How Drugs Act: Concepts for Therapy 22

How many modes of action are there for drug therapy? There are estimations that the currently commercially available drugs exert their action on approximately **500 target structures**. Optimistic prognoses claim that this number can be increased by perhaps a factor of 10. But this number is still small compared to the diversity of proteins that play a role in our organism. Our genome has been sequenced. We know that the number of our genes (about 25,000) is much smaller than it was originally assumed (▶ Sect. 12.3). The number of relevant proteins for which these genes code is, however, significantly larger, because, among other reasons, versatile **posttranslational modification** and **alternative splicing** cause the genetic information to be diversified over multiple protein variants. Accordingly, our genome is mapped, but do we know what function is behind each individual gene? How can predictions about proteins and their functions and possible roles in pathophysiology be extracted from this flood of sequence information? Many of the proteins that have been discovered in the genome can be assigned to protein families based on sequence comparisons. Nonetheless, a significant portion of our genetic information still awaits annotation. The first step has been taken, but how do the spatial structures of these proteins look, for which only sequences are know? Which ligands will be recognized by these proteins, and what biochemical role do they assume in our organism? The **biochemical function**, that is, the assignment of whether a protein represents, for example, a protease, an ion channel, or a transporter, still affords no information at all about what **systemic roles** the protein takes in the functional processes in a cell or in a whole organism. The spatial structure of a protein is responsible for this function. Therefore, the structures of the proteins in our genome are being intensively investigated. The goal is to map the structural space of all proteins as well as possible. Then it could be possible to find a spatially elucidated and adequately homologous reference structure for each discovered sequence. Today, the structures of all members of a few gene families have already been determined. Therefore, it is only a question of time until we have the spatial structure of all relevant proteins at our disposal. The way there may be long and hard, but it is clearly sketched out. Will this revolutionize the market of potential pharmaceuticals and make entirely

G. Klebe, *Drug Design*, DOI 10.1007/978-3-642-17907-5_22,

new therapeutic approaches possible? The chemical space of all imaginable active substances and the biological space of all possible pathology-relevant proteins is discussed in ▶ Sects. 11.4 and ▶ 12.4. Drug design attempts to merge both of these spaces with one another. There are molecules to be found as candidates for potential active substances in the cross section of both spaces.

22.1 The Druggable Genome

In 2002, Andrew Hopkins and Colin Groom published a summary that accurately illuminated the **drug market** at that time (Fig. 22.1). Back then, approximately 20 drugs per year were being launched into the market, and at the moment we are seeing only a very small change in these circumstances. Approximately half of today's drugs inhibit enzymes. Another 30% modulate the behavior of G protein-coupled receptors (GPCRs). About 7% exert their therapeutic effect on ion channels. Transporters, nuclear hormone receptors or other receptors for growth factors, interleukins, or peptides such as insulin are influenced by about 4% of the available drugs each. Then a small portion remains that influences cell-surface integrins or DNA. These market segments in no way cover the frequency of these target structures in our genome. For example, the GPCRs are only 2.3% of our genome if sensory GPCRs are excluded. Approximately 15% of the "druggable" genome, that is, the portion for which the function can be favorably influenced by a pharmaceutical therapy, is assigned to the GPCRs. The kinases make up more than 22% of the genome, but only nine small-molecule inhibitors are commercially available as drugs. However, it is estimated that about 100 substances are in extensive testing. Therefore, it is to be expected that the drug market will change in the next years.

In the next chapters, examples of individual target structures will be introduced that represent potential targets for drug therapy. They are discussed on the basis of their most important structural characteristics because the structure of the target generally defines what is needed to qualify a molecule as an inhibitor, agonist, antagonist, or allosteric modulator. These principles serve as a general concept for the design of new active substances. In modern drug research, the target structure for which a new active substance is sought is usually known. In many historical examples of drug development, this was not initially the case. In the meantime, however, many modes of action are known. Peter Imming and his research group have compiled a summary of the modes of action for a broad collection of drugs that are used today. Furthermore, the database WOMBAT from Tudor Oprea at the University of New Mexico in Albuquerque offers fast access to functionally annotated drugs together with their characteristic properties.

22.2 Enzymes as Catalysts in Cellular Metabolism

All metabolic processes, biosynthetic pathways, and the regulation of important physiological processes are mediated by enzymes. Enzymes are **macromolecular**

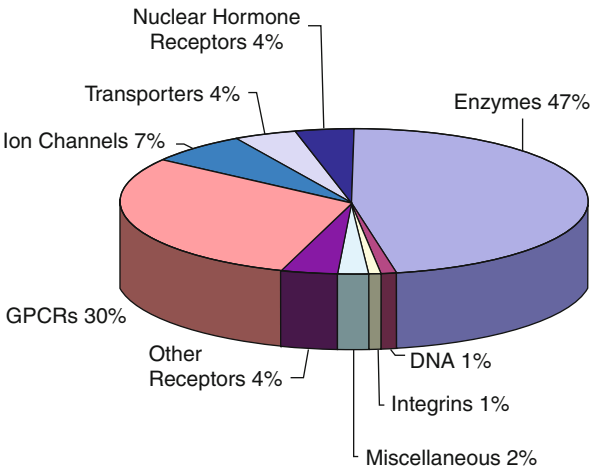

Fig. 22.1 Distribution of the target proteins for drugs that are on the market today.

biocatalysts that allow complex chemical reactions to take place in an aqueous medium, usually at 37°C, and under normal pressure. During the course of evolution, families of enzymes have developed with analogous architecture and identical catalytic sites. Small differences in the structure of the binding sites lead to entirely different substrate specificity, which, according to the required function, render these enzymes either highly specific or profoundly promiscuous.

Enzymes do not bind particularly strongly to their substrates and reaction products. The bound conformation of the ligand is often different from the energetically most favorable conformation in aqueous solution. An enzyme binds the substrate in a geometry that prepares it for the **transition state** of the reaction. Moreover, polar groups can induce the required shifts in charges. The enzyme stabilizes the transition state of a chemical reaction through the spatial arrangement and orientation of its reactive groups. It simultaneously lowers the activation energy of the reaction and makes sometimes very dramatic rate accelerations of chemical reactions possible. After dissociation of the product, the enzyme is available for the transformation of the next substrate molecule.

Enzymes are classified according to the reactions that they catalyze. An international commission has divided enzymes into six classes, each of which is assigned a four-number code (Table 22.1). The main class indicates what type of reaction is catalyzed (redox reactions, transfer reactions, transfer of functional groups to water, cleavage and elimination reactions, isomerization of groups within the substrate, or condensation or linkage of molecular groups). The remaining numbers classify, for example, which group is transferred or whether the protein is regulated by cofactors. The MEROPS database, which is maintained by the Sanger Institute in Cambridge, England, affords fast access and a broad overview of proteases, their substrates, reaction mechanisms, and selectivities. In ► Chaps. 23, "Inhibitors of Hydrolases with an Acyl–Enzyme Intermediate"; ► 24, "Aspartic Protease Inhibitors"; ► 25, "Inhibitors of Hydrolyzing

Table 22.1 Enzyme classification based on the four-digit number code.

Class	Name	Biochemical function	Examples	Coenzymes
EC 1.x.x.x	Oxidoreductases	Catalyze redox reactions; transfer of H- and O-atoms or electrons between molecules	Dehydrogenases, Oxidases, Oxygenases, Hydroxylases	NAD^+, $NADP^+$, FAD, FMD, and Liponic acid
EC 2.x.x.x	Transferases	Transfer functional groups such as methyl, acyl, amino, or phosphate groups from one molecule to another	Phosphotransferases (including kinases) Aminotransferases	*S*-Adenosyl methionine, Biotin, cAMP, ATP, Thiamine pyrophosphate (TPP), Tetrahydrofolic acid
EC 3.x.x.x	Hydrolases	Hydrolytic cleavage of molecules	Esterases, Lipases, Phosphatases, and Peptidases	Not needed
EC 4.x.x.x	Lyases	Non-hydrolytic addition or cleavage of groups on molecules, particularly double bonds, cleavage of C–C, C–N, C–O, and C–S bonds	Decarboxylases, Aldolases, Synthases	TTP, Pyridoxal phosphate
EC 5.x.x.x	Isomerases	Intramolecular rearrangement and isomerization within a molecule	Racemases, Mutases	Glucose-1,6-bisphosphate, Vitamin B12
EC 6.x.x.x	Ligases	Coupling of two molecules by the formation of C–C, C–N, C–O, or C–S bonds by using ATP	Synthestases, Carboxylases	ATP, NAD^+

Metalloenzymes"; ► 26, "Transferase Inhibitors"; and ► 27, "Oxidoreductase Inhibitors," the important enzyme classes for which drugs have been successfully developed will be presented.

22.3 How Do Enzymes Push Substrates Toward the Transition State?

To explain how an enzyme prepares its substrate for the transition state we should consider an example. The crystal structure of creatinase with its natural substrate creatine **22.1** and a very similar inhibitor, carbamoylsarcosine **22.2**, was determined in the research group of Robert Huber at the Max Planck Institute in Martinsried, Germany. The enzyme catalyzes the cleavage of creatine to urea and

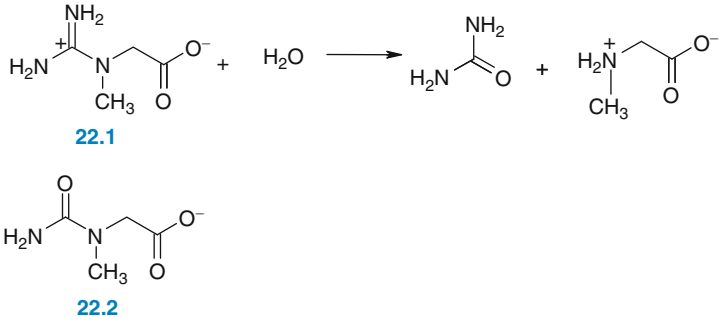

Fig. 22.2 The enzyme creatinase cleaves creatine **22.1** with water into urea and sarcosine. The structurally very similar molecule, carbamoylsarcosine **22.2**, is an inhibitor of this enzyme.

sarcosine (Fig. 22.2). For this, the central carbon in the C–N bond in the guanidinium part of creatine is **nucleophilically attacked by a water molecule**. All three C–N bonds in the guanidinium portion exhibit double-bond character and a planar geometry because of electron delocalization. How does the enzyme manage to distort creatine in the direction of the transition state of the reaction to prepare it for the nucleophilic attack as well as for bond breaking? The zwitterionic creatine is bound through its guanidinium function by two glutamate residues forming two salt-bridge-like hydrogen bonds (Figs. 22.3 and 22.4). The opposite acid function finds strongly polarizing bonding partners in two arginine residues. Furthermore, a water molecule is found near the central imine-like carbon atom in the crystal structure. A histidine is next to it in the binding pocket. This histidine orients the water molecule in exactly the right position and also supports the abstraction of a proton from this water molecule. This increases the nucleophilicity of the water to generate an OH^- group. The vice-like fixation of the guanidine group by the two glutamate residues causes a twisting of this building block, which is planar in the unbound state. Because of this, the conjugation is disrupted and as a consequence, the C–N bond that is to be cleaved is significantly weakened. A nucleophilic attack occurs, and a tetrahedral transition state is formed. At the same time, the now-protonated histidine is able to polarize the methyl-substituted nitrogen atom and involve it in a hydrogen bond. This prepares our substrate for the **bond-breaking transition state**. After transferring the proton from histidine to the substrate, a positive charge is formed on the nitrogen atom of the bond that is to be cleaved. Histidine accepts a proton from the oxygen atom of the tetrahedral transition state as a C=O double bond is formed, and the central C–N bond is cleaved. The products then leave the binding pocket. In this way, the enzyme creates a **stereoelectronically complementary environment** for the cleavage reaction. Its polar groups place the water molecule correctly for the nucleophilic attack, and histidine induces a pyramidalization of the nitrogen atom in the bond to be broken. At the same time, it serves as a proton donor as well as acceptor during the reaction.

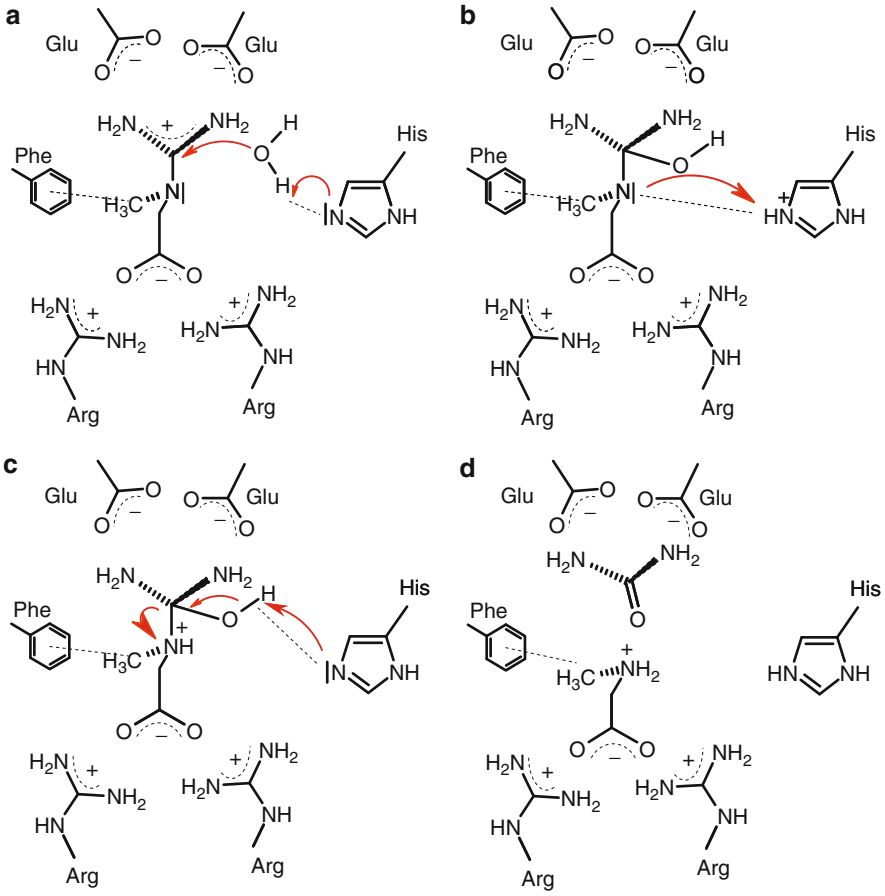

Fig. 22.3 (**a**) In the first step, a water molecule is polarized by a neighboring histidine so that a nucleophilic attack on the imine-like carbon is facilitated. (**b**) Then, the histidine transfers a proton to the central nitrogen atom. (**c**) The substrate reacts further in that a C=O double bond is formed and the C–N bond is cleaved. (**d**) The products urea and sarcosine leave the binding pocket.

The crystal structure in Fig. 22.4 was determined together with carbamoylsarcosine. This molecule is different from the substrate creatine because of an exchange of an oxygen for a nitrogen atom. However, because of this, this part of the molecule does not carry a positive charge as creatine does. The addition of the nucleophilic OH^- leads to decomposition and compensation of the charge in the guanidinium part in creatine. A comparable attack upon carbamoylsarcosine would lead to the formation of a negative charge next to the two negatively charged glutamates. This is energetically unfavorable. As a consequence, the cleavage reaction does not take place on this molecule, instead it blocks the transformation. The example shows how precisely substrate and enzyme must be

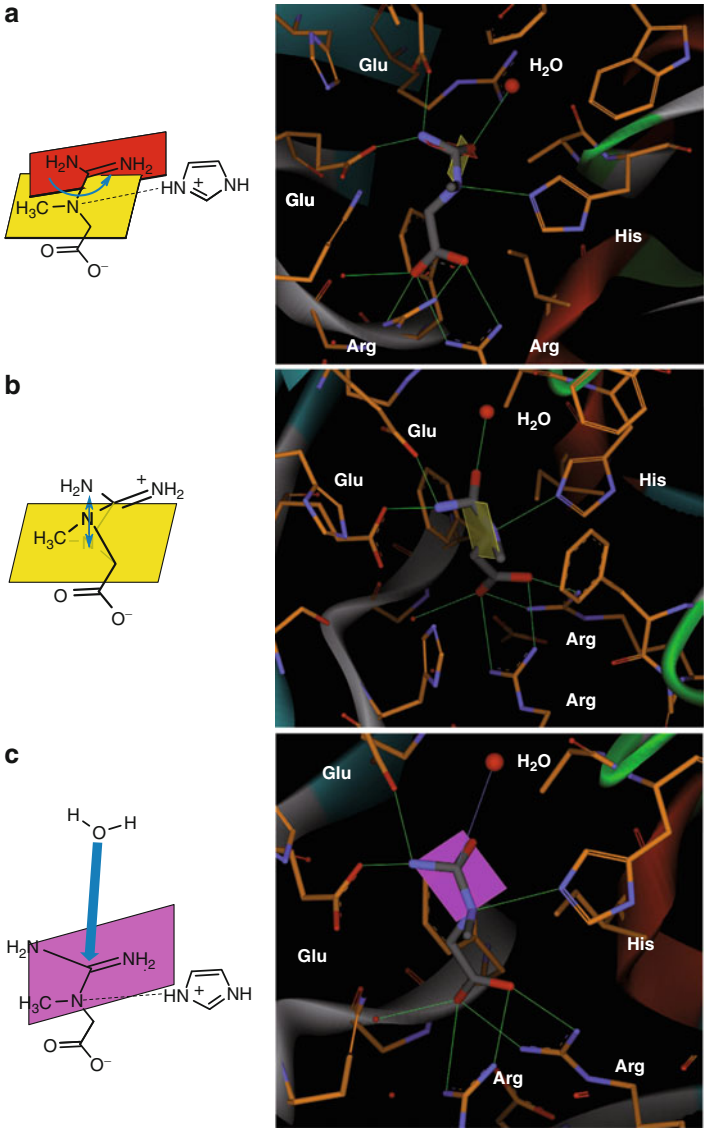

Fig. 22.4 (**a**) The vice-like fixation of the guanidinium group by both glutamate residues causes a twisting in this portion, which is planar in the unbound state. Because of this, the conjugation is disrupted and the C–N bond to be cleaved is weakened. The twisting is indicated by the *red* and *yellow planes* that pass through the atoms of the guanidinium group. (**b**) The neighboring protonated histidine further polarizes the methyl-substituted nitrogen atom, and involves it in a hydrogen bond. In doing so the nitrogen atom takes on a pyramidal configuration, by which it deviates out of the plane (*yellow*) of its next three neighbors. (**c**) In the structure with the substrate-like inhibitor carbamoylsarcosine, a water molecule can be found in the position from which the nucleophilic attack on the substrate creatine is initiated. This occurs from above and diagonally behind the C=N bond.

in harmony with one another. Small changes can drastically change this system and convert a substrate molecule into an inhibitor of the targeted transformation reaction.

22.4 Enzymes and Their Inhibitors

Enzymes can be organized into multienzyme complexes that carry out multiple reactions on one substrate sequentially. They can also form cascades in which one enzyme activates the inactive precursor of the next enzyme. This activation continues to the next enzyme, and the next, and so forth. The coagulation cascade (► Sect. 23.3) is activated by two independent pathways, each along multiple steps, which merge into a common pathway in the end. Because of this, a minor initiating event is amplified by multiple orders of magnitude. This is good for normal coagulation after an injury, but in the context of a coagulopathy (i.e., a tendency to form clots too easily) it can have disastrous consequences!

Quite a number of inhibitors prevent the catalytic effect of an enzyme by occupying the position at which the substrate binds. Such inhibitors are termed **competitive inhibitors**. In addition, there are also **allosteric inhibitors** that bind at another position on the enzyme and cause a change in its three-dimensional structure or dynamic properties. This can prevent the enzyme from adopting the necessary conformation for catalysis and can lead to a weakening of the catalytic activity. Detailed investigations of the enzyme kinetics allow for competitive inhibition to be distinguished **from noncompetitive inhibition**. According to the type of interactions with the enzyme, reversible and irreversible inhibitors can be differentiated. In the case of reversible inhibitors, the binding to the enzyme must be strong so that the transformation of the substrate can be reliably prevented. Some reversible inhibitors form a covalent bond to the catalytic center that is chemically labile, and therefore fully reversible, for instance, a hemiacetal bond. Irreversible inhibitors react with the enzyme by forming a chemically stable bond. The inhibitors or the reacting groups cannot be detached, and for the rest of the lifespan of the enzyme until protein degradation in the organism, the enzyme remains inhibited. Moreover, there are naturally occurring protease inhibitors that indeed reversibly bind, but adhere so strongly that the complex is degraded before the inhibitor is released.

The rational design of an enzyme inhibitor usually starts with the structure of the substrate. One approach that is particularly successful is to imitate the transition state with a chemically analogous group that is not attacked by the enzyme. In the ► Chaps. 23, "Inhibitors of Hydrolases with an Acyl -Enzyme Intermediate"; ► 24, "Aspartic Protease Inhibitors"; ► 25, "Inhibitors of Hydrolyzing Metalloenzymes"; ► 26, "Transferase Inhibitors"; and ► 27, "Oxidoreductase Inhibitors", many examples for the design of such inhibitors are presented. Overall, irreversible enzyme inhibitors play a smaller role than reversible inhibitors, but important drugs such as acetylsalicylic acid (ASA, ► Sect. 3.1), omeprazole (► Sect. 3.5), clopidogrel (a thrombocyte aggregation inhibitor), penicillins and

cephalosporins (▶ Sect. 23.7), and a few monoamine oxidase inhibitors (▶ Sect. 27.8) belong to this group.

22.5 Receptors as Target Structures for Drugs

Receptors are proteins or protein complexes that

- Mediate the information exchange between cells (membrane-bound receptors);
- Regulate hormone-controlled gene expression (soluble receptors or transcription factors);
- Are coupled to ion channels and control the flow of ions into or out of a cell along a concentration gradient.

Important **membrane-bound receptors** are the receptors for adrenaline, serotonin, dopamine, histamine, acetylcholine, adenosine, and thromboxane, and for peptides such as the enkephalins (opiate receptor), neurokinins, and endothelins for glycoproteins, as well as the group of sensory receptors. **Neurotransmitters** are the endogenous agonists of many membrane-bound receptors (▶ Sect. 1.4). Nerve cells are connected to each other through **synapses**; these are zones in which chemical information transfer is accomplished by neurotransmitters. The so-called **synaptic gap** is found between the transmitting cell (**presynaptic neuron**) and the receiving cell (**postsynaptic neuron**). Neurotransmitters are synthesized in the presynaptic neuron and stored in vesicles. Upon nerve stimulation, they are released into the synaptic gap. There, by binding to a specific receptor on the postsynaptic neuron, they effect a change in the membrane potential and consequently stimulate this cell. After reuptake in the cell, containment in vesicles, or after degradation by, for example, the enzyme monoamine oxidase (amines), esterases (acetylcholine), and peptidases, or in glial cells through the effect of catechol-*O*-methyltransferase, the effect subsides again quickly (see Fig. 22.7).

Within the cell, these receptors act upon G proteins (Fig. 22.5), the name of which is derived from guanosine di- and triphosphate. All G protein-coupled receptors (GPCRs) have an identical construction and function principle. They consist of a protein chain with seven hydrophobic segments that penetrate the cell membrane and anchor the receptor. These individual sections are connected to one another by loops. To date around 1,000 different GPCR sequences are known, and new ones are constantly being discovered and characterized (▶ Sect. 29.1).

After an agonist docks, the active conformation of the receptor is stabilized. **Antagonists** prevent the docking of **agonists**, and **inverse agonists** stabilize the inactive conformation of the receptor.

The provoked receptor response is carried out over identical pathways, despite the different types of receptors, and then it branches off again. This economic natural principle is also used in other cases, for example, the regulation of cell proliferation. The more-or-less-pronounced effect specificity is achieved by:

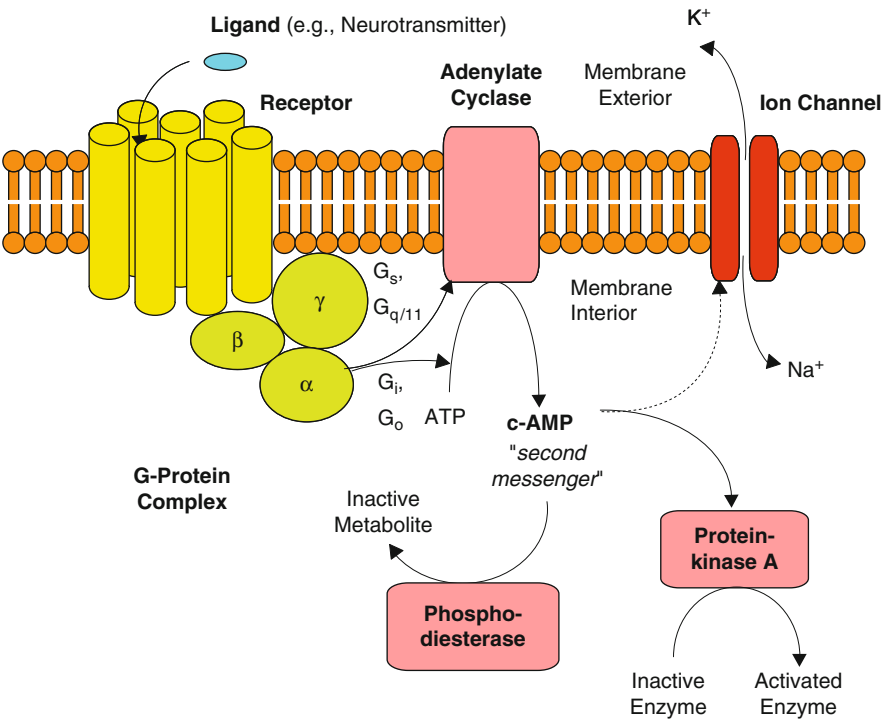

Fig. 22.5 Schematic representation of the structure and function of a G protein-coupled receptor (GPCR). The seven cylinders symbolize the seven transmembrane helices. The extra- and intracellular loops that bind the helices are not shown. After binding an agonist, the α-subunit dissociates from the so-called G protein complex. If a G_s or $G_{q/11}$ protein is present, then an enzyme is activated that generates an internal hormone, a "second messenger." For example, the membrane-bound enzyme adenylate cyclase generates cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). This second messenger can further affect target proteins via protein kinase A, or open an ion channel. To avoid an overreaction, cAMP is constantly being degraded by the enzyme phosphodiesterase. $G_{i/0}$ proteins inhibit enzymes that form second messengers.

- The different structures of the agonists and receptors and the resulting activation of different G proteins and effector proteins;
- The different receptor occupancy and density of different cells;
- The location of the cells that produce and release the hormone or neurotransmitter. This is accomplished in very specific cells; neighboring cells or organs are not involved.

The picture of such receptors can be very complex. For example, in the case of the acetylcholine receptors, two different groups are distinguished that preferably bind either muscarine, a toxin of the toadstool *Amanita muscaria*, or nicotine, the active ingredient of the tobacco plant, *Nicotiana tabacum*. In contrast to the

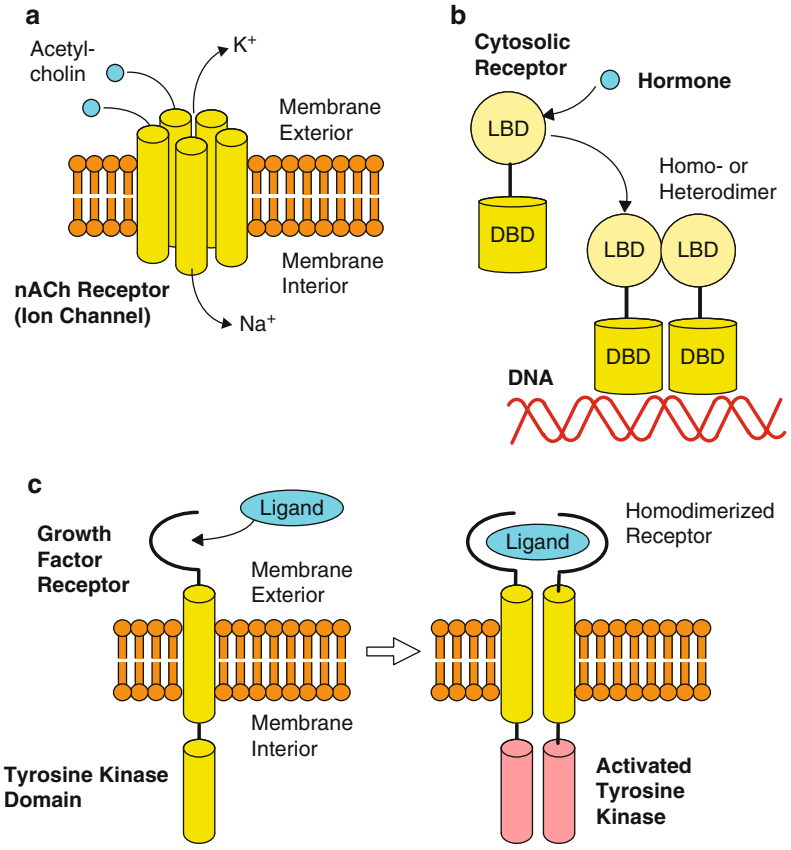

Fig. 22.6 (**a**) The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel (► Sect. 30.4). Here the cylinders do not stand for segments but rather for five separate proteins, each of which has four transmembrane domains. After binding acetylcholine, the channel is quickly opened. (**b**) Soluble receptors dimerize after agonist docking to their ligand-binding domains (LBD). Here homodimers composed of two identical receptors as well as heterodimers of two different receptors can be formed. The so-called zinc fingers of the DNA-binding domains (DBD) recognize very specific sequences of DNA. A particular DNA segment is addressed by dimerizing two receptor units. (**c**) Membrane-bound receptors for growth factors and insulin also dimerize. Two receptors form a complex in the membrane and in doing so activate the intracellular domain of the receptor, in this case, a tyrosine kinase.

muscarinic acetylcholine receptor, the nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel (► Sect. 30.4). It has a complex architecture of five protein chains that are positioned in the cell membrane (Fig. 22.6a). Electron microscopy pictures of the closed and open structure (after activation by acetylcholine) of the 290 kD nAChR protein complex (► Sect. 30.4) are available for the nAChR from the electrical organ of the *Torpedo* electric ray, a fish.

Many hormone receptors, for example, for thyroid hormone, sexual hormones, the corticosteroids, and retinoic acid, are **soluble receptors** that can move freely in

the cytosol, that is, the cell fluid. After binding the agonist, the complex migrates to the nucleus. There, it binds as a dimer to the signal sequences of the DNA, the operator and repressor genes, and induces or suppresses the new synthesis of specific proteins (Fig. 22.6b).

All **cytosolic hormone receptors** or **nuclear receptors** are built from common structural principles (▶ Sect. 28.2). They exhibit domains with a DNA-binding site and a ligand-binding site. The DNA-binding site is highly conserved, that is, its amino acid sequence varies very little between the different receptors. It contains two "zinc fingers" comprising two Zn^{2+}-binding sites that are highly conserved motifs binding to very specific DNA segments, the so-called recognition sequences. The **ligand-binding site** is much more variable. Dimers, either of two identical receptors (homodimers) or from two different receptors (heterodimers) are formed for the interaction with DNA. Four zinc fingers in the dimer recognize 12 base pairs of DNA in total.

Dimerization is also found in other classes of membrane-bound receptors that do not belong to the GPCR type. Among these are the **receptors** for **growth factors**, for example, for human growth hormone (hGH), epidermal growth factor (EGF), and insulin (▶ Sect. 29.8). Upon binding to the factor, these receptors dimerize in the membrane with the extracellular domains. As a consequence, intracellular kinases are activated that are part of the receptor protein (Fig. 22.6c). In addition, there are receptors that must form complexes of more than two units to provoke a receptor response. Among these are a series of immunologically important receptors as well as receptors for the nerve growth factor (NGF) and tumor necrosis factor (TNF).

Multiple examples of proteins are presented in this section that exert their function as oligomers. Indeed, oligomer formation is also common in enzymes. There are many reasons why oligomerization is advantageous. On the one hand, there are functional requirements that demand, as described above, multiple neighboring domains. On the other hand, there can be mechanistical advantages, especially with enzymes. Individual domains of an oligomer are not necessarily independent of one another. Their catalytic efficiency can depend upon what conditions the other domains of the oligomer are currently in. This affords an additional possibility to regulate the protein function. Oligomerization can also have another meaning. The interior of a cell is crowded with proteins, ligands, substrates, and ions. It must be compared with a ticker-tape parade given for a winning football team: hectic pushing and shoving! One way to reduce this number without limiting the catalytic productivity by sacrificing catalytic centers is the formation of oligomers.

22.6 Drugs Regulate Ion Channels: Our Extremely Fast Switches

Ion channels, which are embedded in the cell membrane, allow ions to enter or leave the cell along the corresponding electrochemical concentration gradient when they

are open. The opening or closing of the channel can be either **voltage-**, **ligand-** or **receptor-gated**. All of these processes occur extraordinarily fast (▶ Sect. 30.1).

The intracellular Ca^{2+} ion concentration in all cells is a few factors of 10 below that of the surrounding medium. At the moment of cellular stimulation, all of the voltage-gated calcium channels are momentarily opened by the arrival of an electrical signal. An influx of Ca^{2+} ions into the cell occurs. The intracellular concentration rises swiftly without ever reaching the extracellular concentration. In smooth, skeletal, and heart muscle cells, this process induces a contraction. Then the excess Ca^{2+} ions are pumped out of the cell, and a resting phase follows. This process is repeated very quickly in heart cells in a rhythm of less than a second, corresponding to the length of a heart beat.

Verapamil and nifedipine (▶ Sect. 2.6) affect such voltage-gated **calcium channels** and inhibit the influx of calcium ions. They are called "calcium channel blockers," which describes the mode of action of these substances. By inhibiting the influx of Ca^{2+} ions, the excitability of the cells, for instance, of heart cells, is decreased, less energy is used, and the muscle work becomes more economic. Furthermore, calcium channel blockers offer protection from the high calcium concentrations that are caused by cell demise in poorly perfused areas, for instance, during a heart attack. A particularly favorable therapeutic effect is their blood pressure-lowering properties.

The nicotinic acetylcholine receptor (nAChR, Fig. 22.6a) and the family of glutamate receptors belong to the class of ligand- or receptor-gated ion channels. Here the opening and closing of the channel is not accomplished by an electrical impulse but rather by the binding of a ligand.

Many drugs affect ion channels (▶ Chap. 30, "Ligands for Channels, Pores, and Transporters"). Local anesthetics and antiarrhythmic drugs, which are derived from the former, are sodium channel blockers; they reduce the excitability of nerve cells. The venom of the fugu fish, tetrodotoxin (▶ Sect. 6.2), also blocks this channel. Other antiarrhythmic agents block **potassium channels**. Substances that stabilize the K^+ channel in an open state, so-called K^+ channel openers, act as vasodilators and decrease the blood pressure. The antidiabetic sulfonylureas are K^+ channel blockers that act on the insulin-producing cells in the pancreas (▶ Sect. 30.2).

Tranquilizers of the benzodiazepine type (▶ Sect. 30.6) increase the binding of the neurotransmitter γ-aminobutyric acid (GABA) to **chloride channels**. Prolonged opening of this channel causes an increased influx of chloride ions and with it a change in the response behavior of the nerve cells. Barbiturates and inhaled anesthetics also act on the GABA receptors, but on different domains.

22.7 Blocking Transporters and Water Channels

Transporters are proteins that affect the active uptake of molecules or ions into cells. They play a very decisive role in the digestive process. Because amino acids and sugar cannot cross membranes on their own, they can only be absorbed with the help of **transporters** in the digestive tract.

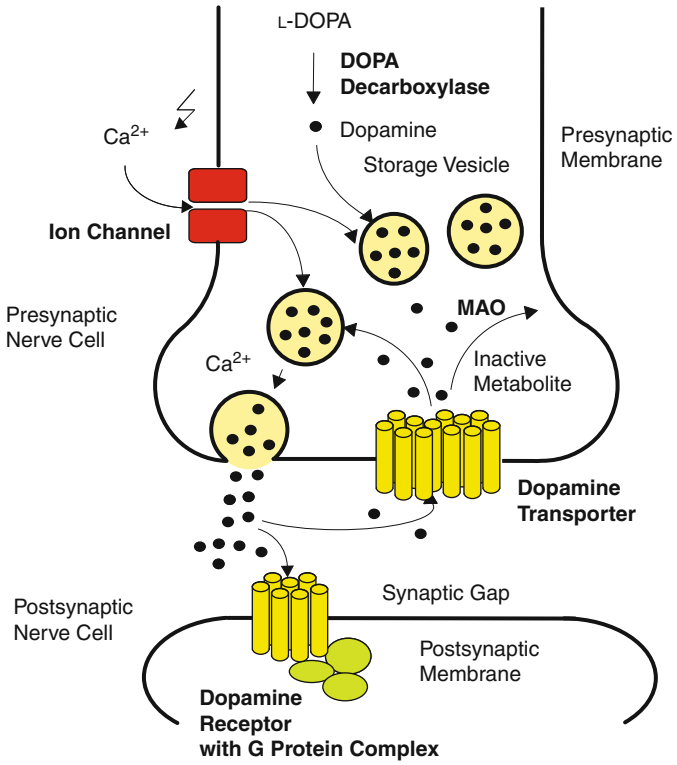

Fig. 22.7 Nerve signal transmission through neurotransmitters is based on a complex interplay of enzymes, receptors, ion channels, and transporters. Dopamine is produced by enzymatic decarboxylation of the amino acid L-DOPA. As with other neurotransmitters, it is stored in special vesicles. Upon electrical stimulation, Ca^{2+} ions flow into the cell. This causes the neurotransmitter to be released into the synaptic gap. The nerve impulse is conducted further by the interaction with the postsynaptic receptor. Finally, the uptake in the presynaptic cell is accomplished by a transporter and the neurotransmitter is stored in a vesicle again, or degraded by the enzyme monoamine oxidase (MAO).

Transporters are also exceedingly important for signal transmission of nerve cells. A neurotransmitter must be rapidly removed from the synaptic gap after its release to prevent a prolonged stimulation of the nerve cell. This is accomplished in part by metabolic degradation, but that is very wasteful for the releasing cell. An uptake (incorrectly called reuptake) with the help of a specific transporter is more economical. The neurotransmitter is stored in vesicles and held at the ready for the next release.

Transporters work against concentration gradients. The transport process is relatively slow, much slower than an ion channel, and it costs energy. The amino acid sequence of the specific transporter is known for many neurotransmitters, amino acids, sugars, and nucleosides. As with the G protein-coupled receptors,

the transporters are differentiated into many families. Most have an even more complex structure with 12 transmembrane domains (▶ Sect. 30.8).

A few active substances directly target the transporters and displace the natural ligands. The euphoric effects of cocaine are due to its binding to the dopamine transporter, which is responsible for the active transport and uptake of dopamine in the nerve cells. A fast flood of cocaine causes a delayed uptake of dopamine from the synaptic gap, and this is responsible for the typical physical and psychiatric effects. A few antidepressants are ligands for the noradrenaline and serotonin transporters (▶ Sect. 1.4). They are bound, but not transported into the cell. In contrast, some analogues of amino acids are brought into nerve cells by transporters and act there as neurotoxins. An overview of the complex interplay of neurotransmitters, enzymes, receptors, and transporters is presented in Fig. 22.7. Some anti-gout drugs bind to the uric acid transporter. They displace uric acid, inhibit its absorption from primary urine, and accelerate the excretion of uric acid with the urine. There are even specific transporters for bile acids.

In addition to the previously described transporters, other representatives of this protein class are also important for the uptake or excretion of foreign substances into or out of cells. Tumor cells often react to therapeutic measures by developing multiple resistance to many structurally diverse substances (▶ Sect. 30.8). Glycoprotein GP 170, also a transporter with 12 transmembrane domains, is responsible for this process.

In contrast to ion channels, **ion transporters** work against the concentration gradients. This is an active process that occurs at the expense of energy. Drugs can influence this too. An example is agents that increase urine production: diuretics. They inhibit different ion transporters. Na^+/K^+ ATPase, a pump that exchanges sodium for potassium ions, is inhibited by the cardiac glycosides, which are prescribed to treat congestive heart failure. Substances of the omeprazole type (▶ Sects. 3.6 and ▶ 9.5) inhibit the H^+/K^+ ATPase, the so-called **proton pump**. Nature uses special water channels to regulate water homeostasis, and also to quickly and selectively transport small, non-charged molecules such as glycerol or urea across the cell membrane. In contrast to the transporters, and analogously to the ion channels, these allow water to flow along the osmotic gradient (▶ Sect. 30.9). Ten isoforms that display different permeabilities have been discovered in mammals. They are tetramers that are composed of six transmembrane helices. Each monomer unit forms a channel. The channels are partially made available for water homeostasis by the release of cytosolic vesicles or activation can be achieved by phosphorylation. Regulation of the water channels by drugs represents a diuretic therapy concept, but the treatment of parasitic infection has also been discussed as an additional indication.

22.8 Modes of Action: A Never-Ending Story

The therapy of viral, bacterial, and parasitic diseases attempts to target a pathogen very specifically. For this, various mechanisms are exploited, for example, biosynthetic pathways that are either not present in humans in an identical form

or that do not play an important role in humans. In this way, the danger of adverse effects can be minimized from the beginning.

Antimetabolites are substances that are incorporated as a false substrate instead of the natural biological reagents, for example, as enzyme cofactors or in DNA. An example is the sulfonamide sulfonamidochrysoidine. Its cleavage product sulfanilamide (▶ Sect. 2.3) is similar to *p*-aminobenzoic acid, which is the starting material in the biosynthesis of an important bacterial cofactor, dihydrofolic acid. Only bacteria are affected by this. Humans are not dependent on this biosynthetic pathway. As with other mammals, humans must obtain dihydrofolic acid from food. A few **virostatics** and **tumor-inhibiting substances** are **nucleoside analogues**. Depending on their structure type, they use a modified base, a modified sugar, or both. All influence the DNA or RNA synthesis. Aciclovir and a few other analogues are taken into the cells as **Trojan horses** in the inactive form, and "armed" once inside the cell. Their activation is carried out by viral enzymes, and this process only occurs inside cells that have been infected by the virus (▶ Sects. 9.5 and ▶ 32.5). Another mechanistic principle tries to interfere with the translation process so that particular proteins are never manufactured in the first place by protein biosynthesis. For this, the translation of the mRNA is blocked by complexation to so-called **antisense oligonucleotides** (▶ Sect. 32.4). The formed double-stranded mRNA cannot be read in the ribosome. Such a therapy can find application for the treatment of exaggerated immune reactions, septic shock, arterial hypertension, pulmonary emphysema, or pancreatitis.

Many **antibiotics**, for example, the penicillins and cephalosporins (▶ Sect. 23.7) inhibit the bacterial **cell-wall biosynthesis**. In the latter process, they block the catalytic center of a transpeptidase that shows a similar mode of action to a serine hydrolase (▶ Sect. 23.7). The antibiotic D-cycloserine, also an inhibitor of the cell-wall construction, penetrates the interior of the bacteria by using a D-alanine transporter. Other antibiotics are **protein biosynthesis inhibitors** (▶ Sect. 32.6). Tetracycline (▶ Sect. 6.3), streptomycin (▶ Sect. 6.3), and chloramphenicol (▶ Sect. 9.2) also inhibit the protein synthesis machinery. They undergo an interaction with the 30S or 50S subunit of the ribosome and block ribosomal peptide synthesis. The elucidation of the spatial structure of the ribosome established fundamentals that allowed the mode of action of a large number of macrolide antibiotics to be understood and afforded a perspective on how the mechanisms of resistance is developed (▶ Sect. 32.6). Antibacterial quinolone carboxylic acids inhibit gyrase. The latter enzyme causes a twisting, and as a consequence enables a dense packing of the DNA in the bacterial cells. Without this twisting, there is simply not enough space in the cell for the genetic material. The so-called polyene antibiotics are used to treat fungal infections. They form channels in the fungal cell membrane that causes a loss in intracellular ions and, consequently, cell death. Azoles inhibit the biosynthesis of ergosterol, which is absolutely required for the construction of the intact cell membrane.

Alkylating agents play an important role in tumor therapy. Reading and writing errors occur because of the alkylation of DNA bases, and these errors have a much

stronger effect on quickly dividing tumor cells than in normal cells, but they also have considerable side effects. **Intercalating tumor therapeutics** are planar molecules that slip between two base pairs of DNA (▶ Sect. 14.9). The disruption that occurs as a consequence also leads to errors in cell division. Other DNA ligands bind in the minor or the major groove on the exterior of the double helix. Taxol (▶ Sect. 6.1) and the epothilones are important active substances for cancer therapy. They bind to tubulin, a protein that forms tube-like structures: so-called microtubuli. Because the formation of such structures is an important prerequisite for cell division, Taxol or the epothilones inhibit this process in a very specific way.

The immunosuppressive ciclosporin (▶ Chap. 10, "Peptidomimetics," Fig. 10.2) blocks the **activation of the immune system**, the so-called helper cells. Two enzymes are involved in this process. One of them, cyclophilin, is a prolyl *cis–trans* isomerase. The other, calcineurin, is a Ca^{2+}/calmodulin-dependent phosphatase. Ciclosporin acts as "putty" between these two proteins. The complex formation prevents the activation of helper cells and therefore stops the stimulation of an immune response. Modern transplant surgery would not be possible without the immunosuppressive ciclosporin and substances with an analogous mode of action.

The so-called RAS proteins play an important role in tumorigenesis. They are a family of enzymes with a relatively low molecular weight. RAS proteins with mutated active centers lose their ability to control cell division, and the cells divide unstoppably. Therefore they are oncogenic, that is, they cause tumors. Around 50% of all lung and colorectal tumors have mutated *ras* genes, and about 95% of the *ras* genes in pancreatic tumors are mutated. There are other approaches for therapy. RAS proteins must migrate from the cytosol, the cell fluid, into the cell membrane to signal the cell division. For this, they are enzymatically equipped with a farnesyl group, which anchors the protein in the cell membrane. The **prevention of the membrane embedding** by inhibiting farnesyltransferase represents an attractive approach for targeted cancer treatment (▶ Sect. 26.10). In the meantime, it has been demonstrated that this principle of blocking the farnesylation of proteins can also be used to treat parasitic infections. For this, the farnesyl transferases of these parasites are the target structures for drug development.

Tumor-suppressor genes produce proteins such as the p53 protein that prevent cell division in the case of DNA damage. Any genetic defect in a cell leading to a reduced concentration of one or more of these proteins has the consequence that cells with defective DNA can proliferate. Cell division runs out of control, and a tumor with additional genetic defects and uncontrolled growth forms.

A vascular occlusion is caused by the aggregation of blood platelets. Proteins on the cell surface play an important role, for example, the adhesion glycoprotein $\alpha_{IIb}\beta_3$. Two of these molecules form a complex with fibrinogen that "glues" the cells together. The targeted development of low-molecular-weight peptidomimetics (▶ Sect. 10.6) starting from an RGD motif (RGD stands for Arg-Gly-Asp) represents a great success in rational drug design (▶ Sect. 31.2). Another system that plays an important role in the cell–cell recognition between leukocytes and endothelial cells are the selectins. In cases of inflammation, the E- and P-selectins are upregulated and presented on the

endothelium, and these prevent leukocytes from rolling along the surfaces of the blood vessels (▶ Sect. 31.3). After adhesion, the leukocytes penetrate the vessel and migrate to the site of the inflammation to fight the infection. In some diseases, an excessive leukocyte infiltration leads to tissue damage. To prevent this, an attempt is made to interfere with the inflammatory cascade with compounds that block the **surface exposition of selectins**. These receptors recognize sugar-like molecular groups on the leukocyte surface, therefore the development of appropriate antagonists based on carbohydrates displays a suitable therapeutic concept.

A surface contact must also be formed between the flu virus and the host cell for infection to take place. The virus docks with its capsule protein, hemagglutinin, to the host cell to initiate **endocytosis**. After gaining entry into the cell, it uses the protein biosynthesis machinery of the infected cell to make copies of itself. After maturation, the new virus must be expelled from the cell again. For this, the new virus buds on the cell surface and the bud is finally cinched off. In the last step, the viral neuraminidase cleaves sialic acid. It is through this acid that the viral hemagglutinin is bound to the host cell. This last step can be blocked by neuraminidase inhibitors (▶ Sect. 31.4). The inhibitors zanamivir and oseltamivir have been very successfully introduced to the market. The CCR5 receptor antagonist maraviroc has been launched for the therapy of HIV; the CCR5 receptor acts as an entry gate for the HI virus, and its inhibition blocks host cell invasion.

The endogenous immune system has developed very efficient defensive mechanisms. Antibodies represent one such defensive weapon. These proteins are able to bind to foreign substances very selectively and with high affinity, and to expose them to phagocytotic cells (i.e., dendritic cells and macrophages) for degradation. This sophisticated, highly specific recognition system for molecules, which ranges from very small low-molecular-weight antigens to complex macromolecular systems, has been tapped for pharmaceutical therapy (▶ Sect. 32.3). Today, numerous artificially manufactured antibodies directed against very different target molecules are found in the therapy of many different diseases. There is no end in sight because currently about 200 newly developed antibodies are in clinical trials.

There are only very few really “unspecifically” acting drugs. Antacids, which neutralize gastric acid purely chemically, belong to this class, as do purely surface-active substances, for instance, amphiphilic bactericides, fungicides, and hemolytics. Specific mechanisms of action have been recognized even for the barbiturates, local anesthetics, inhalation anesthetics, and alcohol, which was long considered to be an unspecific agent. Frequently the evidence of a specific effect was provided over the different effects of pure enantiomers of a racemate. The β-antagonistic effect of an optically active β-blocker is associated with one enantiomer (▶ Sect. 5.5). The unspecific adverse effects with membranes, however, are attributed to both enantiomers equally.

Is there anything new to still be discovered? An absolute surprise was the finding that nitrogen monoxide, NO, a miniscule molecule, is also a neurotransmitter. Substances that release NO or that interfere with the NO biosynthesis lower or raise the blood pressure (▶ Sect. 25.8). New subtypes are constantly being discovered for

already-established receptors. The question of to what extent it is reasonable to optimize an active substance for absolute receptor specificity remains an unsolved problem. It can certainly be the case that some active substances with targeted attacks on multiple receptors or their subtypes are better suited for therapy than highly specific analogues. This is particularly valid for compounds that bind to GPCRs. Here, the activity profile against an entire palette of receptor subtypes is critical for the efficacy of a compound. Numerous GPCRs are even involved in our sense of smell, which follows this principle of multiple graduated receptor responses (▶ Sect. 29.7). It is only in this way that the finely tuned and nuance-rich perception diversity can be achieved. This is a broad field of research. To date, particular in CNS-active substances only clinical research can deliver the results needed to make a decision about the therapeutic usefulness of a compound.

22.9 Resistance and Its Origin

Pathogenic viruses, bacteria, and parasites defend themselves against drug therapy. In the past the inappropriate and too-broad use of antibiotics led to **selection pressure for resistant strains**. Unfortunately, it is the hospitals above all that are the main location for the emergence and spread of resistant strains. The spatial proximity and concentration of the most diverse pathogens is virtually unavoidable. In some cases there are only a few effective weapons left, for example, the glycopeptide antibiotics. They should be used prudently and purposefully, even if that goes against the commercial interests of the manufacturer.

Bacterial pathogens overwhelmingly defend themselves against penicillins and cephalosporins by producing β-lactamases (▶ Sect. 23.7). These are enzymes that open the four-membered lactam ring of these antibiotics into inactive cleavage products. During the long time that this substance class was optimized, metabolically stable analogues as well as specific β-lactamase inhibitors were developed.

The causative agent of the immune deficiency disease AIDS (▶ Sects. 1.3 and ▶ 24.3), the HI virus, a retrovirus, transfers its genetic information from the RNA back into DNA. This process is afflicted with an exceedingly high error rate of about one base mutation per generation. The high mutation rate leads to the fast emergence and selection of resistant strains. In the last 10 years many active substances with entirely different modes of action against the HI virus have been introduced to the market, but resistances to many inhibitors were very quickly observed, for example, against the HIV protease (▶ Sect. 24.3) or reverse transcriptase inhibitors (▶ Sect. 32.5), and even multiple resistances. The mutated viruses are even resistant to multiple, structurally different inhibitors! The combination of different active substances against one and the same target does not help here much further. Only a combination of active substances that hit the virus at completely different instances of its lifecycle offers a reprieve.

Tuberculosis is also reemerging. Resistant pathogens require the development of new therapeutics. After the convincing success of the mosquito extermination

campaign with DDT and therapy with synthetic antimalarials, malaria is again progressing in developing countries.

The largest problem in the therapy of tumors is the development of **multidrug resistance** (MDR) during the treatment. The resistance is not only against the causative agent but rather it occurs simultaneously against entirely different tumor therapeutics. This multidrug resistance is due to the overexpression of a transporter (▶ Sects. 22.7 and ▶ 30.8), glycoprotein 170, which can largely eliminate structurally deviating xenobiotics from the cell. Although GP170 prefers cationic substances, another transporter, the multidrug resistance-associated protein (MRP) eliminates amphiphilic anionic substances, compounds with polar and nonpolar character. But amphiphilic substances are also able to break the resistance of tumor cells. Quantitative structure–activity relationships show that tumor cell resistance to particular drugs is mainly associated with similarities in their molecular weights, that is, the size of the inducing agent, and its lipophilicity.

22.10 Combined Administration of Drugs

Combination drugs are very popular with pharmaceutical manufacturers, doctors, and patients alike. The manufacturers value them because they expand the indication field of a successful substance and bring new life into their sales figures. Some physicians are pleased that the therapy is simplified in many cases, but others reject such combination preparations. An advantage for older patients is that they do not need to take so many different medications at different times of the day and in different doses, rather only one or a few combination drugs. This improves the reliability of the dosing, that is, the compliance. One of the most common reasons for therapy failure is, in fact, the behavior of the patient. Either the regular dose is forgotten, or the patient gives himself or herself a break from the regime over the weekend or while on vacation. These behaviors are particularly pronounced in older patients, with medications that show no obvious immediate success, or with drugs that have side effects that the patient subjectively experiences as unpleasant.

Clinical pharmacologists, academics, and many critically oriented physicians have considerable reservations regarding **combination preparations**. This is understandable if one considers that the attitude of a patient to a particular medication requires the observation of a dose–effect relationship over a long period of time, and finally, an individual therapy. In a combination medication, there is always a fixed relationship between the individual components. Many combinations, for example, analgesics, contain components with different modes of action. These are often misused without a strict medical indication, and are therefore to be judged critically.

There are **reasonable combinations** that even opponents to the general concept of combination therapy would accept without reservations. Among these are

- L-DOPA preparation with which the side effects can be reduced by selective combination (▶ Sects. 9.4, ▶ 26.9, and ▶ 27.8);

- Antihypertensives and diuretics, the different mechanistic principles of which complement one another;
- Antibacterial preparations in which a dihydrofolate reductase inhibitor (▶ Sect. 27.2) is combined with an appropriate sulfonamide;
- Hormonal contraceptives (▶ Sect. 28.5);
- Polyvalent vaccines, with which a single application offers protection against multiple diseases.

In the case of L-DOPA therapy, only combinations of multiple active substances reduce the side effects to a tolerable level. A single principle is often not enough to accomplish the same effect that is achieved with combinations in the case of antihypertensives and diuretics. In the case of the sulfonamide combinations and with antituberculosis compounds, the action over diverse modes of action can prevent or delay the development of resistance. An inhibitor for the P450 family of metabolic enzymes can be justifiable as an adjuvant to expensive medication or with drugs that are used in very high doses. In this way, the concentration of the other drug can be held at a higher level and for a longer time (▶ Sect. 27.7). An important prerequisite for all combination medications are an adequate therapeutic window and adapted pharmacokinetics of the components, at least those that support the actual mode of action.

22.11 Synopsis

- A relatively small portion of the **druggable genome** has been pharmaceutically addressed, and GPCRs overrepresent the targets for which active substances are available. **Protein kinases** represent a particularly promising emerging family of targets.
- **Enzymes** are very popular drug targets, and the natural substrates often provide the starting point for a rational drug-design approach. There are three types of enzyme inhibitors, **competitive inhibitors**, **non-competitive inhibitors**, and **allosteric inhibitors**. Enzyme inhibition can also be classified as **reversible** and **irreversible**. Nowadays reversible inhibition is desired, but some very important drugs are irreversible inhibitors, and some reversible inhibitors have such high affinity that they are *de facto* irreversible inhibitors.
- Receptors are also important drug targets; they can be subdivided into GPCRs, ion channels, hormone receptors, and growth factor receptors. An **agonist** activates the receptor, an **antagonist** prevents the agonist from docking at its binding site, and an **inverse agonist** stabilizes an inactive conformation of the receptor.
- **Ion channels** are extremely fast gateways for ions and can be either voltage- or ligand-gated. Ions can flow only passively with the concentration gradient through an ion channel.
- **Transporters** are special proteins in the membrane that can pump molecules and ions against the concentration gradient at the expense of ATP hydrolysis. Many transporters are attractive drug targets, and others are responsible for the development of drug resistance.

- There are a large variety of known modes of action for drugs. Some of the most diverse modes of action are found in anti-infective drugs. Furthermore, tumor therapeutics exploit diverse, toxic modes of action. The goal in addressing these modes of action in terms of a therapy is to find a pathophysiological process that is unique, or is as unique as possible to the disease to spare healthy tissue from damage.
- **Drug resistance** is an increasingly serious problem and is both an **inevitable** occurrence associated with using a pharmaceutical therapy, and a consequence of the **misuse** of anti-infectives. There are several mechanisms of resistance development in bacteria (i.e., enzyme production), viruses (i.e., fast genetic mutations), and in cancer therapy (i.e., aberrant transporter expression). These mechanisms are not mutually exclusive.
- The issue of combination drugs is a controversial topic. Some physicians are against them, and others are in favor of them, and both sides of the argument have good reasons. Nonetheless, some **drug combinations** are justifiable and help with compliance, clinical efficacy and safety.

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