see lipopolysaccharide

- **enteric coating** encapsulation of a drug with an inert polymer that remains solid at the low pH in the stomach but dissolves in the slightly alkaline milieu of the small intestine 334i
- **entero-hepatic cycling** repeated biliary secretion and intestinal uptake of a drug molecule. Often also involves repeated conjugation in the liver and deconjugation by bacterial enzymes in the large intestine
- **epithelium** cell layer that grows atop a basal membrane. Assumes many different organspecific shapes and functions, such as selective solute transport in intestinal and kidney epithelia, and metabolism and biosynthesis in liver epithelia 334*i*
- **epoxide hydrolase** enzyme that hydrolyzes epoxide groups that may be introduced into xenobiotic molecules by cytochrome P450
- **equilibrium potential** the voltage at which the driving forces associated with a diffusion potential are at equilibrium 334*i*
- **ergosterol** major sterol of fungal and some protozoal cell membranes. Drugs that bind ergosterol or inhibit its synthesis are widely used in antifungal chemotherapy 334i
- **estrogens** steroid hormones that induce development and sustain function of female sexual organs and body attributes. Estradiol and estriol are the most prominent estrogens; they are produced by the ovaries 334*i*

excitable cell see action potential

- excitation-conduction system population of specialized cells in the heart that spontaneously generate action potentials and distribute them throughout the heart muscle. The system's topmost part is the sinoatrial node; it usually sets the heart rhythm 334*i*
- **excitation-contraction coupling** the functional connection in a muscle cell between its excitation by action potentials and its contraction 334*i*
- **excitatory postsynaptic potential** localized, partial and short-lived depolarization of a postsynaptic membrane, caused by a single firing of the presynaptic terminal 334*i*
- **exocrine** (gland) secreting to the exterior. Also includes secretion across mucous membranes, for example into the intestines 334i
- **extracellular signal-regulated kinase** protein kinase that is activated downstream of various types of receptors. ERKs affect transcription and cell proliferation
- **false transmitter** a drug that accumulates in presynaptic transmitter storage vesicles and thereby excludes the true transmitter from storage and subsequent release 334i
- fast block see channel block
- favism see glucose-6-phosphate dehydrogenase
- **firing level** threshold voltage at which a voltage-gated channel will open. Usually below neutral, but differs substantially between different channels 335*i*
- **first pass effect** extent of metabolic inactivation of a drug as it passes through the liver immediately after intestinal uptake. Commonly stated as percentage of the total
- **fluorescence resonance energy transfer** (FRET) nonradiative transfer of excitation energy between two fluorophores. The emission spectrum of the donor fluorophore must overlap the absorption spectrum of the acceptor fluorophore. FRET occurs over distances of no more than a few nanometers, which makes it useful for studying binding and dissociation of proteins 335*i*
- **follicle-stimulating hormone** hypophyseal peptide hormone that stimulates ovary follicle development in women and spermatogenesis (sperm cell formation) in men 335*i*
- FRET see fluorescence resonance energy transfer
- full agonist a drug that achieves maximal activation of its receptor

G protein see G protein-coupled receptor

- **G protein-coupled receptor** receptor protein in the cytoplasmic membrane that binds a ligand on the extracellular side and then activates a heterotrimeric G protein on the intracellular side 335*i*
- **GABA**_A **receptor** ligand-gated chloride channel in the brain that is activated by GABA (*y*-aminobutyric acid). Inhibits neuronal excitation; major drug target
- **genetic knockout** inactivation of a specific gene using recombinant DNA techniques. Widely used in experimental cell biology and for target validation in drug discovery 335*i*
- **glaucoma** eye disease characterized by excessive pressure within the eye; can lead to blindness in extreme cases
- **glia cell** cell in brain tissue that is not a nerve cell. Among the various types of glia cells, astrocytes are the most common ones

glomerulus see nephron

- **glucocorticoids** steroid hormones that affect metabolic regulation and inhibit inflammation. Produced in the adrenal gland; cortisol and cortisone are the most important of these hormones 335*i*
- **glucose-6-phosphate dehydrogenase** first enzyme in the hexose monophosphate shunt. Mutations of this enzyme cause lack of NADPH in erythrocytes and favism
- **glutathione**-*S*-transferase enzyme that couples free glutathione to an electrophilic center on a substrate molecule, most commonly a xenobiotic
- glycinergic see adrenergic
- **Goldman equation** Equation that relates the magnitudes of multiple ion gradients across a membrane to the overall diffusion potential. Generalization of the Nernst equation 335i
- gonadal relating to the gonads, that is, the ovaries and testicles

GPCR shorthand for G protein-coupled receptor

- **GPCR kinase** protein kinase that phosphorylates activated GPCRs and thereby primes them for inactivation by β -arrestin 335*i*
- **Gram-negative** class of bacteria with cell walls that have an outer membrane containing lipopolysaccharide 335*i*
- **Gram-positive** class of bacteria, characterized by a comparatively simple cell wall structure that lacks an outer membrane 335*i*
- **green-fluorescent protein** protein, isolated originally from a jellyfish species, which autocatalytically forms an internal fluorophore that emits visible (green) light. Translational fusion with GFP, or mutant variants thereof, is widely used in experimental cell biology to track proteins of interest
- **growth hormone** hypophyseal peptide hormone that promotes production of growth factors in several tissues. Promotes growth, raises blood glucose 335*i*

half-life see drug elimination

- **hemagglutinin** protein that causes clumping (agglutination) of red blood cells. The hemagglutinins of influenzavirus and related viruses bind neuraminic acid residues on cell surfaces, including those of red blood cells; this causes cell clumping because one virus particle contains multiple copies of the hemagglutinin protein and thus can bind to multiple cells 335*i*
- **hemodynamic shock** acute drop of blood pressure, usually accompanied with counterregulatory rise of the heart rate. Major causes are blood volume loss, vasodilation in septicemia, or acute heart muscle failure in myocardial infarction 335*i*

- **hemolytic anemia** lack of red blood cells due to their premature destruction. In the context of pharmacology, most commonly triggered by drugs in conjunction with glucose-6-phosphate dehydrogenase deficiency (favism)
- **hepatic** concerning or belonging to the liver 335*i*
- Her2/neu growth factor receptor. Excessive activation promotes growth of breast cancer and some other cancers. Therapeutically targeted with inhibitory monoclonal antibodies 335*i*
- **HMG-CoA reductase** key enzyme in the biosynthesis of cholesterol. Converts hydroxymethylglutaryl-CoA (HMG-CoA) to mevalonic acid. Inhibited by "statin" drugs 335*i*
- **humanized antibody** hybrid monoclonal antibody that combines a mouse-derived antigen recognition site with a human antibody scaffold. Less immunogenic in humans than conventional mouse-derived monoclonal antibodies 335i
- **hyperpolarization** deviation of the membrane potential from its normal value to a more strongly negative (inside) value 335*i*
- hypertension pathologically increased arterial blood pressure 335*i*
- **hypertensive crisis** a medical emergency, typically occurring in patients with known chronic hypertension, characterized by spiking yet rapidly changing arterial blood pressure 335i
- **hypophyseal gland** small endocrine gland connected to the hypothalamus that secretes multiple peptide hormones, many of which control other endocrine glands 335*i*
- **hypothalamus** portion of the brain located close to the brain stem. It is anatomically and functionally connected to the hypophyseal gland and controls many activities of the autonomic nervous system 335i
- **inferior vena cava** major vein that collects all venous blood from the entire body below the heart. There is a superior vena cava as well that drains venous blood from the upper part of the body 335i
- **inhibitory postsynaptic potential** localized and short-lived hyperpolarization of a postsynaptic membrane, caused by a single firing of a presynaptic terminal that releases an inhibitory transmitter 335*i*
- **inositoltriphosphate** second messenger released from phosphatidylinositol-bis-phosphate by phospholipase C. Activates a cognate receptor in the endoplasmic reticulum that is a calcium channel 335*i*
- **interstitial space** the entirety of the fluid-filled extracellular space outside of the circulation 335*i*
- intravascular within the blood vessels 335*i*
- **inverse agonist** an inhibitory drug that reduces the activity of its receptor to a level below that of the unbound state 335*i*
- **ion channel** membrane protein that selectively conducts one or several specific ion species 335*i*
- **ionophore** small molecule that reversibly binds specific ions; the bound complex is able to efficiently cross cell membranes 335*i*
- ionotropic receptor synonymous with ligand-gated ion channel
- **irreversible inhibition** inhibition by a drug that binds irreversibly, usually by forming a covalent bond, to a receptor or enzyme and displaces the physiological ligand or substrate
- K_{ATP} channel ATP-regulated potassium channel

- **lead compound** molecule that binds and acts on a given drug target that serves as the starting point for drug development, which usually involves increasing affinity and improving **pharmacokinetics** 336*i*
- **leak channels** ion channels that are continually open. K⁺ leak channels are important in stabilizing the resting potential in human cells 336*i*
- **leukemia** malignancy derived from any of the cell lines of the bone marrow. Grows diffusely in the bone marrow and, in advanced cases, in the liver and spleen. Mostly treated with chemotherapy 336*i*

leukocytes white blood cells. Consist of granulocytes, lymphocytes, and monocytes 336*i*

- leukotrienes class of eicosanoids involved in inflammation and allergy
- **ligand-gated ion channel** ion channel that opens—or, in some cases, closes—in response to the binding of a ligand molecule. The ligand may bind from the extracellular side, as is the case with neurotransmitter receptors, or from the intracellular side, which happens with cyclic nucleotide-gated (CNG) channels 336*i*
- **lipopeptide** a peptide that is modified with a fatty acyl residue. Many lipopeptides are antibiotics 336*i*
- **lipopolysaccharide** key component of the outer membrane of Gram-negative bacteria. Consists of a core oligosacharide carrying 6–7 fatty acyl residues, as well as a typically very long linear polysaccharide chain. Powerful trigger of non-specific immune reactions 336*i*
- **liposomes** artificial membrane vesicles, consisting of a lipid bilayer that encloses an aequeous interior volume 336*i*
- liver lobule anatomical and functional unit of liver tissue

loop of Henle part of the nephron

- **low density lipoprotein** plasma lipoprotein species rich in cholesterol. Forms from very low density lipoprotein (VLDL) through extraction of triacylglycerol by lipoprotein lipase 336*i*
- **luteinizing hormone** hypophyseal peptide hormone that stimulates ovulation and progestin formation in women and androgen formation in men 336*i*
- **lysosome** specialized cellular vesicle carrying hydrolytic enzymes and other molecules that aid in the inactivation and degradation of phagocytosed microbes and particles

macrophage see phagocytosis

- **malaria** infectious disease caused by the unicellular eukaryotic parasite *Plasmodium falciparum* and related species, and transmitted by *Anopheles* mosquitoes. Parasites propagate in red blood cells and sometimes in the liver 336*i*
- **malignancy** behavior of tumors, characterized by genetic instability, local invasiveness, and metastasis formation 336*i*
- **malignant lymphoma** cancer derived from lymphatic cells. Multiple forms; major distinction according to B-cells or T-cells. May grow as a single solid tumor mass or diffusely. Typically treated with radiation or chemotherapy, not surgery 336*i*
- **membrane potential** electrical potential across the cell membrane, caused by ion concentration gradients across and ion channels within the membrane. Most often on the order of -60 to -70 mV inside relative to outside 336i
- **metastasis** (pl. metastases) secondary tumor. Arises from single cells that are released by the primary tumor and carried with the blood, lymph or otherwise to remote locations, where they settle and proliferate. Formation of metastases is a hallmark of malignant tumors

- **mineralocorticoids** steroid hormones that promote excretion of K⁺ and retention of Na⁺. Produced in the adrenal gland; aldosterone is the most important such hormone 336*i*
- **monoamine oxidase** enzyme involved in the oxidative degradation of monoamine transmitters, and also in the metabolism of some drugs 336*i*
- **monoamine transmitter** this term comprises catecholamines, serotonin, and histamine 336*i*
- motor endplate synapse between a motoneuron and skeletal muscle cell 336*i*
- muramyl-transpeptidase see peptidoglycan
- murein see peptidoglycan
- **muscarinic acetylcholine receptor** cholinergic G protein-coupled receptor. Multiple subtypes, occurs mostly in the parasympathic nervous system 336*i*
- **muscle relaxant** drug that induces muscle relaxation by blocking synaptic transmission in the motor endplate
- **myasthenia gravis** autoimmune disease. Autoantibodies directed at the nicotinic acetylcholine receptor in motor endplates compromise synaptic transmission, resulting in muscle weakness 336*i*
- **mycobacteria** class of bacteria with thick, multilayered cell walls. Includes causative agents of tuberculosis and leprosy 336*i*
- mycolic acid fatty acid with very long alkyl chain found in mycobacterial cell walls 336i
- **myocardial infarction** acute occlusion of a coronary artery caused by a blood clot, typically atop an atherosclerotic lesion, with subsequent degeneration of the part of the heart muscle that has been deprived of perfusion 336*i*
- **nephron** functional unit of the kidneys. Consists of a glomerulus that produces primary filtrate from blood plasma and of several successive tubular segments that post-process the filtrate into urine 336*i*
- **Nernst equation** equation that relates the magnitude of an ion gradient across a membrane to the resulting diffusion equilibrium potential 336*i*
- neuron nerve cell 336i
- **neurotransmitter** small molecule that is released by a nerve cell and activates or inhibits another nerve cell or other excitable cell nearby 337i
- **neutral antagonist** a drug that neither increases nor decreases the activity of its receptor relative to the receptor's unbound state
- **nicotinic acetylcholine receptor** ligand-gated cation channel that is activated by acetylcholine and causes cell excitation. Found in motor endplates, autonomic ganglia, and the brain 337*i*
- **nitric oxide synthase** enzyme that produces NO from arginine, using NADPH and O_2 as cosubstrates. Occurs in three isoforms, namely endothelial NOS, neuronal NOS, and inducible NOS 337i
- **non-ionic diffusion** The transport across membranes of an ionizable drug molecule in its non-ionized form
- **nuclear hormone receptor** receptor protein that binds a hormone or metabolite and then binds to cognate DNA sequences and regulates gene transcription through transactivation or transrepression. In transactivation, hormone-bound receptors act as transcription factors, that, is they bind to cognate DNA motifs and induce gene transcription. In transrepression, hormone-bound receptors interact directly with other transcription factors and prevent them from binding to DNA. Nuclear hormone receptors mediate the

effects of steroids, thyroid hormones, and retinoic acid, as well as enzyme induction in drug metabolism 337*i*

- **oncoprotein** mutant protein (encoded by a corresponding *oncogene*) that initiates or sustains malignant behavior of tumor cells 337*i*
- **organic anion transporter** class of transport protein important in drug transport; operates either by facilitated diffusion or secondary active transport 337*i*
- **organic cation transporter** class of transport protein important in drug transport; operates either by facilitated diffusion or secondary active transport
- **orphan receptor** putative receptor protein, identified as such by sequence homology to known receptors, with as yet unknown ligand and function
- **orthosteric** binding mode of a drug that binds "in the right place", *i.e.* within the same binding pocket that is also used by a receptor's physiological ligand. The receptor can therefore only bind either its physiological ligand or the drug, not both 337*i*

osteoblast see bone matrix

osteoclast see bone matrix

- **oxytocin** hypophyseal peptide hormone involved in regulating mammary gland and sexual organ function 337*i*
- **pancreatic islets** groups of endocrine gland cells that are interspersed in the tissue of the exocrine pancreas tissue 337*i*
- parasympathetic ganglia see parasympathetic nervous system
- parasympathetic nervous system part of the autonomic nervous system. Lowers the heart rate and blood pressure, promotes interior organ perfusion and function. Transmission prominently mediated by acetylcholine. Antagonistic to the sympathetic nervous system 337*i*

parathyroid gland see parathyroid hormone

- **parathyroid hormone** peptide hormone produced by the parathyroid glands, which are four small nodes of gland tissue attached to the thyroid gland. Mobilizes calcium and phosphate from bone matrix and increases their resorption in kidneys and intestine 337*i*
- **Parkinson's disease** CNS disease with motor symptoms, caused by degeneration of dopamine-producing cells in the brain stem
- **partial agonist** a drug that increases the activity of its receptor to a level above that of the unbound state but below that achieved by a **full agonist** 337*i*
- **patch clamp technique** experimental setup to study the conductivity of ion channels in small numbers or singly on live cells 337*i*
- **pathogenesis** the biochemical or physiological mechanism by which the ultimate cause of a disease causes its clinical manifestations

penicillins see beta lactam antibiotics

peptidoglycan matrix material of the bacterial cell wall, consisting of linear strands of Nacetylglucosamine and muramic acid that are crosslinked by oligopeptides; crosslinks are formed extracellularly by muramyl-transpeptidase 337*i*

phagocyte see phagocytosis

phagocytosis uptake of particles by cells, mostly specialized phagocytes such as neutrophil granulocytes and macrophages. After uptake, the particles are enclosed, and often destroyed, within phagosomes. Important part of immune defence against bacteria and fungi 337*i*

phagosome see phagocytosis

- **pharmacodynamics** as a solitary term: general principles of drug action; when applied to a specific drug: that drug's mode of action 337*i*
- **pharmacokinetics** study of drug movement and turnover, that is, rates and mechanisms of uptake into, distribution within and elimination from the body. When applied to a single drug, denotes that drug's characteristics of uptake, distribution, and elimination 337*i*
- **pharmacology** scientific discipline concerned with the modes of action and therapeutic uses of drugs
- **pharmacophore** consensus set of molecular properties such as charge distribution and shape required for activation or inhibition of a specific drug target 337*i*
- pharmacotherapy medical treatment using drugs 337*i*
- **phase I metabolism** initial reaction in the metabolic transformation of a drug. Often performed by cytochrome P450 enzymes 337*i*
- **phase II metabolism** conjugation of a drug that involves a reactive site on the drug molecule that was created or exposed in a phase I reaction. Typically consists in conjugation with a polar moiety that facilitates excretion 337*i*
- **phase III metabolism** a bit of a misnomer; denotes no actual chemical reaction but excretion of drug metabolites. The transporters that mediate excretion often recognize substrates that are conjugated with glucuronic acid and glutathione 337i
- **phenotypic screening** strategy for drug discovery in which the desired functional response of a cell or organism is observed directly after application of each candidate drug, and the search is not limited to a specific molecular target 337*i*
- **phosphodiesterase** enzyme that inactivates cAMP or cGMP by cleaving them to AMP or GMP, respectively. Several isoforms differ in preference for one or the other cyclic nucleotide
- **phospholipase** A_2 enzyme that cleaves the fatty acyl residue connected to the second (central) carbon of the glycerol backbone
- **phospholipase C** enzyme that cleaves the headgroup, including the phosphate, from a phospholipid substrate, leaving diacylglycerol in the membrane 337*i*
- **photoaffinity labeling** experimental technique for identifying binding sites of specific ligands 337*i*

physiology scientific study of the physical and chemical principles of organ function 337*i* **pituitary** see **hypophyseal gland**

planar lipid bilayer experimental setup to study the conductivity of ion channels in small numbers or singly 337*i*

plasmodia see malaria

- **polyene antibiotics** class of antibiotics that contain an extended polyene moiety and bind to ergosterol or other sterols in cell membranes 337*i*
- **polyprotein** translation product that is destined to be proteolytically cleaved into multiple functionally unconnected proteins. Many viruses, including HIV and hepatitis C virus, encode polyproteins and a protease that processes it 337*i*
- **portal circulation** the part of the circulation that involves the portal vein. Venous blood from the intestinal organs, the spleen, and the pancreas is drained into the portal vein and passed through the liver before reentering the general circulation

portal vein see portal circulation

postsynaptic see synapse

potassium channel potassium-selective ion channel. Structurally and functionally diverse 337*i*

potency concentration of a drug that achieves 50% of that drug's maximal effect 337i

pregnane X receptor nuclear hormone receptor involved in induction of enzymes in drug metabolism 338*i*

presynaptic see synapse

primary filtrate see nephron

- **prodrug** drug precursor molecule that must undergo metabolic transformation in order to become activated 338*i*
- **progestins** class of steroid hormones that maintain the mucous membrane of the uterus in a state ready for accepting the fertilized egg cell, and sustain the function of the placenta throughout pregnancy. Produced in the ovaries and, during pregnancy, the placenta itself 338*i*
- prostaglandin H synthase synonymous with cyclooxygenase
- **prostaglandins** class of eicosanoids involved in pain perception, inflammation, blood coagulation, and other physiological and pathological phenomena 338*i*
- **protease-activated receptors** GPCRs that are activated by proteolytic cleavage, rather than by binding of an extrinsic ligand
- **protein binding** nonspecific adsorption of drugs to proteins, most prominently albumin and other plasma proteins
- **protein phosphorylation** transfer of a phosphate group from ATP or GTP to a substrate protein by a protein kinase. Usually results in either activation or inactivation of the substrate protein. Can be undone by protein phosphatases 338*i*
- **protozoa** taxonomic category that includes single-celled, eukaryotic pathogens. Phylogenetically heterogeneous 338*i*
- proximal tubule part of the nephron
- pulmonary concerning the lungs 338*i*
- **reactive oxygen species** radicalic or ionic oxygen species or oxygen compounds. Important examples are O_2^- and H_2O_2 . May arise as side products in normal metabolism, through ionizing radiation, or be formed by dedicated enzymes that are particularly abundant in phagocytes. Important for immune defense but also involved in tissue destruction in inflammatory diseases 338*i*
- **receptor** macromolecule that is bound and activated, inhibited, or otherwise functionally influenced by a drug or other ligand. Note that this definition excludes proteins such as albumin that merely bind drugs but are not functionally affected by them 338*i*
- **receptor desensitization** inactivation of a receptor in response to prolonged exposure to ligand 338*i*
- **receptor occupancy** fraction (percentage) of a **receptor** that is saturated with a cognate drug or ligand 338*i*
- **receptor tyrosine kinase** a receptor that is also a protein kinase. Binding of the hormone or other mediator to the receptor activates the kinase activity, which attaches phosphate to tyrosine residues on substrate proteins 338*i*
- **refractory state** functional state of a receptor molecule, or of a cell, in which it is inactive and at the same time not amenable to activation
- renal concerning or belonging to the kidneys 338i
- **renal clearance** parameter that governs the renal elimination of a drug; defined as volume flow of urine times urine concentration over plasma concentration of the drug

- **resorption ester** a prodrug in which a polar moiety such as a carboxyl or hydroxyl group has been converted to an ester, so as to make the molecule less polar and improve its intestinal absorption 338*i*
- **resting potential** membrane potential in excitable cells that prevails in the absence of action potentials 338*i*
- **retinoid X receptor** nuclear hormone receptor that binds retinoic acid and, after forming a heterodimer with of of several other receptors, affects gene transcription 338*i*
- **rheumatism** group of autoimmune diseases affecting the joints and sometimes other organs 338*i*
- rhodopsin light-activated GPCR that acts as the light sensor in the eye 338*i*
- **RNA interference** Sequence-specific inhibition of gene expression at the level of mRNA, induced by double-stranded RNA sequences 338*i*
- **ryanodine receptor** a calcium-activated calcium channel in the endoplasmic reticulum. Involved in excitation-contraction coupling in muscle cells 338i
- **salvage pathway** metabolic pathway that diverts purine and pyrimidine bases or nucleosides from complete degradation and converts them back to intact nucleotides 338*i*
- **sarcoma** malignant tumor derived from non-epithelial tissues such as bones, cartilage and muscle 338*i*
- **Scatchard plot** a plot format for ligand binding data that is useful for distinguishing single from multiple binding sites 338*i*
- **second messenger** signaling molecule that is formed intracellularly downstream of the activation of a hormone or **neurotransmitter** receptor. Usually regulates multiple proteins in the cell. Examples: cAMP, cGMP, $IP_3 338i$
- **selective toxicity** toxic action of a drug that is limited to pathogenic microbes or to tumor cells 338*i*
- **semisynthesis** organic-synthetic process that starts with a complex molecule obtained from some biological source, typically a plant or microbe. This approach may be used for economical reasons even if the target product can in principle be synthesized from scratch 338*i*
- **septicemia** severe infection, in which the bacterial or fungal pathogen is carried in the bloodstream and may settle in multiple organs. Often leads to septic shock, which is one form of hemodynamic shock 338*i*

serotoninergic see adrenergic

serum see blood plasma

sinoatrial node see excitation conduction system

sleeping sickness disease caused by infection of the CNS with certain *Trypanosoma* parasites, which are transmitted by tsetse flies 338*i*

slow block see channel block

- smooth muscle muscle tissue with cells that lack the characteristic striated pattern found in skeletal and heart muscle. Smooth muscle is always controlled by the autonomic nervous system 338*i*
- **sodium channel** ion channel that selectively transports sodium. Activated by membrane depolarization 338i
- **sodium-potassium ATPase** ATP-dependent membrane transporter that exchanges two extracellular K⁺ ions for 3 intracellular Na⁺ sodium ions; plays a key role in maintaining ion gradients and membrane potential 338*i*

- **soluble guanylate cyclase** cytosolic enzyme that converts GTP to the second messenger cyclic GMP (cGMP). Activated by nitric oxide 338*i*
- **spatial summation** cumulative depolarization of a postsynaptic membrane by simultaneous activity in several adjoining synapses 338*i*
- statins see HMG-CoA reductase
- **steroid hormones** Hormones biosynthetically derived from cholesterol. Sexual hormones, glucocorticoids and mineralocorticoids belong to this class of hormones 338*i*
- stimulus trafficking synonymous with agonist-specific coupling
- **streptomycetes** soil bacteria that are genetically related to mycobacteria. Important as sources of many antibiotics 338*i*
- **stroke** acute occlusion or rupture of an artery of the brain, typically arising at an atherosclerotic lesion of that artery 338*i*
- substance P peptide neurotransmitter involved in pain perception 339*i*

sulfonamides class of antimicrobial drugs that inhibit folic acid synthesis 339i

sympathetic ganglia see sympathetic nervous system

- **sympathetic nervous system** part of the **autonomic nervous system**. Increases heart rate and blood pressure, inhibits interior organ perfusion. Uses both acetylcholine and norepinephrine as transmitters. Antagonistic to the **parasympathetic nervous system** 339*i*
- **synapse** connection between two nerve cells or other excitable cells. Transmitter is released from the presynaptic nerve terminal into the synaptic cleft and binds to receptors on the postsynaptic cell, which either increases or decreases its membrane potential. The transmitter is inactivated by degradation or reuptake into the presynaptic cell 339*i*
- **tachyphylaxis** rapid, usually partial desensitization of a receptor in response to sustained stimulation by an agonistic drug 339*i*
- **target validation** experimental evaluation of a hypothetical drug target by means of surrogate stimulation or inhibition, often by means of genetic knockout or RNA interference 339*i*
- **temporal summation** cumulative depolarization of a postsynaptic membrane by a rapid succession of excitatory postsynaptic potentials occurring in the same synapse 339*i*
- **therapeutic index** ratio of a drug's toxic concentration over its therapeutic concentration 339*i*
- therapeutic range synonymous with therapeutic index 339i
- **thioredoxin** small enzyme molecule that reduces multiple substrates, including xenobiotics and disulfide bonds in proteins. Contains two vicinal cysteine residues in the active site, which are converted to a disulfide in the reaction and subsequently reduced again at the expense of NADPH 339*i*
- **thrombocytes** also called blood platelets. Small, **anucleate** cells that are formed in the bone marrow as cytoplasmic fragments pinched off from megakaryocytes. Play a key role in blood coagulation 339*i*
- thromboxanes class of eicosanoids involved in blood coagulation and inflammation
- **thyroid gland** endocrine gland located in the front of the neck that produces thyroid hormones (thyroxine, triiodothyronine) and calcitonin 339*i*

thyroid hormones see thyroid gland

- thyroid peroxidase key enzyme for hormone production in the thyroid gland
- **thyroid-stimulating hormone** hypophyseal peptide hormone that stimulates proliferation and hormone secretion in the the thyroid 339*i*

- **thyrotropin-releasing hormone** hypothalamic peptide hormone that stimulates the secretion of thyroid-stimulating hormone from the hypophyseal gland 339*i*
- **tight junction** complex protein structure that tightly connects the cytoplasmic membranes of two neighboring cells in an endothelial or epithelial cell layer and restricts the passage of fluid between the them 339*i*
- transactivation see nuclear hormone receptor
- **transient receptor potential channel** ion channel that conducts calcium and other cations. Different types are activated by heat, pressure or other physical and chemical stimuli 339*i*

transrepression see nuclear hormone receptor

- **trypanosomes** protozoal parasites, causative agents of sleeping sickness and Chagas disease 339*i*
- **tubular secretion** active transport of solutes into the nascent urine. Occurs mostly in the proximal tubule of the nephron
- **two-state model of receptor activation** theoretical model of receptor behavior. The receptor is assumed to spontaneously alternate between an active and an inactive conformation. Agonists selectively bind and stabilize the active conformation, antagonists selectively bind the inactive conformation

use-dependent block see channel block

- vascular part of, or concerning blood vessels 339i
- vasodilation widening of blood vessels, caused by relaxation of vascular smooth muscle cells (vasorelaxation). Excessive systemic vasodilation occurs can lead to hemodynamic shock 339*i*
- **vasopressin** hypophyseal peptide hormone involved in regulating blood pressure and urine volume 339*i*
- venule small vein 339i
- **viral protease** virus-encoded protease that cleaves a viral polyprotein precursor to mature, functional proteins 339*i*
- **voltage-gated ion channel** ion channel that is opened in response to membrane depolarization 339*i*
- **volume of distribution** ratio of the number of molecules of a drug in the body, divided by the plasma concentration of this drug
- xanthine oxidase enzyme that oxidizes hypoxanthine to xanthine and then uric acid in the degradation pathway for adenine and guanine. Also contributes to the metabolism of some drugs 339*i*
- **xenobiotic response element** regulatory DNA sequence motif recognized by the pregnane X receptor
- **xenobiotics** small molecules that originate outside the human body and are not required in human metabolism. The term includes drugs, poisons and inert substances, of both natural and synthetic origin 339*i*
- yellow-fluorescent protein see green-fluorescent protein

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