2DE

►Two-dimensional Electrophoresis
►Proteomics

14-3-3σ

The 14-3-3 proteins constitute a family of abundant, highly conserved and broadly expressed acidic polypeptides. One member of this family, the 14-3-3σ isoform {sigma}, is expressed only in epithelial cells and is frequently down-regulated in a variety of human cancers and plays a role in the cellular response to DNA damage. The 14-3-3s generally form heterodimers with other family members, but 14-3-3σ preferentially forms homodimers in cells. Three amino acids that are completely conserved in all other 14-3-3s, are not present in 14-3-3σ. These amino acids unique to 14-3-3σ confer a second ligand-binding site involved in 14-3-3σ-specific ligand discrimination.

►14-3-3 Proteins

A Kinase Anchoring Proteins (AKAPs)

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Synonyms

Protein kinase A anchoring proteins

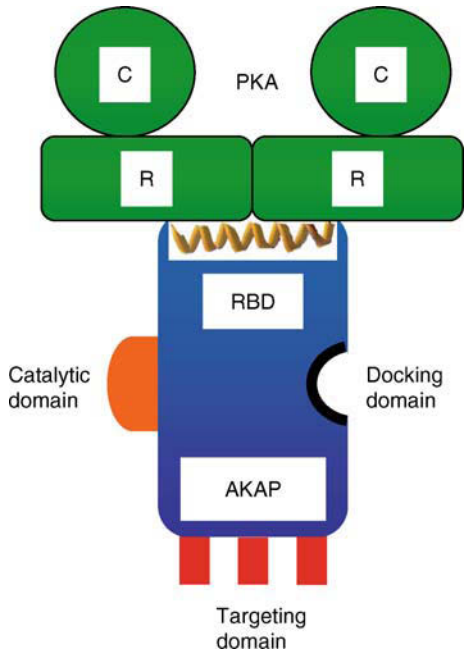
Definition

AKAPs are a diverse family of about 75 scaffolding proteins. They are defined by the presence of a structurally conserved protein kinase A (PKA)-binding domain. AKAPs tether PKA and other signalling proteins to cellular compartments and thereby limit and integrate cellular signalling processes at specific sites. This compartmentalization of signalling by AKAPs contributes to the specificity of a cellular response to a given external stimulus (e.g. a particular hormone or neurotransmitter).

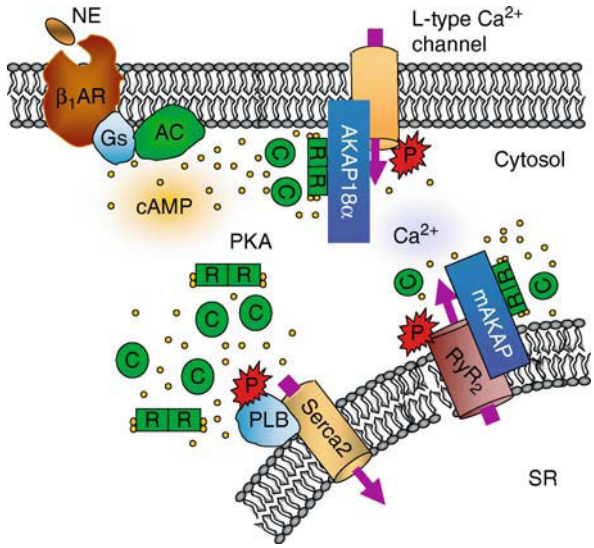
Basic Mechanisms

AKAP-dependent Control of cAMP/PKA Signalling

A large variety of extracellular stimuli including hormones and neurotransmitters elicit the generation of the second messenger cyclic adenosine monophosphate (cAMP). Cyclic AMP binds to several effector proteins including ion channels, cAMP-dependent guanine-nucleotide-exchange factors (Epacs) and PKA. The latter is the main effector of cAMP. Binding of four molecules of cAMP activates the kinase. Activated PKA transfers a phosphate group from adenosine triphosphate (ATP) to consensus sites on many different substrate proteins and thereby modulates their activity. It appears that different external stimuli mediate activation of specific pools of PKA located at defined sites within cells (compartments) including, for example mitochondria, nuclei, exocytic vesicles, sarcoplasmic reticulum and the cytosol [1]. A kinase anchoring proteins (AKAPs; Fig. 1) tether PKA to such cellular compartments and allow for its local activation, and consequent phosphorylation of particular substrates in close proximity [2]. Spatial and temporal coordination of PKA signalling through compartmentalization by AKAPs is considered essential for the specificity of PKA-dependent cellular responses to a particular external stimulus [3, 4]. AKAP–PKA interactions play a role in a variety of cellular processes including β-adrenoceptor-dependent regulation of cardiac myocyte contraction (Fig. 2), vasopressin-mediated water reabsorption, proton secretion from gastric parietal cells, modulation of insulin secretion from pancreatic β cells and T cell receptor signalling. A typical AKAP is AKAP18α, also termed AKAP15. It tethers PKA to ►L-type Ca^{2+} channels in



A Kinase Anchoring Proteins (AKAPs).
Figure 1 Model of an A kinase anchoring protein (AKAP). The unifying characteristic of AKAPs is the presence of a structurally conserved binding domain for the dimer of regulatory (R) subunits of PKA (RBD, regulatory subunit binding domain). In the inactive state, PKA forms a tetramer consisting of a dimer of R subunits each bound to one catalytic subunit (C). Binding of two molecules of cAMP to each R subunit causes a conformational change and release of the C subunits, which in the free form phosphorylate substrate proteins in close proximity. The RBD in all AKAPs with pericentrin as the only exception forms an amphipathic helix that docks into a hydrophobic pocket formed by the dimerization and docking domain of R subunits. The targeting domain, which tethers the AKAP complex to cellular compartments and docking domains, which bind further signalling proteins (e.g. phosphodiesterases, phosphatases or other kinases) are specific for individual AKAPs. A few AKAPs possess catalytic activity such as the RhoGEF-activity in AKAP-Lbc conferred by a DH domain. The proteins within the AKAP family are without obvious sequence homology.



A Kinase Anchoring Proteins (AKAPs).
Figure 2 β-adrenoceptor-induced increases in cardiac myocyte contractility depend on AKAP–PKA interactions. Stimulation of β-adrenoceptors (β_1AR) on the surface of cardiac myocytes by binding of adrenergic agonists such as norepinephrine (NE), epinephrine or isoproterenol increases contractility of the heart. Agonist binding to the receptors activates the G protein G_s and adenylyl cyclase (AC), and consequent synthesis of cAMP which binds to regulatory (R) subunits of protein kinase A (PKA) inducing dissociation of catalytic (C) subunits (see also Fig. 1). The C subunits phosphorylate L-type Ca^{2+} channels located in the plasma membrane (plasmalemma) and ryanodine receptors (RyR_2) embedded in the membrane of the sarcoplasmic reticulum (SR). Phosphorylation of the two channel proteins increases their open probability and leads to an increase in cytosolic Ca^{2+} causing increased contractility. For the relaxation of cardiac myocytes, Ca^{2+} has to be removed from the cytosol. A pivotal role in this plays sarcoplasmic Ca^{2+} ATPase (SERCA). It pumps Ca^{2+} back into the SR. SERCA is inhibited when bound to phospholamban (PLB) and activated upon dissociation from PLB, which is induced by β-adrenoceptor-mediated PKA phosphorylation. Collectively, the PKA phosphorylation events increase cardiac myocyte contractility. The efficient phosphorylation of L-type Ca^{2+} channels occurs only if PKA is anchored to the channel by AKAP18α. For the phosphorylation of RyR, PKA anchoring to this channel by mAKAP is a prerequisite. Further AKAPs are likely to be involved in PKA-dependent phosphorylation events in response to β-adrenoceptor stimulation (e.g. PLB).

cardiac myocytes and skeletal muscle cells and facilitates their phosphorylation in response to β-adrenoceptor activation. The phosphorylation increases the open probability of the channel.

The tethering of PKA through AKAPs by itself is not sufficient to compartmentalize and control a cAMP/PKA-dependent pathway. Cyclic AMP readily diffuses throughout the cell. Therefore, discrete cAMP/PKA signalling compartments are only conceivable if this diffusion is limited. ►Phosphodiesterases (PDE) establish gradients of cAMP by local hydrolysis of the second messenger and thereby regulate PKA activity locally. Several AKAPs interact with PDEs and thus play a role at this level of control. For example, the interaction of muscle-specific mAKAP with cAMP-specific PDE4D3 and the ►ryanodine receptor (RyR)

facilitates hydrolysis of cAMP in the vicinity of RyR at the sarcoplasmic reticulum of cardiac myocytes. Local cAMP hydrolysis keeps mAKAP-associated PKA activity low. An increase in the cAMP level exceeding the PDE4D3 hydrolyzing capacity activates PKA, which phosphorylates RyR and increases the open probability of this Ca^{2+} channel. PKA also phosphorylates mAKAP-bound PDE4D3 and thereby enhances PDE4D3 activity. This again increases local cAMP hydrolysis, switches off PKA, and eventually reduces RyR phosphorylation. This negative feedback loop regulating RyR phosphorylation is completed by association of mAKAP with protein phosphatase 2A (PP2A), dephosphorylating RyR. Dephosphorylation decreases the channel open probability of RyR.

AKAP-dependent Integration of Cellular Signalling

In addition to PKA, PDEs and protein phosphatases involved in cAMP signalling, AKAPs interact with other signalling proteins whose activation depends on second messengers other than cAMP, e.g. Ca^{2+}. AKAPs may bind additional kinases such as protein kinases C (PKC) and D (PKD), and further protein phosphatases such as calcium/calmodulin-dependent phosphatase (calcineurin, protein phosphatase 2B, PP2B). This scaffolding function allows AKAPs to integrate cellular signalling processes. For example, rat AKAP150 and its human ortholog AKAP79 bind PKA, PKC and calcineurin. In neurons, AKAP150-bound PKC is activated through a M_1 muscarinic receptor-induced pathway that depends on the G protein G_q and leads to elevation of cytosolic Ca^{2+} and diacylglycerol. AKAP150 interacts directly with M channels (K^+ channel negatively regulating neuronal excitability) and facilitates PKC phosphorylation and thereby inhibition of this channel. AKAP79 coordinates the phosphorylation of ►AMPA channels. Cyclic AMP-activated AKAP79-bound PKA phosphorylates and thereby activates the channels. A raise of cytosolic Ca^{2+} activates AKAP79-bound calcineurin, which in turn dephosphorylates the channels. The dephosphorylation mediates the rundown of AMPA channel currents.

AKAP-Lbc binds PKA, PKC and PKD and possesses intrinsic catalytic activity (Rho guanine nucleotide exchange factor (RhoGEF) activity). Through its RhoGEF activity it catalyses the exchange of GDP for GTP on the ►small GTPase Rho. The GTP form of Rho is active and induces the formation of F-actin-containing stress fibres. Agonists stimulating receptors coupled to the G protein G_s may mediate activation of AKAP-Lbc-bound PKA, which in turn phosphorylates AKAP-Lbc. Subsequently, a protein of the ►14–3–3 family binds to the phosphorylated site and inhibits the RhoGEF activity. In contrast, agonists stimulating receptors coupled to the G protein G_{12} increase the RhoGEF activity.

AKAPs Optimise the Limited Repertoire of Cellular Signalling Proteins

Intriguingly, the same AKAP may coordinate regulation of different target proteins. In hippocampal neurons, AKAP150 positions PKA and calcineurin to modulate AMPA channels and maintains PKC inactive. In superior ganglial neurons, AKAP150 facilitates PKC phophorylation of M channels while keeping PKA and calcineurin inactive. The difference is due to the interaction of AKAP150 with the scaffolding protein SAP97, which occurs in hippocampal neurons but not in superior ganglial neurons. SAP97 positions AKAP150 such that PKA and calcineurin are in close proximity to AMPA channels. Thus by variation of a single interacting partner an AKAP optimises the usage of the limited set of cellular signalling proteins.

In summary, the function of AKAPs goes far beyond controlling cAMP/PKA signalling by simply tethering PKA to cellular compartments and confining the access of PKA to a limited set of local substrates. AKAPs are scaffolds forming multiprotein signal transduction modules, recently termed “AKAPosomes” that coordinate and integrate cellular signalling processes.

Pharmacological Intervention

Disturbances of compartmentalized cAMP signalling in processes such as the ones mentioned above cause or are associated with major diseases including congestive heart failure, diabetes insipidus, diabetes mellitus, obesity, diseases of the immune system (e.g. AIDS), cancer and neurological disorders including schizophrenia. However, AKAPs participating in compartmentalized cAMP signalling networks are not targeted by drugs which are currently applied for the treatment of such diseases.

Recently, clinically relevant intracellular protein–protein interactions have gained much interest as potential drug targets. The cell-type specificity of such interactions and the finding that mostly only selected isoforms of proteins interact with each other offers great opportunities for highly selective pharmacological intervention. For targeting AKAP-dependent protein–protein interactions, peptides non-selectively displacing PKA from all AKAPs have been developed so far. For example, the peptides functionally uncouple, PKA from L-type Ca^{2+} channels in cardiac myocytes by disruption of the AKAP18α–PKA interaction that facilitates L-type Ca^{2+} channel phosphorylation (see above). This prevents β-adrenoceptor-induced increases in cytosolic Ca^{2+}, an effect resembling that of β-blockers. In renal collecting duct principal cells, vasopressin regulates water reabsorption from primary urine by triggering the PKA phosphorylation and subsequent redistribution of aquaporin-2 (AQP2) from intracellular vesicles into the plasma membrane. The redistribution depends on

the compartmentalization of PKA by AKAPs, one of which is AKAP18δ. The PKA anchoring disruptor peptides displace PKA from AQP2-bearing vesicles and inhibit vasopressin-mediated water reabsorption, i.e. have an aquaretic effect. These examples suggest that cell-type specific pharmacological intervention at selected AKAP–PKA interactions is a feasible concept for the treatment of human diseases (e.g. cardiovascular disease or diseases associated with water retention).

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ABC-proteins

►ABC Transporters

ABC Transporters

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Synonyms

ATP-binding cassette proteins; ABC-proteins

Definition

The ►ABC-transporter superfamily represents a large group of transmembrane proteins. Members of this family are mainly involved in ATP-dependent transport processes across cellular membranes. These proteins are of special interest from a pharmacological point of view because of their ability to transport numerous drugs, thereby modifying intracellular concentrations and hence effects.

Basic Characteristics

ATP-binding cassette (ABC-) proteins have been identified in all living organisms; they are present in plants, bacteria, and mammalians. In humans the ABC-superfamily comprises about 50 members; on the basis of homology relationships this superfamily is organized in several subfamilies named ABCA to ABCF. Not all of them are pharmacologically important, for example, members of the A branch are mainly involved in lipid trafficking. ABCB2 as well as ABCB3, which are also termed as *transporter associated with antigen processing* (TAP), are involved in the transport of peptides presented by Class I HLA molecules. However, other ABC-transporters like ABCB1 (►P-glycoprotein, P-gp), ABCG2 (*breast cancer resistance protein*, BCRP) and several members of the C-branch are of high pharmacological relevance because they are involved in transport of several drugs; thereby affecting pharmacokinetic parameters.

ABCB1, for example, which is also known as P-glycoprotein (P-gp) and probably the best characterized ABC-transporter, has been identified as the underlying mechanism of a cancer-related phenomenon called ►multidrug resistance (therefore, P-gp is also termed *multidrug resistance protein (*MDR1)), which is characterized by the resistance of cancer cells against drug therapy. Interestingly, this phenomenon is not directed against a single drug or structurally related entities, but comprises unrelated compounds with different target structures. Meanwhile, besides P-gp further ABC-transporters have been identified to be involved in this process. For example, in 1992 Cole et al. identified the first member of the so-called *multidrug resistance related proteins* (MRP). The ►MRP-proteins (MRP1–MRP9) belong to the C-branch of the ABC-superfamily, which currently consists of a total of 13 members (ABCC1–ABCC13). In addition to ABCB1 and the ABCC family, a member of the ABCG family has recently been demonstrated to confer drug resistance. This protein called *breast cancer resistant protein* (►BCRP/ABCG2) was first identified in mitoxantrone resistant cell lines, which lack expression of P-gp or MRP1.

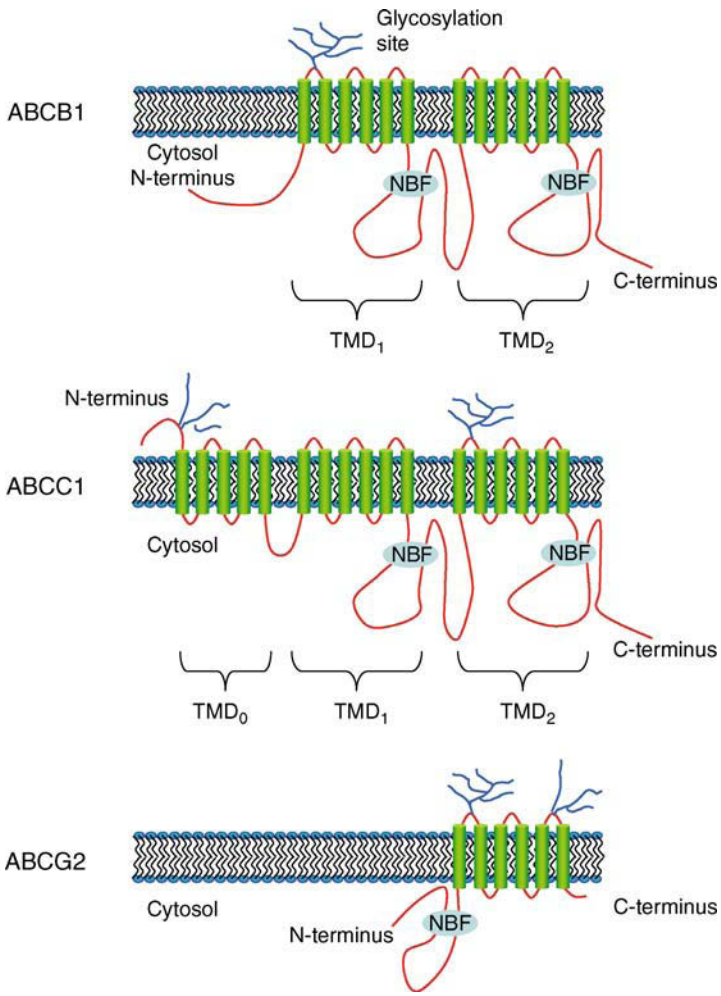
Topology and Structure

Most ABC-transporters, especially those located in the plasma membrane, are phosphorylated and glycosylated transmembrane proteins of different molecular weights (e.g., P-gp: 170 kDa; MRP2: 190 kDa; BCRP: 72 kDa). Topologically, most ABC-transporter show a similar structure: they are organized in two transmembrane domains (TMD), each consisting of six

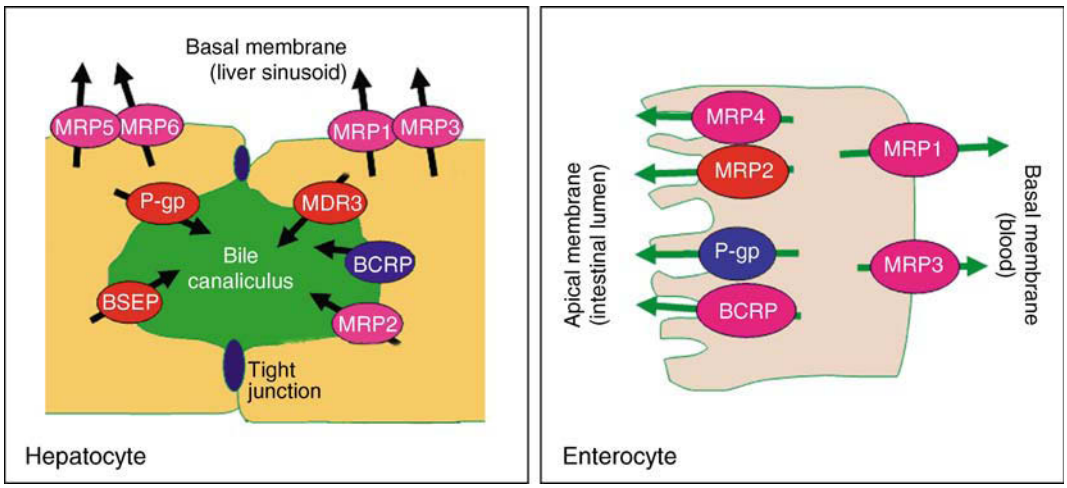
α-helical, transmembranal segments and two ATP binding domains linked to the C-terminus of the TMDs. These domains, which are also termed *nucleotide binding folds* (NBFs), contain the highly conserved Walker A and B consensus motifs and the LSGGQ motif (also called C- or signature motif). While the Walker A and B motifs are also found in other ATP-hydrolyzing ATP proteins, the LSGGQ motif is unique for the ABC-transporters. The ATP hydrolysis catalyzed by the NBFs is a prerequisite for substrate binding and enables transport against a substrate gradient. In addition to these general characteristics, several members of the ABCC-family (e.g., ABCC1–3) contain a further N-terminal TMD, which, however, is not required for transport activity. In contrast to the other TMDs, this N-terminal TMD contains five transmembranal segments and lacks the NBF. Besides this structural variant some other ABC-transporters (e.g., ABCB2, 3 (TAB1 and 2), as well as ABCG2) contain only one TMD and NBF (Fig. 1). Therefore, these transporters are termed *half transporter* (in contrast to *full transporter*); however, to achieve functional activity they have to form hetero- or homodimers.

Tissue Distribution and Expression

Although initially detected in cancer cell lines ABC-transporters show a wide tissue distribution. Several members of drug transporting ABC-proteins, for example, are highly expressed in physiological barriers such as the apical membrane of gut enterocytes, the endothelial cells of the blood–brain barrier or the maternal facing (apical) membrane of the placental syncytiotrophoblast. In all of these organs they protect sensitive tissues like brain or the growing fetus against potentially toxic compounds. In addition, ABC-transporter expression is highly abundant in hepatocytes (Fig. 2). Here, ABC-transporters are involved in detoxification of many endogenous and exogenous



ABC Transporters. Figure 1 Structure of ABCB1, ABCC1, and ABCG2 (NBF: *nucleotide binding fold*; TMD: *transmembrane domaine*. Modified according to ►www.iwaki-kk.co.jp/bio/specialedition/se02.htm).



ABC Transporters. Figure 2 ABC-transporter expression in hepatocytes and enterocytes (modified according to ►www.iwaki-kk.co.jp/bio/specialedition/se02.htm).

agents and are therefore expressed both in the canalicular and sinusoidal membrane. The canalicular expression is a prerequisite for bilary elimination. For example, the *bile salt export pump* (BSEP/ABCB11) is transporting bile salts, MRP2 (ABCC2) is involved in the elimination of organic anions like bilirubin-glucuronide or glutathione-conjugates and finally, P-gp (ABCB1) eliminates a wide variety of drugs into the bile. In contrast, other ABC-transporters like MRP1 (ABCC1) and 3 (ABCC3) are mainly located in the basal membrane of hepatocytes. They transport xenobiotics and several conjugates back to the blood and seem to be important under certain pathophysiological conditions, for example, hepatic expression of both transporters is enhanced during cholestasis, thereby protecting the hepatocytes against toxic bile acid concentrations by transport into the blood followed by increased renal elimination.

Various ABC-transporters are expressed in organs like heart, lung, pancreas, or cellular blood compounds. They may be important both for physiological processes and local drug concentrations. In this context, it is noteworthy that many of these transporters not only eliminate xenobiotic and toxic compounds from the cell, but also endogonous compounds. For example, MRP4, 5, and 8 (ABCC4, 5, and 11), which are expressed in many tissues and cancer cells, not only transport xenobiotics like nucleotide-based anticancer drugs but also the second messenger molecules cAMP and cGMP. Thereby, these transporters may play a role in regulating intra- and extracellular cyclic nucleotide concentrations.

ABC-Transporters and Disease

Based on their physiological function it is not surprising that genetic polymorphisms affecting expression and function of ABC-proteins have been identified as the underlying mechanisms for some diseases. For example, mutations in the MRP2 (ABCC2) gene, which lead to the loss of this protein from the canalicular membrane of hepatocytes, are the mechanism of the ►Dubin–Johnson Syndrome. Here, the bilary elimination of MRP2 substrates like bilirubin and bilirubin-glucuronide is blocked; therefore the respective plasma levels are elevated leading to the disease. Another example is ABCC7, which is also called *cystic fibrosis transmembrane conductance regulator* (CFTR) and forms an anion channel in different tissues like the epithelial surfaces of the respiratory and intestinal tract. As its alias indicates, ABCC7 is involved in the pathogenesis of *cystic fibrosis*, because mutations in the ABCC7 gene associated with dysfunction or epithelial absence of the transporter are the underlying reason for the incorrect ion homeostasis, especially for chloride, which is the predominate anion transported by ABCC7 under physiological conditions.

Drugs

In this context, two aspects are important. First, many drugs are substrates of ABC-transporters and therefore these transporters might affect the bioavailability of these substances. Tissues like liver, intestine, and kidney exhibit high expression levels of different transport proteins. Therefore, substrates of these transporters may be intensively eliminated to the bile and urine or transported back to the intestine, thereby limiting oral bioavailability. Besides these pharmacokinetic important organs, ABC-transporters are expressed in target tissues of certain drugs. As already mentioned this point carries an unsolved problem in chemotherapy because many anticancer drugs are ABC-transporter substrates and tumor cells often show an enhanced

transporter expression and therefore MDR. However, this problem is not restricted to cancer therapy. For example, ABC-transporters are also expressed in the ►blood–brain barrier; thereby limiting the access of drugs to the brain. While this is useful for drugs like loperamide, a morphine-based drug against diarrhea, it might be a problem in the case of antipoychotic and antiepileptic drugs. A list of ABC-transporters and their substrates is given in Table 1.

Second, as already shown for P450 enzymes before, there is also a drug-interaction potential on the transporter level. The promoter regions of some ABC-transporter genes (e.g., P-gp) contain transcription factor binding sites like the *pregnane X receptor* (PXR), the *constitutive androstane receptor* (CAR), the *farnesoid X receptor* (FXR), the *steroid and xenobiotic receptor* (SXR) or the *peroxisome proliferator-activated receptor* (PPAR). Therefore, these proteins are not only regulated by endogenous compounds like bile acids or steroid hormones but also by therapeutic agents like phenobarbital, rifampicin, or dexamethasone. This regulation might be accompanied by an altered bioavailability of transporter substrates, when coadministered with these compounds. For example, the decreased bioavailability of digoxin, a P-gp substrate, after coadministration of rifampicin is due to an enhanced intestinal P-gp expression. On the other hand, many compounds are inhibitors of ABC-transporters (in the case of P-gp, for example, verapamil, ketoconazole, amiodarone, progesterone, indinavir, clarithromycin, cyclosporine, chlorpromazine, or methadone), which in turn leads to higher plasma levels after coadministration of substrates for these transporters.

In addition, ABC-transporters demonstrate interindividual variability caused by genetic polymorphisms. Again, the *ABCB1* (P-gp) is the best characterized transporter in this field. Here, various synonymous and nonsynonymous polymorphisms as well as deletions and insertions have been described. Some of the nonsynonymous *single nucleotide polymorphisms* (SNPs) have already been shown to be associated with an altered transport activity of the protein. Interestingly, this observation has also been made for the C- to T-variant at position 3435, which represents the most frequent synonymous SNP of *ABCB1*. This C3435T polymorphism could be associated with an altered protein expression and function of P-gp, because individuals homozygous for this polymorphism show a significant lower intestinal P-gp expression. This finding was underlined by elevated digoxin plasma levels in patients homozygous for this SNP in comparison with the wild type. Recent data suggest that the altered protein expression and function of this variant may be due to the presence of a rare codon, which affects the timing of cotranslational folding and insertion of the protein into the membrane.

Taken together ABC-transporters represent a large family of proteins affecting the pharmacokinetic parameters of various drugs. Here, P-gp is currently the best characterized member and it may also be one of the most important ABC-transporters with regard to drug transport. However, it becomes more and more apparent that ABC-transporter act in a coordinated

ABC Transporters. Table 1 Substrates of ABC-transporters involved in multidrug resistance (MDR)

ABC-transporter	Transporter substrates
P-gp (ABCB1)	Verapamil, digoxin, mitoxantrone, vinblastine, doxorubicin, losartan, talinolol, cortisol, dexamethasone, colchicine, loperamide, domperidone, indinavir, erythromycin, tetracycline, itraconazole, cyclosporine, methotrexate, amitryptyline, phenobarbital, morphine, cimetidine, and others
MRP1 (ABCC1)	Glucuronides and sulfate conjugates of steroid hormones and bile salts, colchicine, doxorubicin, daunorubicin, epirubicin, folate, irinotecan, methotrexate, pacitaxel, vinblastine, vincristine, and others
MRP2 (ABCC2)	LTC4, bilirubin-glucuronide, estradiol 17β-glucuronide, dianionic bile salts, anionic conjugates, glutathione disulfide, and others
MRP3 (ABCC3)	Organic anions including bile salts
MRP4 (ABCC4)	PMEA, PMEG, ganciclovir, AZT, 6-mercaptopurin, thioguanine, methotrexate, cAMP, cGMP, estradiol 17β-glucuronide, DHEAS, sulphated bile acids, glutathione, PGE1, PGE2, and others
MRP5 (ABCC5)	PMEA, PMEG, cladribine, gemcitabine, cytarabine, 5-FU, 6-mercaptopurine, thioguanine, cAMP, cGMP, glutathione, DNP-SG, $CdCl_2$, and others
BCRP (ABCG2)	Cisplatin, folate, methotrexate, mitoxantrone, topotecan, irinotecan, steroids (cholesterol, testosterone, progesterone), certain chlorophyll metabolites, and others

fashion with other detoxification systems like P450 enzymes and ►uptake transporters. In particular, P-glycoprotein and Cytochrome P450 3A4 are closely intertwined in terms of regulation and function. Thus, further reviews have to address the combined action of various systems.

►MDR-ABC-Transporters
►ATP-dependent K^+ Channel
►Antracyclins

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ABPs

►Actin Binding Proteins

Absence Epilepsy

Absence Epilepsies are a group of epileptic syndromes typically starting in childhood or adolescence and characterized by a sudden lack of attention and mild automatic movements for some seconds to minutes. Absence epilepsies are generalized, i.e. the whole neocortex shifts into a state of sleep-like oscillations.

►Antiepileptic Drugs

Absorption

Absorption is defined as the disappearance of a drug from the site of administration and its appearance in the blood ("central compartment") or at its site of action. The main routes of administration are oral or parenteral (injection). After oral administration, a drug has to be taken up (is absorbed) from the gut. Here, the main site of absorption is the small intestine. In this case, only a portion of drug reaches the blood and arrives at its site of action.

►Pharmacokinetics

Abstinence Syndrome

The abstinence syndrome (synonym, withdrawal symptom) is observed after withdrawal of a drug to which a person is addicted. For example, the abstinence syndrome after alcohol withdrawal is characterized by tremor, nausea, tachycardia, sweating and sometimes hallucinations.

►Drug Addiction
►Dependence

Abused Drugs

►Drug Addiction/Dependence

Acadesine

5-Aminoimidazole-4-carboxamide ribonucleoside (also known as AICA riboside or AICAR). An adenosine analogue that is taken up into cells by adenosine transporters and converted by adenosine kinase to the monophosphorylated nucleotide form, ZMP. ZMP is an analogue of AMP that activates the AMP-activated protein kinase (AMPK), for which acadesine or AICAR can be used as a pharmacological activator.

►AMP-activated Protein Kinase

ACE Inhibitors

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Synonyms

Angiotensin-converting enzyme inhibitors

Definition

Angiotensin converting enzyme (ACE) plays a central role in cardiovascular hemostasis. Its major function is the generation of angiotensin (ANG) II from ANG I and the degradation of bradykinin. Both peptides have profound impact on the cardiovascular system and beyond. ACE inhibitors are used to decrease blood pressure in hypertensive patients, to improve cardiac function, and to reduce work load of the heart in patients with cardiac failure.

Mechanism of Action

ACE inhibitors inhibit the enzymatic activity of ►angiotensin converting enzyme (ACE). This enzyme cleaves a variety of pairs of amino acids from the carboxy-terminal part of several peptide substrates. The conversion of ANG I to ANG II and the degradation of bradykinin to inactive fragments are considered the most important functions of ACE [1–3]. ACE inhibitors are nonpeptide analogues of ANG I. They bind tightly to the active sites of ACE, where they complex with a zinc ion and interact with a positively charged group as well as with a hydrophibic pocket. They competitively inhibit ACE with Ki values in the range between 10^{-10} and 10^{-11} [3].

Effects of ACE Inhibitors Mediated by the Inhibition of ANG II Generation

ANG II is the effector peptide of the renin–angiotensin system [1, 2]. ANG II is one of the most potent vasoconstrictors, fascilitates norepinephrine release, stimulates aldosterone production, and increases renal sodium retention. In addition, ANG II is considered to be a growth factor, stimulating proliferation of various cell types. The actions of ANG II are mediated through two ►angiotensin receptors, termed AT_1 and AT_2. Most of the cardiovascular functions of ANG II are mediated through the AT_1 receptor.

In some patients with hypertension and in all patients with cardiac failure, the renin–angiotensin system is activated to an undesired degree, burdening the heart. The consequences of diminished ANG II generation by ACE inhibitors are multiple. In patients with hypertension, blood pressure is reduced as a result of (i) decreased peripheral vascular resistance, (ii) decreased sympathetic activity, and (iii) reduced sodium and water retention. In patients with cardiac failure, cardiac functions are improved as a result of (i) reduced sodium and water retention (preload and afterload reduction), (ii) diminished total peripheral resistance (afterload reduction), and (iii) reduced stimulation of the heart by the sympathetic nervous system. A reduction of cardiac hypertrophy appears to be another desired effect of ACE inhibitors. It is mediated at least partially by the reduction of intracardiac ANG II levels. ACE inhibitors furthermore protect the heart from arrhythmia during reperfusion after ischemia, and improve local blood flow and the metabolic state of the heart. These effects are largely mediated by Bradykinin (see below).

In the vasculature, ANG II not only increases contraction of smooth muscle cells, but is also able to induce vascular injury. This can be prevented by blocking ►NFκB activation [3] suggesting a link between ANG II and inflammation processes involved in the pathogenesis of arteriosclerosis (see below). Thus, ACE inhibitors not only decrease vascular tone but probably also exert vasoprotective effects.

In the kidney, ANG II reduces renal blood flow and constricts preferentially the efferent arteriole of the glomerulus with the result of increased glomerular filtration pressure. ANG II further enhances renal sodium and water reabsorption at the proximal tubulus. ACE inhibitors thus increase renal blood flow and decrease sodium and water retention. Furthermore, ACE inhibitors are nephroprotective, delaying the progression of glomerulosclerosis. This also appears to be a result of reduced ANG II levels and is at least partially independent from pressure reduction. On the other hand, ACE inhibitors decrease glomerular filtration pressure due to the lack of ANG II-mediated constriction of the efferent arterioles. Thus, one important undesired effect of ACE inhibitors is impaired glomerular filtration rate and impaired kidney function.

Another effect of ANG II is the stimulation of ►aldosterone production in the adrenal cortex. ANG II increases the expression of steroidogenic enzymes, such as aldosterone synthase and stimulates the proliferation of the aldosterone-producing zona glomerulosa cells. Aldosterone increases sodium and water reabsorbtion at the distal tubuli. More recently it has been recognized that aldosterone is a fibrotic factor in the heart. ACE inhibitors decrease plasma aldosterone levels on a short-term scale, thereby not only reducing sodium retention but also preventing aldosterone-induced cardiac fibrotic processes. On a long-term scale, however, patients with cardiac failure exhibit high aldosterone levels even when taking ACE inhibitors.

In this context, it is important to note that circulating ANG II levels do not remain reduced during long-term treatment with ACE inhibitors. This is likely the result of activation of alternative, ACE-independent pathways of ANG II generation. The protective effects of ACE inhibitors on a long-term scale, therefore, are not explained by a reduction of circulating ANG II levels. They are either unrelated to inhibition of ANG II generation, or a result of the inhibition of local generation of ANG II. Indeed, due to the ubiquitous presence of ACE in endothelial cells, large amounts of ANG II are generated locally within tissues such as kidney, blood vessels, adrenal gland, heart, and brain, and exert local functions without appearing in the circulation [2]. Membrane-bound endothelial ACE, and consequently local ANG II generation, has been proved to be of greater significance than ANG II generated in plasma by the circulating enzyme. Experimental evidence also indicates that plasma ACE may infact not be relevant to blood pressure control at all.

Effects of ACE Inhibitors Mediated by the Inhibition of Bradykinin Degradation

Kinins are involved in blood pressure control, regulation of local blood flow, vascular permeability, sodium balance, pain, inflammation, platelet aggregation and coagulation. Bradykinin also exerts antiproliferative effects [4]. In plasma, bradykinin is generated from high molecular weight (HMW) kininogen, while in tissues lys-bradykinin is generated from HMW and low molecular weight (LMW) kininogen. Several effects of bradykinin are explained by the fact that the peptide potently stimulates the NO-pathway and increases prostaglandin synthesis in endothelial cells. In smooth muscle cells and platelets, NO stimulates the soluble guanylate cyclase, which increases cyclic GMP that in turn activates protein kinase G. As a consequence, vascular tone and subsequently systemic blood pressure is decreased, local blood flow is improved, and platelet aggregation is prevented.

ACE inhibitors inhibit the degradation of bradykinin and potentiate the effects of bradykinin by about 50–100-fold. The prevention of bradykinin degradation by ACE inhibitors is particularly protective for the heart. Increased bradykinin levels prevent postischemic reperfusion arrhythmia, delays manifestations of cardiac ischemia, prevents platelet aggregation, and probably also reduces the degree of arteriosclerosis and the development of cardiac hypertrophy. The role of bradykinin and bradykinin-induced NO release for the improvement of cardiac functions by converting enzyme inhibitors has been demonstrated convincingly with use of a specific bradykinin receptor antagonist and inhibitors of NO-synthase.

In the kidney, bradykinin increases renal blood flow, whereas glomerular filtration rate remains unaffected. Bradykinin stimulates natriuresis and, through stimulation of prostaglandin synthesis, inhibits the actions of antidiuretic hormone (ADH), thereby inhibiting water retention. Bradykinin further improves insulin sensitivity and cellular glucose utilization of skeletal muscle cells in experimental models. This, however, appears not to be relevant in the clinical context.

Bradykinin exerts its effects via B_1 and B_2 receptors. The inhibition of bradykinin degradation by ACE inhibitors compensatory leads to increased conversion of bradykinin to des Arg-9-bradykinin by kininase I. This peptide still has strong vasodilatatory properties and a high affinity to the B_1 receptor. The clinical relevance of this aspect is not clear. The cardioprotective effects of bradykinin are mediated via B_2 receptors, since they can be blocked by a specific B_2 receptor antagonist [4]. On the other hand, kinins increase vascular permeability with the consequence of edema, exhibit chemotactic properties with the risk of local inflammation and they are involved in the manifestation of endotoxic schock. Increased bradykinin levels are thus thought to cause some of the undesired effects observed with ACE inhibitors, such as cough, allergic reactions, and anaphylactic responses, for instance angioneurotic edema [5].

Clinical Use (Including Side Effects)

ACE inhibitors are approved for the treatment of hypertension and cardiac failure [5]. For cardiac failure, many studies have demonstrated increased survival rates independently of the initial degree of failure. They effectively decrease work load of the heart as well as cardiac hypertrophy and relieve the patients symptoms. In contrast to previous assumptions, ACE inhibitors do not inhibit aldosterone production on a long-term scale sufficiently. Correspondingly, additional inhibition of aldosterone effects significantly reduces cardiac failure and increases survival even further in patients already receiving diuretics and ACE inhibitors. This can be achieved by coadministration of spironolactone, which inhibits binding of aldosterone to its receptor.

In the treatment of hypertension, ACE inhibitors are as effective as diuretics, β-adrenoceptor antagonists, or calcium channel blockers in lowering blood pressure. However, increased survival rates have only been demonstrated for diuretics and β-adrenoceptor antagonists. ACE inhibitors are approved for monotherapy as well as for combinational regimes. ACE inhibitors are the drugs of choice for the treatment of hypertension with renal diseases, particularly diabetic nephropathy, because they prevent the progression of renal failure and improve proteinuria more efficiently than the other drugs.

More than 15 ACE inhibitors are presently available. They belong to three different chemical classes: sulfhydryl compounds such as captopril, carboxyl compounds such as enalapril, and phopshorus compounds

such as fosinopril. Sulfhydryl compounds exert more undesired, but also desired effects, since they additionally interact with endogenous SH groups. For instance, these compounds may potentiate NO-actions or act as scavengers for oxygen-derived free radicals. Carboxyl compounds are in general more potent than captopril. Phosporous compounds are usually characterized by the longest duration of action.

Most ACE inhibitors are prodrugs, with the exceptions of captopril, lisinopril, and ceranapril. Prodrugs exert improved oral bioavailability, but need to be converted to active compounds in the liver, kidney, and/or intestinal tract. In effect, converting enzyme inhibitors have quite different kinetic profiles with regard to half time, onset and duration of action, or tissue penetration.

In general, ACE inhibitors at the doses used to date are safe drugs. In contrast to many antihypertensive drugs, ACE inhibitors do not elicit a reflectory tachycardia and do not influence lipid or glucose metabolism in an undesired manner. Glucose tolerance is even increased. Most undesired effects are class-specific and related to the inhibition of ACE. Less dangerous, but often bothersome, are dry cough, related to increased bradykinin levels and loss of taste or impaired taste. The more severe undesired effects are hypotension, hyperkaliemia, and renal failure, but those can be easily monitored and appropriately considered. The risk for hypotension increases in combination with diuretics, particularly when ACE inhibitors are initiated in patients who already receive diuretics. The risk of hyperkaliemia increases with coadministration of spironolactone and the risk of renal failure is higher in volume-depleted patients or those already exhibiting impaired renal function. Seldom (0.05%) the development of angioneurotic edema occurs (usually) during the first days of treatment and is life threatening. Allergic responses and angioneurotic edema are related to bradykinin. Recently, specific AT_1 receptor antagonists have become available and are used in the management of hypertension and are presently tested for use in cardiac failure. They are believed not to exhibit the bradykinin-related undesired effects. Indeed, undesired effects of AT_1 receptor antagonists are lower than seen with ACE inhibitors. On the other hand, AT_1 receptor antagonists are probably less effective since the patients do not profit from the cardioprotective effects of bradykinin. Studies comparing the effects of ACE inhibitiors with AT_1 receptor antagonists are presently underway. ACE inhibitors are contraindicated in pregnancy (risk of abortion, acute renal failure of the newborn) and patients with bilateral stenosis of the renal artery. Special caution should be taken if patients have autoimmunolocial systemic diseases.

►Blood Pressure Control
►Renin–Angiotensin–Aldosteron System

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ACE2

►Angiotensin-converting Enzyme-2

Acetyl β-methylcholinesterase

►Cholinesterase

Acetylcholine

Acetylcholine (Ach) is an ester of acetic acid and choline with the chemical formula $CH_3COOCH_2CH_2N^+(CH_3)_3$. ACh functions as a chemical transmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in a wide range of organisms, humans included. Neurotransmitter involved in behavioral state control, postural tone, cognition and memory, and autonomous parasympathetic (and preganglionic sympathetic) nervous system.

►Cholinesterases
►Emesis

►Orexins
►Muscarinic Receptors
►Nicotinic Receptors

Acetylcholine Hydrolase

►Cholinesterase

Acetylcholinesterase

Acetylcholine serves as a neurotransmitter. Removal of acetylcholine within the time limits of the synaptic transmission is accomplished by acetylcholinesterase (AChE). The time required for hydrolysis of acetylcholine at the neuromuscular junction is less than a millisecond (turnover time is 150 μs) such that one molecule of AChE can hydrolyze 6×10^5 acetylcholine molecules per minute. The K_m of AChE for acetylcholine is approximately 50–100 μM. AChE is one of the most efficient enzymes known. It works at a rate close to catalytic perfection where substrate diffusion becomes rate limiting. AChE is expressed in cholinergic neurons and muscle cells where it is found attached to the outer surface of the cell membrane.

►Neurotransmitter Transporters
►Cholinesterase

Acetylthiocholinesterase

►Cholinesterase

Acetyltransferase

Acetyltransferase is an enzyme that catalyses the transfer of an acetyl group from one substance to another.

►Histon Acetylation

N-Acetyltransferases

N-Acetyltransferases (NATs) catalyze the conjugation of an acetyl group from acetyl-CoA on to an amine, hydrazine or hydroxylamine moiety of an aromatic compound. NATs are involved in a variety of phase II-drug metabolizing processes. There are two isozymes NAT I and NAT II, which possess different substrate specificity profiles. The genes encoding NAT I and NAT II are both multi-allelic. Especially for NAT II, genetic polymorphisms have been shown to result in different phenotypes (e.g., fast and slow acetylators).

►Pharmacokinetics
►Pharmacogenetics

α1-Acid Glycoprotein

One of the plasma proteins which is mainly responsible for the plasma protein binding of drugs. Its level is known to be elevated in some pathological states, such as inflammation.

►Drug Interaction

Acidosis

State of deviation of plasma pH (systemic acidosis) or tissue extracellular pH (tissue or local acidosis) from normal (ca. pH 7.4) towards lower values. Deviation of 0.1 pH units is significant. Systemic acidosis can be caused by lung or kidney failure. Local acidosis can be the consequence of injury, inflammation, or tumor growth, due to disruption of blood supply. Local acidosis is normally associated with hypoxia.

►Proton-Sensing GPCRs

ACPD

ACPD (1-aminocyclopentane-1,3-dicarboxylic acid) is a selective agonist for metabotropic glutamate (mGlu)

receptors. Within the 4 stereoisomers, 1S,3R-ACPD activates group-I and group-II mGlu receptors as well as some group-III receptors (mGlu8) at higher concentrations. The 1S,3S-ACPD isomer is one of the first selective group-II mGlu receptor agonists described. These molecules have been widely used to identify the possible physiological functions of mGlu receptors.

►Metabotropic Glutamate Receptors

ACTH

Adrenocorticotrope Hormone.

►Gluco-Mineralocorticoid Receptors

Actin

►Cytoskeleton

Actin Binding Proteins

By binding to F-actin, actin binding proteins (ABPs) stabilize F-actin or regulate its turnover. Known ABPs are proteins such as α-actinin, talin, tensin, filamin, nexilin, fimbrin, and vinculin.

►Cytoskeleton

Actin Filaments

►Cytoskeleton

Action Potential

An Action Potential is a stereotyped (within a given cell) change of the membrane potential from a resting (intracellular negative) value to a depolarized (intracellular positive) value and then back to the resting value. The durations of Action Potentials range from a couple of milliseconds in nerve cells to hundreds of milliseconds in cardiac cells. Action Potentials may be propagated along very elongated cells (skeletal muscles, axons of neurons, etc) or from one cell to another via electrical gap junctions (e.g. in cardiac tissue).

►Inwardly Rectifying K^+ Channels
►Voltage-dependent Na^+ Channels
►Antiarrhythmic Drugs

Activated Partial Thromboplastin Time

Activated partial thromboplastin time (aPTT) is a coagulation assay, which measures the time for plasma to clot upon activation by a particulate substance (e.g., kaolin) in the presence of negatively charged phospholipids.

►Anticoagulants

Activator Protein-1

Activator Protein-1 (AP1) comprises transcriptional complexes formed by dimers of members of the Fos, Jun, and ATF family of transcription factors. These proteins contain basic leucine zipper domains that mediate DNA binding and dimerization. They regulate many aspects of cell physiology in response to environmental changes.

►NFAT Family of Transcription Factors

Active Site

Active site of an enzyme is the binding site where catalysis occurs. The structure and chemical properties of the active site allow the recognition and binding of the substrate. The active site is usually a small pocket at the surface of the enzyme that contains residues responsible

for the substrate specificity (charge, hydrophobicity, and steric hindrance) and catalytic residues which often act as proton donors or acceptors or are responsible for binding a cofactor such as pyridoxal, thiamine, or NAD. The active site is also the site of inhibition of enzymes.

Active Transport

Permeation of a drug through biological membranes against the electrochemical gradient. This type of drug transport requires energy produced by intracellular metabolic processes.

►Drug Interaction
►ABC Transporters
►MDR-ABC Transporters

Active Transporters

Active Transporters use the energy of ATP for vectorial transport through a biological membrane against concentration gradient of the transported substrate.

►ABC Transporters
►MDR-ABC Transporters

Activins

Activins are growth and differentiation factors belonging to the transforming growth factor-β superfamily. They are dimeric proteins, consisting of two inhibin-β subunits. The structure of activins is highly conserved during vertebrate evolution. Activins signal through type I and type II receptor serine/threonine receptor kinases. Subsequently downstream signals such as Smad proteins are phosphorylated. Activins are present in many tissues of the mammalian organism, where they function as autocrine and/or paracrine regulators of various physiological processes, including reproduction. In the hypothalamus, activins are thought to stimulate the release of gonadotropin-releasing hormone. In the pituitary, activins increase follicle-stimulating hormone secretion and up-regulate gonadotropin-releasing hormon receptor expression. In the ovaries, activins regulate processes such as folliculogenesis, steroid hormone production and oocyte maturation. During pregnancy, activin-A is also involved in the regulation of placental functions.

►Receptor Serine/Threonine Receptor Kinase
►Transforming Growth Factor-β Superfamily

Acute Phase Reactants

Acute phase reactants (e.g., C-reactive protein) are proteins that increase during inflammation and are deposited in damaged tissues. They were first discovered in the serum, but are now known to be involved in inflammatory processes in the brain (e.g., found in the brain of Alzheimer patients and associated with amyloid plaques).

►Inflammation
►Neurodegeneration

Acyl-CoA

Acyl-CoAs are the activated intermediates of fatty acid metabolism formed by the condensation of fatty acids with Coenzyme A.

►Lipid Modifications
►Fatty Acid Transporters

Acyl-CoA Synthetase

Acyl-CoA synthetases are enzymes (i.e., ligases) that convert fatty acid molecules into acyl-Coenzyme A molecules for their subsequent oxidation.

►Fatty Acid Transporters
►Lipid Modification

Adaptive Immunity

The adaptive or specific arm of the immune system consists of T- and B-lymphocytes and antibodies. T- and

B-cells carry antigen receptors that are generated by random genetic rearrangement during the ontogeny of lymphocytes in the bone marrow (B cells) or the thymus (T-cells). The hallmarks of adaptive immunity are the improved and specific defenses by T and B memory cells and antibodies after repeated exposure (immunological memory) to the eliciting antigen.

►Immune Defense

Adaptor Proteins

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Synonyms

Scaffold; Docking protein; Anchoring protein

Definition

Adaptor proteins are multi-domain proteins (Fig. 1) that interact with components of signaling pathways [1]. As a consequence of these interactions, adaptor proteins are able to regulate signaling events within the cell, providing spatiotemporal control and specificity, and influencing how a cell responds to a particular stimulus.

Basic Mechanisms

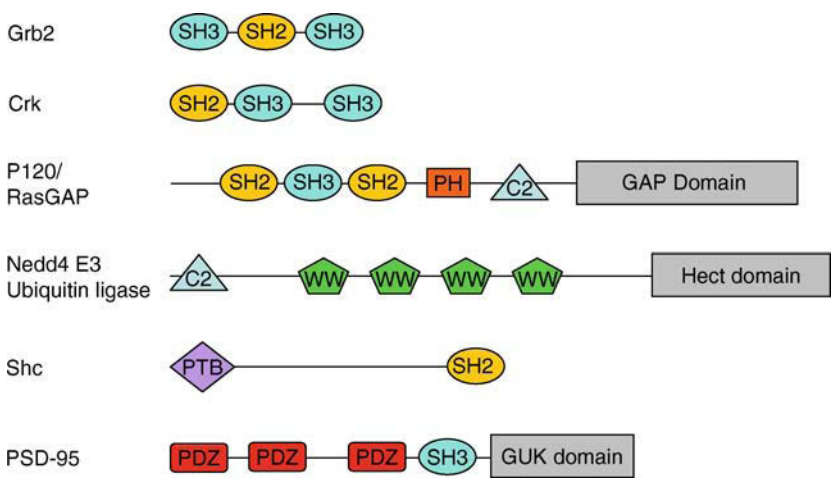
Adaptor proteins function by simultaneously interacting with multiple components of a signaling pathway (Fig. 2). In order to be able to bind to more than one target protein at the same time, adaptor proteins contain at least two specific protein-protein interaction domains. These domains recognize specific motifs in the target proteins and can act completely independently, like beads on a string, or interact with another domain within the same molecule. Such intramolecular interactions can regulate the ability of each domain to bind to its target.

Adaptor Protein Function

In their simplest form, adaptor proteins perform a straightforward function: the formation of multi-protein complexes. However, they often provide more than a static scaffold support for signaling components, instead enabling dynamic regulation to control propagation of pathways and networks. Consequently, adaptor proteins can act as signaling modules, directing propagation of the pathway, influencing downstream events and even modifying the cellular response to a specific stimulus. Some of the different roles played by adaptor proteins are described below. These functions are not mutually exclusive and more than one of these roles can be performed by a particular adaptor protein at one time.

Assembly of Signaling Complexes

This is perhaps the simplest function provided by adaptor proteins and involves bringing together



Adaptor Proteins. Figure 1 Adaptor protein domains. A scheme of the domain structures of some well-characterized adaptor proteins is shown. Descriptions of domain characteristics are in main text except: C2, binds to phospholipids; GTPase activating protein (GAP) domain, inactivates small GTPases such as Ras; Hect domain, enzymatic domain of ubiquitin ligases and GUK domain, guanylate kinase domain. For clarity, not all domains contained within these proteins are shown.

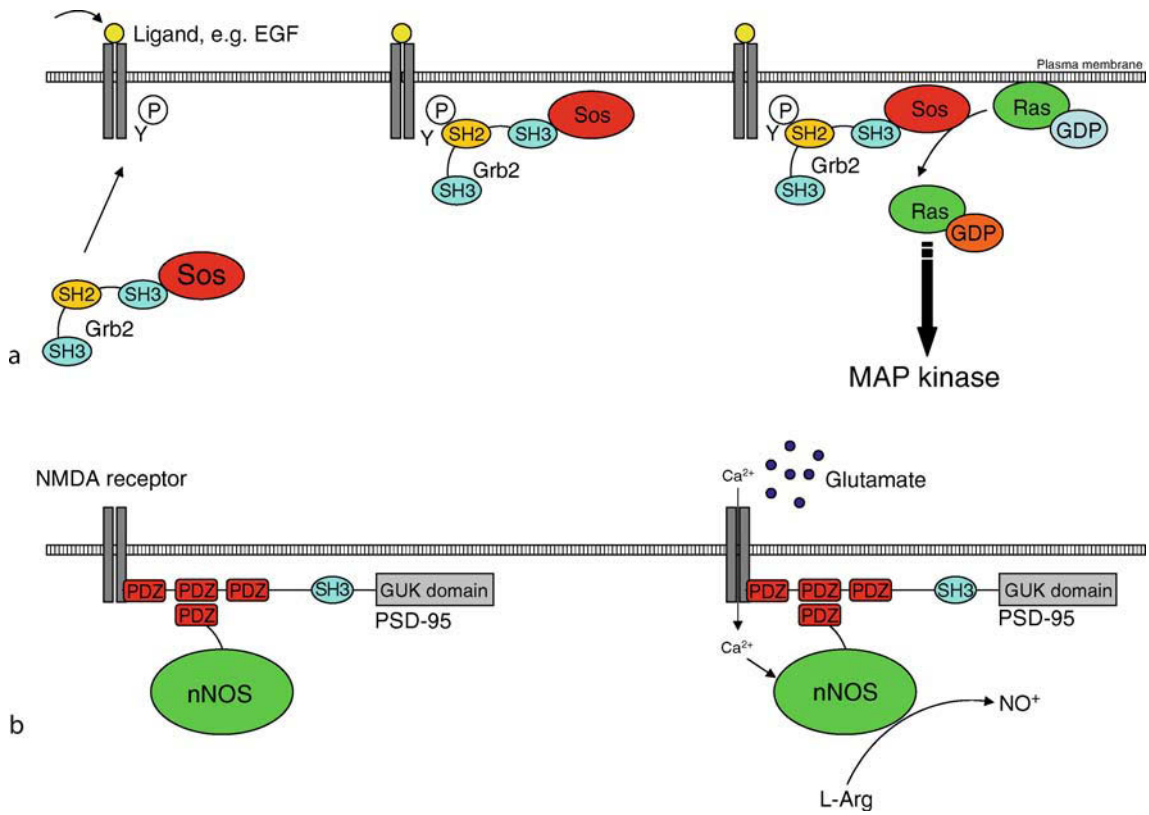
individual components of a pathway. These complexes promote propagation, and often amplification, of the signal. Examples of this are found in the ►MAP kinase cascade pathway, where the adaptor proteins IQGAP1 and kinase suppressor of Ras-1 bind to multiple ►kinases. Consequently, they enable efficient signaling from one kinase to the next.

Spatial Regulation

Adaptor proteins can assemble the complexes in particular subcellular compartments. For example, following activation of a ligand-bound receptor, adaptor proteins can localize downstream signaling targets to the intracellular domains of the receptor (Fig. 2a), thereby facilitating propagation of the signal through the cell. Note that signaling events occur in all cell organelles and subcellular compartments and adaptor proteins appear to function throughout the cell. Through their ability to localize signaling targets to specific subcellular compartments, adaptor proteins not only facilitate signaling events, but also influence how these signals are interpreted by the cell, and consequently the cellular response. For example, in the MAP kinase pathway, signaling from the plasma membrane activates different signaling components to those activated when the signal originates from the Golgi.

Temporal Regulation

The duration of signaling influences how a cell responds to a particular stimulus. For example, brief activation of the MAP kinase cascade in the neuronal cell line, PC12, results in proliferation, while sustained



Adaptor Proteins. Figure 2 Examples of adaptor protein function. Selected examples of how adaptor proteins facilitate signaling are shown. (a) Growth factor signaling. Many growth factor receptors contain an intrinsic tyrosine kinase. Following stimulation by cognate ligand binding, the intracellular domains of the transmembrane receptor autophosphorylate on key tyrosine (Y) residues. The SH2 domain of the adaptor protein Grb-2 associates with the phosphorylated tyrosine residues on the receptor, while the SH3 domain binds to the Ras activator Sos. Consequently, Sos is localized to the receptor and the plasma membrane where it activates the small GTPase Ras, which results in activation of the mitogen-activated protein (MAP) kinase cascade. By this mechanism, Grb-2 facilitates the transduction of an extracellular stimulus to an intracellular signaling pathway. (b) The adaptor protein PSD-95 associates through one of its three PDZ domains with the N-methyl-D-aspartic acid (NMDA) receptor. Another PDZ domain associates with a PDZ domain from neuronal nitric oxide synthase (nNOS). Through its interaction with PSD-95, nNOS is localized to the NMDA receptor. Stimulation by glutamate induces an influx of calcium, which activates nNOS, resulting in the production of nitric oxide.

MAP kinase signaling promotes differentiation of PC12 cells. Adaptor proteins are able to regulate the time course of signaling events, and therefore the cellular outcome.

Activation of Signaling Components

The binding of an adaptor protein may activate the target molecule. An example of this is the adaptor protein insulin receptor substrate-1 (IRS-1), which is activated during insulin signaling. Following binding by insulin, the insulin receptor (which contains an intrinsic tyrosine kinase) catalyzes phosphorylation of selected tyrosine residues on IRS-1. A subset of these phosphorylated tyrosines act as docking sites for SH2 (see below) domains on p85, the regulatory subunit of phosphoinositide 3-kinase. As a consequence of the interaction between IRS-1 and p85, phosphoinositide 3-kinase is activated.

Inactivation of Kinases

►A-kinase anchoring proteins (AKAPs) are a well-studied class of adaptor proteins that regulate protein kinase A (PKA) signaling [2]. PKA is activated in response to 3′5′-cyclic-adenosine monophosphate (cAMP). ►AKAPs provide spatiotemporal specificity for PKA activity by forming multi-protein complexes and localizing them to the appropriate subcellular compartment. These complexes contain kinases and phosphatases, as well as phosphodiesterases, which catalyze the hydrolysis of cAMP to AMP. Therefore AKAPs anchor both positive and negative regulators of PKA signaling.

Sequestering Signaling Components

Specificity in signal transduction is also achieved by selective separation of signaling components. By associating with specific proteins and bringing them together, adaptor proteins can determine how the pathway propagates through the cell. This is important for ►Ras signaling, for example. Ras, which can be activated in response to large number of growth factors and signaling cues, regulates multiple pathways. Therefore, by sequestering specific proteins, adaptor proteins ensure that a particular stimulus activates the appropriate pathway(s). Sequestration of proteins also enables adaptor proteins to negatively regulate signaling. An example of this can be found in T cell activation. The adaptor protein, c-Cbl, sequesters the tyrosine kinase, Syk, preventing recruitment to IgE receptors. Consequently, T cell activation is attenuated.

Domains

In order to bind to target proteins, adaptor proteins contain protein-protein interaction domains which recognize specific target motifs (Fig. 1). Through combinations of these domains, adaptor protein can interact with multiple target proteins, potentially forming large signaling complexes. Whilst many different protein binding domain have been identified, adaptor proteins often utilize the well characterized domains described below.

Src Homology 2 (SH2) and Src Homology 3 (SH3)

►SH2 domains are common protein modules that recognize short motifs containing a tyrosine residue that has been phosphorylated by a tyrosine kinase. Other residues outside of this motif provide specificity to determine which SH2 domain-containing protein associates with that particular site. ►SH3 domains bind to polyproline motifs with the sequence PXXP. These two domains are often found independently in adaptor proteins such as ►Src-homologous and collagen (Shc) and ►PSD-95 (Fig. 1). However, the SH2/SH3 adaptors ►Crk and ►Grb-2 contain both SH2 and SH3 domains (Fig. 1). In these proteins, the SH2 domain recognizes a binding motif in activated transmembrane receptors, while the SH3 recognizes other signaling proteins, such as the Ras activator ►Sos (Fig. 2). Consequently, these proteins couple an activated receptor to signaling components downstream, thereby facilitating signal propagation.

Post Synaptic Density Protein/Drosophila Disc Large Tumor Suppressor/Zonula Occludens-1 Protein (PDZ)

►PDZ domains bind to short peptide motifs at the C-terminal end of target proteins, and are particularly important in spatial organization of receptors and ion channels [3]. Many adaptor proteins contain multiple PDZ domains, which have important implications for their functions. By interacting with subunits from different receptors, adaptor proteins containing multiple PDZ domains can promote formation of homogenous receptor complexes. This clustering can be further enhanced through the ability of PDZ domains to self-associate, enabling oligomerization of the adaptor proteins. Conversely, individual PDZ domains can associate with different target proteins, enabling the formation of large heterogeneous complexes of proteins. Examples of PDZ containing adaptors proteins include ►PSD-95 (Fig. 1), Lim kinase and membrane-associated guanylate kinases (MAGUKs).

Phosphotyrosine-binding (PTB)

►PTB domains recognize small peptides containing a phosphotyrosine, usually with the consensus sequence, NPXpY. Some PTB-containing proteins, such as Numb, are able to bind to the consensus peptide in the absence of phosphorylated tyrosine, suggesting phosphotyrosine is dispensable for the function of certain PTB domains. Hydrophobic residues N-terminal to the phosphotyrosine provide some specificity of target and distinction from SH2 domains. PTB domains appear to be particularly important in docking

proteins to activated receptors. Examples of PTB containing proteins include Numb, IRS-1 and Shc [4] (Fig. 1).

WW

►WW domains (named after the one letter abbreviation for the amino acid tryptophan) are small regions of around 30 residues, which, like SH3 domains, bind to polyproline sequences. These sequences often contain the consensus sequence PPXY or PPLP. Examples of proteins that contain WW domains include Nedd4 E3 ubiquitin ligase (Fig. 1) and IQGAP1.

Pleckstrin Homology (PH)

►PH domains consist of about 120 amino acid residues. They do not interact with other proteins, but associate with specific polyphosphoinositides. Consequently, PH domains appear to be important for localizing target proteins to the plasma membrane. Examples of PH domain-containing proteins include phospholipase C and p120/RasGAP (Fig. 1).

Regulation of Adaptor Proteins

The interactions between adaptor proteins and their targets are often regulated (see below). By these mechanisms, specific signals are able to control which adaptor proteins, and consequently which target proteins, are recruited to a particular signaling complex. Common methods of adaptor protein regulation are described here.

Phosphorylation

Phosphorylation is a common method of regulation. As described above, SH2 domains bind to phosphorylated tyrosine residues. Conversely, phosphorylation of serines and threonines proximal to SH3 and PDZ domains uncouples them from their target motifs. Therefore modulation of protein kinase activity in cells regulates interactions between adaptor proteins and their target proteins.

Chemical Regulation

Cellular messengers, such as calcium, also regulate adaptor protein function. The adaptor protein IQGAP1 binds multiple members of the MAP kinase cascade, including B-Raf, MAPK/extracellular-regulated kinase (MEK) and extracellular-regulated kinase (ERK). Binding of calcium to its effector, calmodulin, increases the association of calmodulin with IQGAP1 and inhibits the interaction with B-Raf.

Intramolecular Interactions

Another way in which the function of adaptor proteins is regulated is through modulation of intramolecular interactions. Often one domain will bind to another domain in the same adaptor molecular, preventing further interactions with other proteins. An example of this is found in the adaptor protein, Crk. Crk contains an N-terminal SH2 domain, followed by two SH3 domains. The SH3 domains are separated by a linker region containing a tyrosine residue. When Crk is phosphorylated by a tyrosine kinase, such as Abl, the intrinsic SH2 domain binds to the phosphotyrosine, attenuating Crk signaling activity.

Conformational Changes

Changing the conformation of adaptor proteins can also alter their function. p130Cas, which is a target for ►Src kinase, serves as an example. The central region of p130Cas contains multiple ►Src phosphorylation sites that, when phosphorylated, promote the binding and recruitment of other targets proteins. Under resting conditions, these phosphorylation sites are hidden due to the folded conformation of p130Cas. When mechanical stress is applied to the cell, this central region is stretched exposing the Src phosphorylation sites. This alteration in conformation results in increased phosphorylation of p130Cas and recruitment of the adaptor protein ►CrkII, leading to activation of the Ras family GTPase, Rap1.

Pharmacological Intervention

Adaptor proteins are attractive targets for the design of new therapies against diseases in which signaling pathways are deregulated. For example, many cancers and inflammatory disorders display hyperactive MAP kinase signaling. This may be due to increased growth factor/cytokine stimulation or increased intracellular kinase activity. Adaptor proteins play well-established roles in controlling MAP kinase activation, and so provide a potential target for novel therapies [5]. For example, Grb-2 has been the focus of research to identify compounds which target either its SH2 or SH3 domains. The importance of Grb-2 in the activation of Ras, and therefore stimulation of the MAP kinase pathway, suggests compounds that inhibit Grb-2 function could potentially be useful in the treatment of many cancers.

At present no compounds targeting adaptor proteins have been approved for clinical use.

Therapies for treatment of diseases caused by hyperactive intracellular signaling may utilize inhibitors targeted against a specific kinase. However, this approach has several problems, such as inhibition of other non-target kinases and inhibition of signaling events not related to the disease. Consequently, the use of kinase inhibitors is not always effective and often produces side effects. However, as described above, adaptor proteins provide specificity to signaling pathways. Therefore, disrupting adaptor protein function may allow more specific targeting of the aberrant cellular response which contributes to the disease, such

as cell proliferation or cytokine production. By selectively interfering with adaptor interactions, these agents are likely to have fewer side effects and increased efficacy.

►A Kinase Anchoring Proteins (AKAPs)

References

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Addiction

►Dependence
►Drug Addiction

Addison's Disease

An endocrine disorder first described by the British Physician Thomas Addison in the mid 1800's. The adrenal glands fail to produce sufficient amounts of glucocorticoid hormones (cortisol) and sometime mineralocorticoid (aldosterone). If left untreated it is life-threatening, the patient will show muscle weakness, hyperpigmentation and even depression. Typical treatment is hydrocortisone replacement therapy.

►Melanocortin

Additive Interaction

Interaction in which the combined effect is the sum of the effects of each drug administered separately.

►Drug Interaction

Adenosine

Adenosine is produced by many tissues, mainly as a by-product of ATP breakdown. It is released from neurons, glia and other cells, possibly through the operation of the membrane transport system. Its rate of production varies with the functional state of the tissue and it may play a role as an autocrine or paracrine mediator (e.g. controlling blood flow). The uptake of adenosine is blocked by dipyridamole, which has vasodilatory effects. The effects of adenosine are mediated by a group of G protein-coupled receptors (the $G_{i/o}$-coupled A_1- and A_3 receptors, and the G_s-coupled A_{2A}-/A_{2B} receptors). A_1 receptors can mediate vasoconstriction, block of cardiac atrioventricular conduction and reduction of force of contraction, bronchoconstriction, and inhibition of neurotransmitter release. A_2 receptors mediate vasodilatation and are involved in the stimulation of nociceptive afferent neurons. A_3 receptors mediate the release of mediators from mast cells. Methylxanthines (e.g. caffeine) function as antagonists of A_1 and A_2 receptors. Adenosine itself is used to terminate supraventricular tachycardia by intravenous bolus injection.

►Purinergic System
►Adenosine Receptors
►Sterol Transporters

Adenosine Receptors

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Synonyms

Ado receptors; A receptors; P1 receptors

Definition

Extracellular adenosine acts through a class of ►G protein-coupled receptors (GPCRs), defined across mammalian species as A_1, A_{2A}, A_{2B}, and A_3ARs (adenosine receptors). Adenosine has a cytoprotective role in the body, both in the periphery and in the central nervous system. Following binding of adenosine, or another naturally occurring ►agonist, the receptor

interacts with heterotrimeric G proteins to stimulate or inhibit downstream signaling cascades.

Basic Characteristics

The purine nucleoside adenosine, as a natural local modulator of cell action, increases the ratio of oxygen supply to demand, suppresses excessive inflammation, and promotes tissue protection against apoptosis or ischemic damage. ARs also have effects on proliferation and differentiation. Nearly every cell type in the body expresses one or more of these receptors, indicating the fundamental nature of adenosine as a cytoprotective mediator. In the cardiovascular system, ARs present on myocardial, vascular, and inflammatory cells respond to the stress of ischemia and other damaging conditions. Adenosine has been termed a "retaliatory" metabolite of ATP, in that its extracellular level rises in response to excessive energy demand in relation to the available energy supply, i.e., utilization of intracellular ATP. Adenosine aids in correcting this imbalance between energy supply and demand by causing vasodilation, increasing vascular integrity and angiogenesis, and counteracting the lethal effects of prolonged ischemia on cardiac myocytes. In the brain, activation of presynaptic A_1ARs suppresses the release of excitatory neurotransmitters and counteracts excitotoxicity by both presynaptic and postsynaptic mechanisms.

The affinity and selectivity of nucleosides as adenosine ►agonists has been extensively explored, resulting in thousands of selective agonists, of which many are useful in pharmacological studies [1]. Selected agonists of A_1, A_{2A}, and A_3 receptors are shown in Fig. 1. Highly selective ►antagonists of A_1, A_{2A}, A_{2B}, and A_3 receptors have been reported both as research tools and as experimental therapeutic agents (Fig. 2) [3, 4]. Caffeine and other naturally occurring alkylxanthines act as nonselective competitive antagonists of adenosine with respect to at least three of the four subtypes of the ARs. In fact, antagonism of ARs is the most likely mechanism of action of ingested caffeine. These simple xanthine antagonists are of micromolar affinity, but these lead molecules have been optimized for affinity and selectivity. Library screening has pointed the way for the identification of a variety of chemically novel lead structures that have been optimized by medicinal chemists for affinity and selectivity as adenosine antagonists.

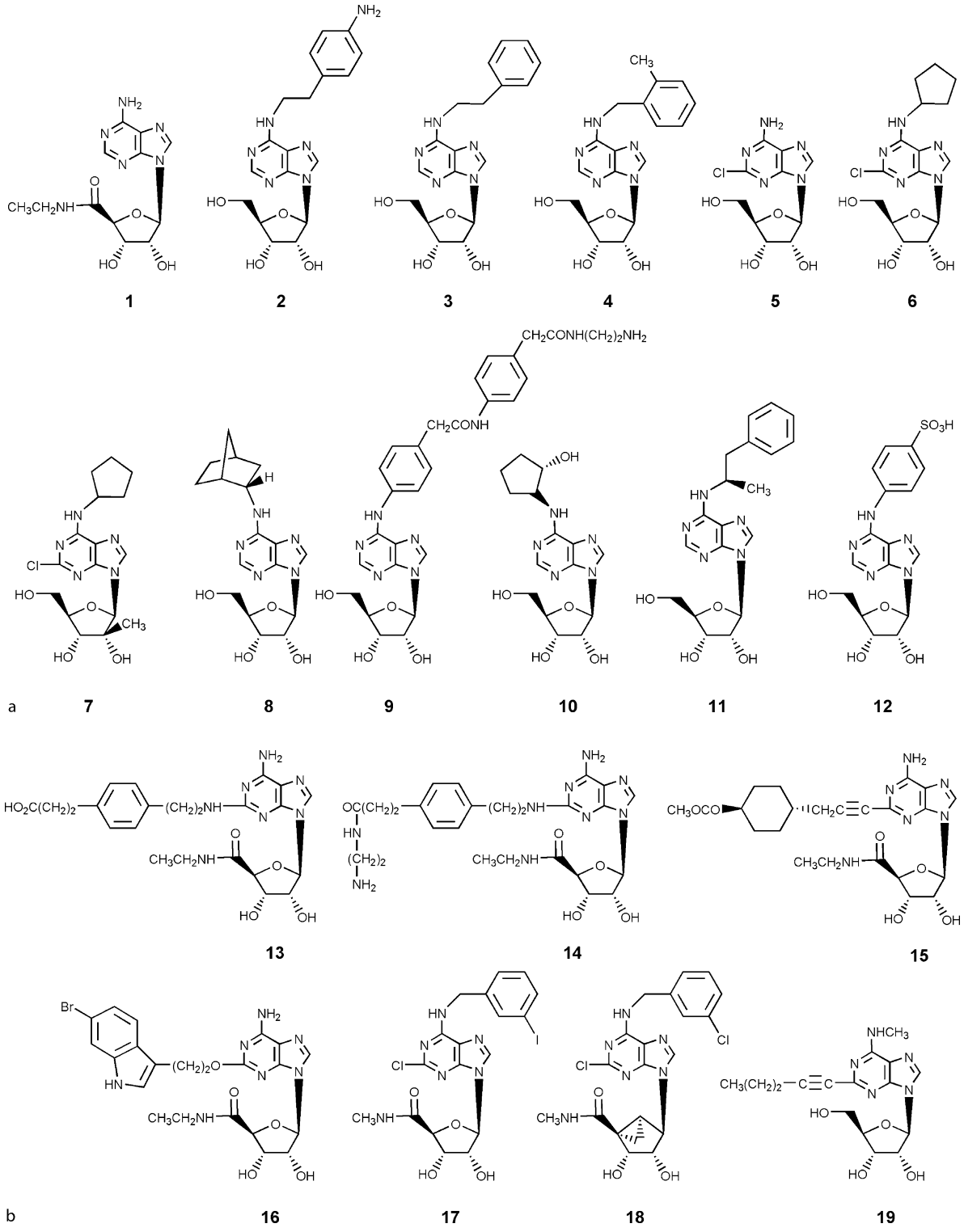
Sources of Adenosine, its Transport Mechanisms, and Metabolism

Adenosine production in the synapse is not through vesicular release in response to nerve firing, as is the case for classical neurotransmitters. Rather, adenosine acts as a local autacoid, the release of which increases upon stress to an organ or tissue. Most cells in culture and in situ produce and release adenosine extracellularly. This endogenous adenosine released tends to influence the outcome of pharmacological studies and can cause misleading results if not properly controlled. Depending on stress factors present, the levels of extracellular adenosine in a given tissue or organ may vary widely, leading to highly variable basal levels of stimulation of the ARs by endogenous adenosine. The level of adenosine may be as low as ~20 nM in the resting brain and as high as 100 μM in severe ischemic conditions. The half-life of adenosine in the blood is short (~1 s), and the peripheral administration of adenosine has no effect on the extracellular concentration of adenosine in the brain. Both neuronal and glial cell function are regulated by adenosine.

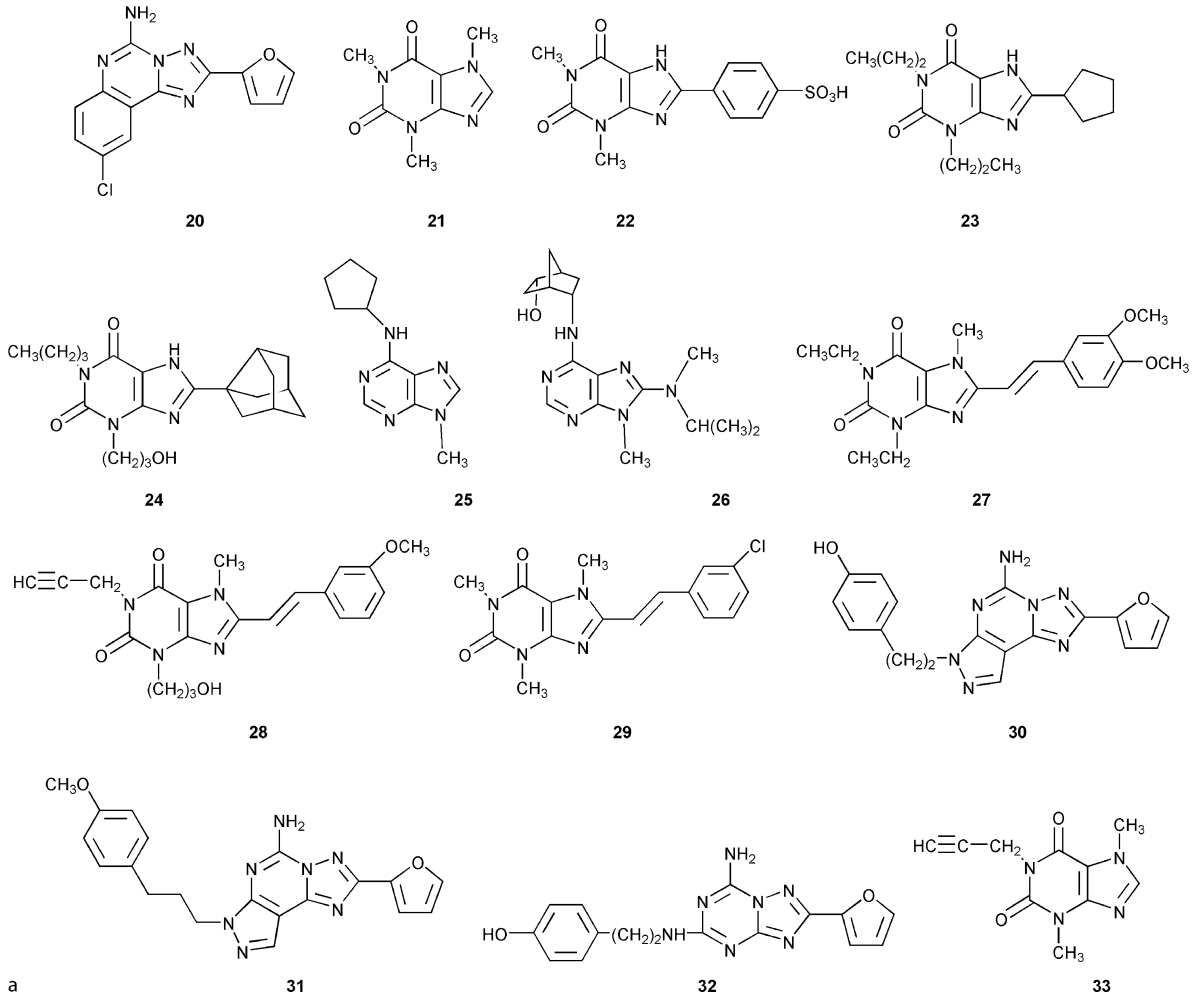
One source of extracellular adenosine may be both from inside the cell, where it is present in millimolar concentrations. As a hydrophilic small molecule, adenosine does not diffuse freely through the intact plasma membrane; rather, it may pass through an equilibrative transporter such as the ENT1 nucleoside transporter, for which there are well characterized inhibitors [7]. Levels of extracellular adenosine may also rise as a result of the enzymatic hydrolysis of extracellular adenine nucleotides or cell lysis. Nucleotide precursors of adenosine, notably ATP and ADP, have their own extracellular signaling properties that are mediated by P2 receptors. ►Ectonucleotidases, which are also ubiquitously expressed on the cell surface, but with characteristic distribution patterns, cleave adenine nucleoside 5′-phosphate derivatives (including activators of P2X and P2Y nucleotide receptors) to eventually form adenosine. There are many classes of ectonucleotidases; however, the most relevant species in the family of ectonucleoside triphosphate dihydrolases (E-NTPDases) that act to breakdown P2 agonists are apyrase or NTPDase1 (which converts ATP and ADP to AMP) and NTPDase2 (which converts ATP to ADP). A separate enzyme, ecto-5′-nucleotidase (CD73), converts AMP to adenosine. CD73 is characteristically found on the surface of astrocytes but not neurons.

Unlike classical neurotransmitters, adenosine does not have a rapid synaptic uptake system (as for the biogenic amines), and its chemical inactivation system is not as rapid as for the transmitter acetylcholine, for example. Adenosine may be metabolized extracellularly and inactivated with respect to the ARs in a more general fashion by the widespread enzymes adenosine kinase (AK, to produce AMP) and adenosine deaminase (AD, to produce inosine). Both AMP and inosine are only weakly active at ARs, depending on the subtype.

Inhibition of the metabolism of extracellular adenosine or its uptake proteins is being explored for therapeutic purposes. AK inhibitors have been proposed for the treatment of pain and seizures; however, the promising clinical development of these efficacious compounds was discontinued due to toxicity.



Adenosine Receptors. Figure 1 Structures of widely used AR agonists, both nonselective and selective. Affinities/potencies at the ARs are found in Table 2. (a) Nucleoside derivatives that are either nonselective or selective for A_1 receptors (**1**–**12**). (b) Nucleoside derivatives that are selective for A_{2A}, A_{2A}/A_{2B} (mixed), or A_3 receptors (**13**–**19**).



Adenosine Receptors. Figure 2 Structures of widely used AR agonists, both nonselective and selective. Affinities/potencies at the ARs are found in Table 2. (a) Nonnucleoside derivatives that are either nonselective or selective for A_1 or A_{2A} receptors. (b) Nonnucleoside derivatives that are selective for A_{2B} or A_3 receptors.

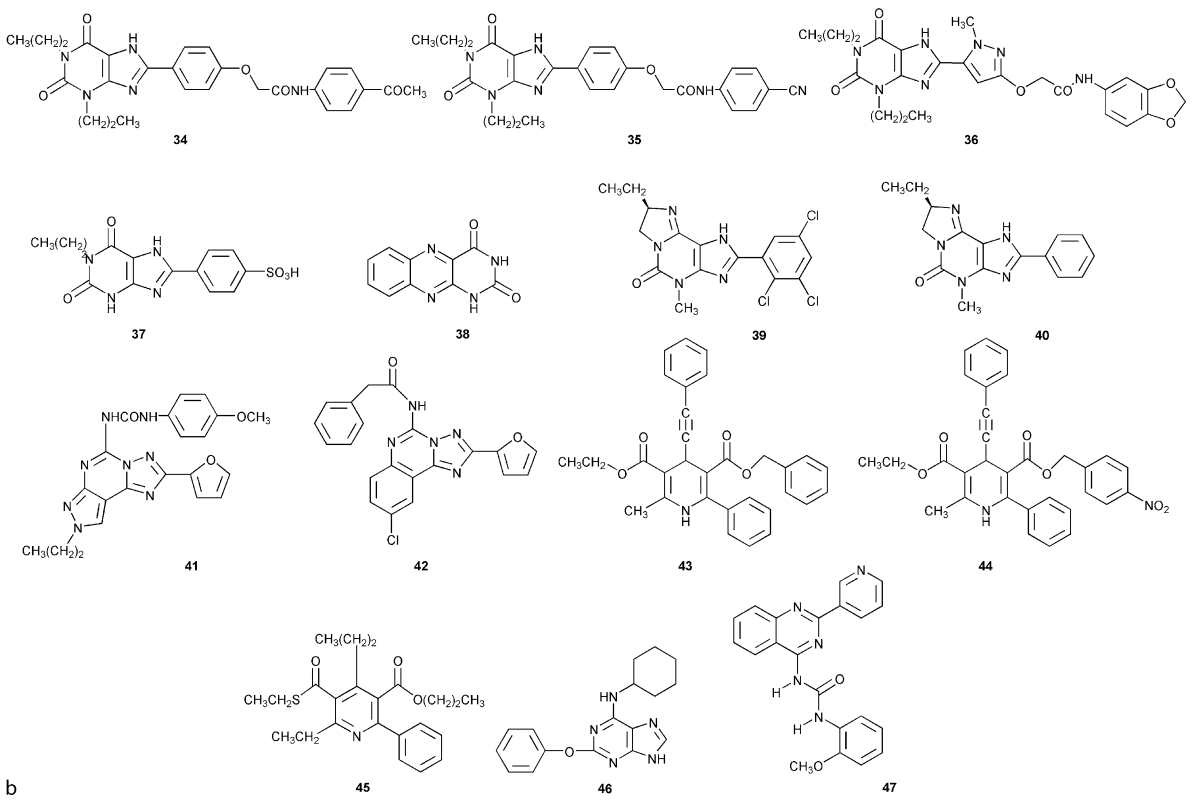
Receptor Structure, Signaling Pathways Coupled to ARs, and AR Regulation

The four mammalian ARs are members of the rhodopsin-like Class A family of ►GPCRs, which contain seven transmembrane helical domains (►TMs). Characteristics of the four subtypes of the human ARs, length of their primary sequences, their chromosomal localization, and their signaling pathways are given in Table 1. The A_{2A} receptor is considerably longer than the other three subtypes, due to its extended carboxy-terminal.

Two AR subtypes, A_1 and A_3, couple through G_i to inhibit adenylate cyclase, while the other two subtypes, A_{2A} and A_{2B}, stimulate adenylate cyclase through G_s or G_{olf} (for A_{2A}). The A_{2B}AR is also coupled to the activation of PLC through G_q. Furthermore, each of these receptors may couple through the β,γ subunits of the G proteins to other effector systems, including ion channels and phospholipases. Levels of intracellular calcium increase upon stimulation of ARs, which interact with other second messenger systems. ARs have been found to couple to mitogen-activated protein kinases (MAPKs) in a variety of circumstances, leading to effects on differentiation, proliferation, and cell death.

Crosstalk occurs between ARs and other receptors. For example, an otherwise subthreshhold concentration of acetylcholine, as might be present in the Alzheimer's brain, still produces a strong calcium signal when the A_1 AR is costimulated. Crosstalk occurs with the striatal dopamine receptor system, in which a direct physical association (dimerization) occurs between A_{2A} and D_2 receptors, and between other subtypes. The A_1AR forms functional heterodimers with the $P2Y_1$ nucleotide receptor, which may be stimulated by $P2Y_1$ receptor agonists but is not blocked by $P2Y_1$ receptor antagonists.

Downregulation of ARs should be considered in pharmacological studies and in the development of



Adenosine Receptors. Figure 2 (Continued)

agonists for therapeutic purposes. Responses of all four subtypes have been found to desensitize, and downregulated the receptors. The most rapid downregulation among the AR subtypes is generally seen with the A_3AR.

Ligands and Mechanisms Involved in Ligand Binding

The structure activity relationships (►SAR) of newly synthesized analogues of nucleosides, xanthine heterocycles, and nonxanthine heterocycles have been explored at the ARs. Potent and selective AR antagonists have been prepared for all four subtypes [3, 4], and selective agonists are known for three subtypes [1]. Thus, numerous pharmacological tools are available for in vitro and in vivo use (Table 2). Potent and selective A_{2B} AR agonists are yet to be reported, although several research groups have identified lead compounds.

Agonists: Medicinal chemists have extensively explored the ►SAR of adenosine derivatives as agonists of the ARs. Until recently, with the synthesis of atypical adenosine agonists that are pyridine-3,5-dicarbonitrile derivatives, nearly all AR agonists have been purine nucleoside derivatives. In general, for the adenine moiety of adenosine, modifications at the N^6 position have led to selectivity for the A_1AR, and modifications at the 2 position, especially with ethers, secondary amines, and alkynes, have led to selectivity for the A_{2A}AR. Commonly used A_1AR agonists that are N^6-cycloalkyl derivatives are the 2-chloro analogue CCPA **6** and S(-)-ENBA **8**, which are more highly selective than R-PIA **11**. When using the human ARs, S(-)-ENBA is more highly A_1AR-selective than CCPA or its 2-H analogue CPA. Substitution of the 2′ position of the ribose moiety of CCPA with a methyl group results in **7**, with a high selectivity for the A_1AR. The hydroxy derivative **10** of CPA is a clinical candidate selective for the A_1AR. The A_1AR agonist SPA **12** contains a sulfonate group, which tends to prevent passage across the blood–brain barrier. Some N^6 derivatives are not selective for the A_1AR; for example, the arylamino derivative APNEA **2**, which is an analogue of **3**, and metrifudil **4**, are relatively nonselective. 2-Chloroadenosine **5** is a nonselective agonist that is subject to cellular uptake and nonreceptor-mediated effects.

The substitution at the 2 position may also lead to A_{2A}AR enhancing effects, which are further boosted with an uronamido substitution at the 5′ position of adenosine. When the 5′-*N*-alkyluronamide group alone is present, high affinity [at the A_{2A}AR but not selectivity] is typically observed, similar to the nonselective agonist NECA **1**. By combination of this-uronamide group with the appropriate 2 position substitution, selectivity may be achieved for the A_{2A}AR. For example, the 5′-*N*-ethyl derivatives CGS21680 **13** and ATL-146e **15** are both selective in

Adenosine Receptors. Table 1 Characteristics of the four subtypes of adenosine receptors (human, unless noted)

Receptor subtype	A_1	A_{2A}	A_{2B}	A_3
Genbank accession number	S45235	S46950	X68487	L22607
Amino acids	326	412	332	318
Theoretical molecular model (PBD ID)	–	1UPE	–	1OEA; 1R7N
Chromosomal localization	1q32.1	22q11.2	17p11.2–12	1p13.3
G protein coupling selectivity	$G_{i/o}$	G_s; G_{olf}	G_s; G_q	$G_{i/o}$
Key residues for ligand recognition	H278 (7.43)	E151, E169 (EL2); F182 (5.43); H250 (6.52); N253 (6.55); H278 (7.43); S281 (7.46)	F59 (2.56)	H95 (3.37); N250 (6.55); H272 (7.43)
Mutations specific for reduced agonist potency	E16A (1.39); C85S (3.30); T277A (7.42); L65T (2.60) (rat); I69S (2.64) (rat)	T88A (3.36); S277A (7.42); H278Y (7.43)	–	
Mutations specific for reduced antagonist potency	H251L (6.52) (bovine)	V84L (3.32)	–	K152A (EL2); W243A (6.48)

The A_1AR is found in the brain (cortex, cerebellum, hippocampus), dorsal horn of the spinal cord, eye, adrenal gland, heart (atrium), skeletal muscle, liver, kindly, adipose tissue, salivary glands, esophagus, colon, antrum, and testis. The A_{2A}AR is found in spleen, thymus, leukocytes (both lymphocytes and granulocytes), and blood platelets. In the brain it is restricted to the striatum, nucleus accumbens, olfactory tubercle, and large striatal cholinergic interneurons. The message for A_{2B} and ARs occurs throughout the CNS and in many peripheral tissues.

binding to the rat A_{2A}AR, but are less selective at the human subtypes. CGS21680 crosses the blood–brain barrier to only a small degree. The aminoethyl derivative APEC **14**, however, crosses the blood–brain barrier and is more potent than CGS21680 in causing locomotor depression through a central mechanism.

There are not yet any agonists that are truly selective for the A_{2B}AR, known as the "low affinity" adenosine A_2 receptor. A novel agonist, the 2-(6-bromotryptophol) ether derivative MRS3997 **16**, is a full agonist with mixed selectivity at A_{2A} and A_{2B}ARs.

The A_3 agonists Cl-IB-MECA **17** and its corresponding 2-H analogue IB-MECA are widely used as selective agonists of the A_3AR, although even more selective agents are now known, including those in which the ribose-like ring has been conformationally locked in the receptor-preferring north conformation, such as MRS3558 **18**. HEMADO **19** is a human A_3AR-selective agonist, but its selectivity does not generalize across species.

Antagonists: The classical AR ►antagonists are ►xanthines derivatives such as caffeine **21** and theophylline (1,3-dimethylxanthine). The micromolar affinity of the naturally occurring antagonists has been greatly exceeded with the introduction of selective antagonists, even reaching subnanomolar affinity. For example, the A_1AR-selective antagonists **23** and **24** are xanthine derivatives. The 8-cyclopentyl derivative DPCPX **23** is highly A_1AR selective in the rat and less A_1AR selective among the human AR subtypes. DPCPX is an ►inverse agonist at the A_1AR, while the adenine derivative N-0840 **25** is a ►neutral antagonist. In general, modifications of the xanthine scaffold at the 8 position with aryl or cycloalkyl groups have led to selectivity for the A_1AR, although the water soluble 8-sulfophenyl derivative **22** is nonselective. Persistent problems in the use of xanthine derivatives as AR antagonists of the A_1AR are their low aqueous solubility and their interaction at the A_{2B}AR. Use of adenine derivatives, such as the inverse agonist WRC-0571 **26**, provides A_1AR-selective antagonists that have low affinity at the A_{2B}AR.

Modifications of ►xanthines at the 8 position with alkenes (specifically styryl groups) have led to selectivity for the A_{2A}AR. The 8-styrylxanthine derivatives KW6002 **27**, MSX-2 **28,** and CSC **29** are moderately potent A_{2A}AR antagonists [3]. Some 8-styrylxanthine derivatives, especially CSC, have been discovered to inhibit monoamine oxidase-B as well. High selectivity of ►xanthines at the A_1, A_{2A}, and A_{2B} (e.g., MRS1754 **35** and its *p*-$COCH_3$ analogue MRS1706) ARs has been achieved. ►Xanthines that are selective for the A_3AR remain to be designed, however, PSB-II **40** contains an elaborated xanthine ring system.

In recent years, an enormous diversity of heterocyclic structures has been reported as AR antagonists (Table 2) [2]. For example, the nonselective triazoloquinoline antagonist CGS15943 **20**, first introduced in the early 1990s, has given rise to numerous derivative

Adenosine Receptors. Table 2 Affinity of commonly used adenosine receptor agonists and antagonists for defining pharmacologically adenosine receptor subtypes

		K_i (nM), unless noted			
Selectivity	**Compound**	**A_1AR[a]**	**$A_{2A}AR$[a]**	**$A_{2B}AR$[a]**	**A_3AR[a]**
	Agonists				
None	1 NECA	14	20	140[c]	25
	2 APNEA	14[b]	172[b]	n.d.	116[b]
	3 PhEt-Ado	12.9	676	67,000[c]	2.1
	4 Metrifudil	59.6[b]	24.1[b]	>100,000[c]	47.2
	5 CADO	7.5	630	24,000[c]	87
A_1	6 CCPA	0.83	2270	18,800[c]	38
	7 2′-MeCCPA	3.3	9580	37,600	1150
	8 *S*-ENBA	0.38	>10,000	>10,000[c]	915
	9 ADAC	0.85[b]	210[b]	n.d.	13.3
	10 GR79236	3.1[b]	1300[b]	n.d.	n.d.
	11 R-PIA	2.0	860	58,000[c]	8.7
	12 SPA	70[b]		52,000[c]	
A_{2A}	13 CGS21680	289	27	>10,000[c]	67
	14 APEC	400[b]	5.7[b]	50[b]	
	15 ATL-146e	77	0.5	n.d.	45
A_{2B}	16 MRS3997		128	150	
A_3	17 Cl-IB-MECA	220	5360	>100,000[d]	1.4
	18 MRS3558	260	2300	>10,000[d]	0.29
	19 HEMADO	327	1230	>10,000	1.1
	Antagonists				
None	20 CGS15943	3.5	4.2	16	51
	21 Caffeine	29,000	48,000	10,400	13,300
	22 SPT	4020	7050	1330	5890
A_1	23 DPCPX	3.9	129	56	3,980
	24 PSB36	0.12[b]	552[b]	187	2300
	25 N-0840	750			
	26 WRC-0571	1.7	105	n.d.	7940
A_{2A}	27 KW6002	2830	36	1800	>3,000
	28 CSC	28,000[b]	54[b]	n.d.	n.d.
	29 MSX-2	2500	8	>10,000	>10,000
	30 SCH58261	725	5.0	1110	1200
	31 SCH442416	1110	0.048	>10,000	>10,000
	32 ZM241,385	774	1.6	75	743
	33 DMPX		11,000		
A_{2B}	34 MRS1706	157	112	1.39	230
	35 MRS1754	403	503	2.0	570
	36 MRE 2029-F20	245	>1000	3.0	>1000
	37 PSB1115	>10,000	24,000[b]	53.4	>10,000
	38 Alloxazine			2400	

compounds, including $A_{2A}AR$-selective antagonists (**30**, and **31** and the triazolotriazine **32**), $A_{2B}AR$-selective antagonists (**34**–**37**), and A_3AR-selective antagonists (**41** and **42**). The triazolotriazine – ZM241,385 **32** – and the pyrazolotriazolopyrimidines – SCH-58261 **30** and SCH442,416 **31** – are highly potent and selective $A_{2A}AR$ antagonists [3]. SCH442,416 displays > 23,000-fold selectivity for the human $A_{2A}AR$ (K_i 0.048 nM) in comparison to human A_1AR and IC_{50} > 10 μM at the A_{2B} and A_3 ARs.

Adenosine Receptors. Table 2 Affinity of commonly used adenosine receptor agonists and antagonists for defining pharmacologically adenosine receptor subtypes (Continued)

		K_i (nM), unless noted			
Selectivity	**Compound**	**A_1AR[a]**	**$A_{2A}AR$[a]**	**$A_{2B}AR$[a]**	**A_3AR[a]**
A_3	39 PSB-10				0.43
	40 PSB-11	1640	1280	2100[d]	3.5
	41 MRE 3008-F20	1200	141	2100	0.82
	42 MRS1220	305[b]	52.0[b]	n.d.	0.65
	43 MRS1191	>10,000	>10,000	>10,000[c]	31.4
	44 MRS1334	>100,000	>100,000	n.d.	2.7
	45 MRS1523	15,600[b]	2050[b]	n.d.	18.9
	46 MRS3777	>10,000	>10,000	>10,000[c]	47
	47 VUF5574	>10,000	>10,000		4

n.d., not determined.
[a]Affinity at human A_1, A_{2A}, A_{2B}, and A_3ARs, unless noted, expressed as K_i (nM).
[b]Affinity determined at rat ARs.
[c]Potency in a cyclic AMP functional assay.

Alloxazine **38** is a weak, nonxanthine antagonist that is slightly selective for the A_{2B}AR.

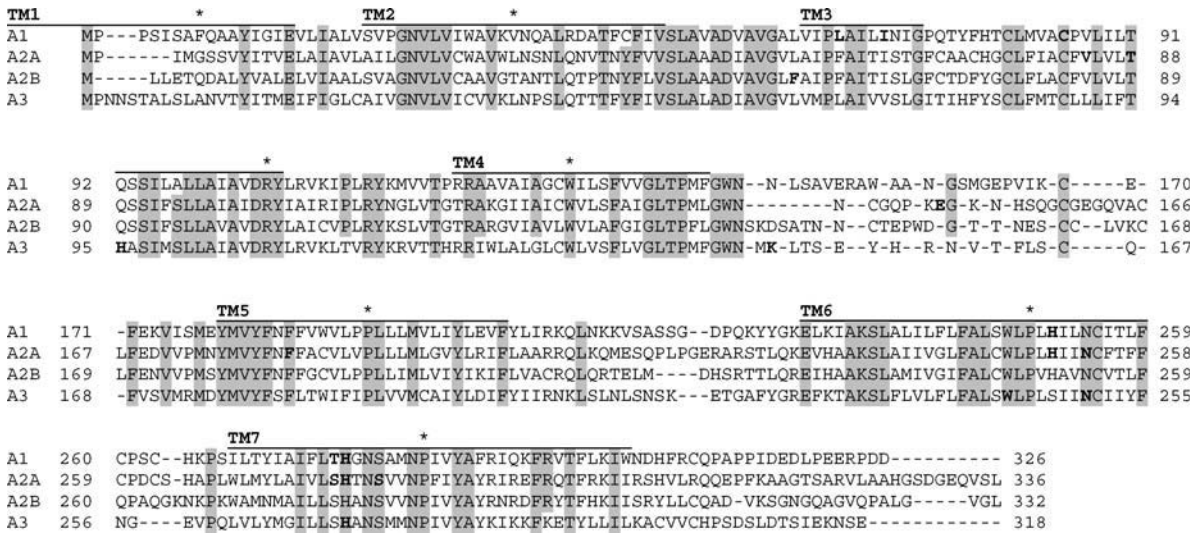
Although the simple xanthine antagonists have not provided suitable analogues with A_3AR selectivity, a cyclization of the xanthine nucleus, leading to imidazopurinones **39**, **40**, and their congeners has. For A_3AR, most of the successful leads have come from chemically diverse heterocycles. The dihydropyridine derivatives MRS1191 **43**, its nitro analogue MRS1334 **44b**, and the pyridylquinazoline derivative VUF5574 **47** are potent, selective A_3AR antagonists in the human, but are weak at the rat A_3AR. Nevertheless, MRS1191 has been used successfully in murine species. MRS1220 **42** is very potent and selective at the human A_3AR but not at the rat or the mouse receptor. There is a marked species dependence of antagonist affinity at the A_3AR. Commonly used A_3AR antagonists must be treated with caution in non-human species other than human. In general, one must be cognizant of potential species differences for both AR agonists and antagonists. The pyridine derivative MRS1523 **45** is a moderately selective A_3AR antagonist units for both the rat and human.

Variations in the relative efficacy of nucleosides, depending on structure, have been noted. This is especially pronounced for the A_3AR, at which changes on the adenine moiety (N^6 and 2 positions) and ribose moiety can either reduce efficacy to the point of pure antagonism (i.e., combination of 2-Cl and N^6-(3-iodobenzyl)) or guarantee a robust, nearly full activation of the A_3AR (i.e., 5′-uronamide) [2]. Such nucleoside-derived A_3AR antagonists tend to have selectivity that is more general across species.

Radioligands: Radioligands commonly used for the ARs are: A_1 agonist [^{3}H]CCPA **6**, antagonist [^{3}H]DPCPX **23**; A_{2A} agonist [^{3}H]CGS21680 **13**, antagonist [^{3}H]ZM241,385 **32** or [^{3}H]SCH58261 **31**; A_3 agonist [^{125}I]I-AB-MECA (the N^6-(4-amino-3-iodobenzyl) 2-H analogue of **17**) and antagonist [^{3}H]PSB-11 **40**. Ligands for in vivo positron emission tomographic imaging of A_1 and A_{2A}ARs have been developed [3]. Potent fluorescent ligands have been reported for A_1 and A_{2A}ARs.

Allosteric modulation: In addition to AR agonists and antagonists that interact directly with the primary (orthosteric) site of the receptor, ►allosteric modulators of agonist action are also under consideration for disease treatment. Such modulators, either positive enhancers or negative allosteric inhibitors might have advantages over the directly-acting (orthosteric) receptor ligands. The action of the allosteric compounds would depend on the presence of a high local concentration of adenosine, which often occurs in response to a pathological condition [4]. In some cases (dependent on tissue, receptor subtype, and other conditions), one would wish to boost the adenosine effect, and therefore, an allosteric enhancer would be useful. In other cases, the elevated adenosine may be detrimental, in which instance one would want to apply a negative modulator. ►Allosteric modulators have been explored and are under development for the A_1 and A_3 AR subtypes [4].

Receptor modeling: Each of the ARs has been modeled based on homology to bovine rhodopsin (the only GPCR with an available crystal structure) or by other methods. Several such theoretical models are now available (Table 1). Molecular modeling of the ARs and ►ligand docking have provided insights into the putative binding sites of all of the subtypes, which has aided in ligand design [1, 3]. Essential residues for the binding of ligand and activation of the receptors have



Adenosine Receptors. Figure 3 An alignment of the primary sequences of the four human AR subtypes. Regions of conservation are highlighted. * indicates the most conserved (X.50) residue in each *TM* region. Bold residues correspond to those indicated in Table 1. The A2A receptor is truncated in the carboxy-terminal region.

been defined through both modeling and site-directed mutagenesis. Similar to other GPCRs having small molecular ligands, ►TMs 3, 5, 6, and 7 of the ARs are thought to be most closely associated with bound agonists and antagonists. An alignment of the primary sequences of the four human AR subtypes (Fig. 3) indicates a high degree of homology within the ►TMs. The second extracellular loop (EL2) is highly variable in sequence and contains a relatively high content of charged residues, in addition to the connection point (Cys residue) of a conserved disulfide bridge.

Key functional residues for ligand binding and activation that have been identified are listed in Table 1 (also in bold in 3). Extensive mutagenesis of the ARs has helped in defining a putative, hydrophilic ribose-binding region spanning ►TMs 3 and 7. The putative adenine-binding region contains more hydrophobic residues, particularly in the vicinity of the N^6 substituent. The EL2 also is thought to coordinate the ligands.

The binding and activation steps of receptor action have been dissected computationally, although not yet in a global fashion. The conformational dynamics of the activation of the A_3AR have been approximated with respect to isolated portions of the receptor.

Drugs

Presently, only adenosine itself is approved for clinical use. It is used widely in the treatment of supraventricular tachycardia and in cardiac stress imaging to assess coronary artery disease [5]. Other agonists and antagonists and an ►allosteric modulator of the A_1 receptor are in clinical trials for a variety of indications. Selective AR agonists are undergoing clinical trials for cardiac arrhythmias and pain (A_1); cardiac imaging and inflammation (A_{2A}); colon cancer, rheumatoid arthritis, psoriasis, and dry eye (A_3). Selective AR antagonists are either in or advancing toward clinical trials for kidney disorders (A_1); Parkinson's disease (A_{2A}); diabetes and asthma (A_{2B}); cancer and glaucoma (A_3).

►Purinergic System

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Adenoviruses

►Gene-Therapy Vectors

Adenylate Cyclase

►Adenylyl Cyclases

Adenylyl Cyclases

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Synonyms

Adenylyl cyclase (preferred); Adenylate cyclase; Adenyl cyclase (original); ATP:pyrophosphate lyase; Cyclizing (E.C.4.6.1.1.)

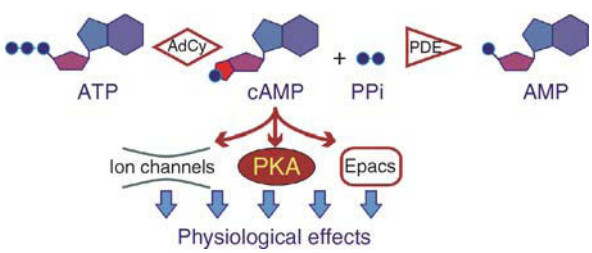
Definition

Adenosine 3′:5′-monophosphate (►cAMP) regulates effects in all eukaryotic cells, principally through activation of cAMP-dependent protein kinase (►PKA), but also through cAMP-gated ion channels (►CNGs) and select guanine nucleotide exchange factors (►Epacs) (Fig. 1). Cellular levels of cAMP levels reflect the balance of activities of adenylyl cyclases (AdCy: 5′-ATP ⟨ cAMP + PP_i) and cAMP ►phosphodiesterases (PDE: cAMP ⟨ 5′-AMP). Adenylyl cyclases occur throughout the animal kingdom and play diverse roles in cell regulation [1].

Basic Characteristics

Classes of Adenylyl Cyclases

Adenylyl cyclases belong to the larger class of purine nucleotide cyclases. These have been divided into classes I–VI [2]. Class I cyclases include those in gram negative bacteria, e.g. *Escherichia coli* and *Yersinia pestis*, in which cAMP levels respond to external nutrient levels and mediate effects on transcription factors and gene expression. Class II cyclases comprise the extracellular soluble toxins of certain pathogens (see below under "*Bacterial and other adenylyl cyclases*"), e.g. those of *Bacillus anthracis*, *Bordetella pertussis*, and *Pseudomonas aeroginosa*. Class III cyclases (with subclasses IIIa–IIId) include most adenylyl and guanylyl cyclases (Fig. 2). These enzymes respond to changes in the extracellular environment. In mammals this occurs via hormones, neurotransmitters, odorants, or tastes. In lower organisms influences may be via changes in ionic factors, glucose, bicarbonate, or serum factors, and through osmoregulation, chemotaxis, phototaxis, or pH, in various bacteria. Class IV–VI cyclases are tentative assignments with few members each, but contain soluble and smallest adenylyl cyclases (~180 amino acids). The Class IV adenylyl cyclase of *Y. pestis* has been crystallized and its structure determined. The enzyme in *Prevotella ruminicola* was designated as a Class V cyclase [2]. And the enzyme found in the nitrogen-fixing bacterium *Rhizobium etli* was designated as a Class VI adenylyl cyclase.

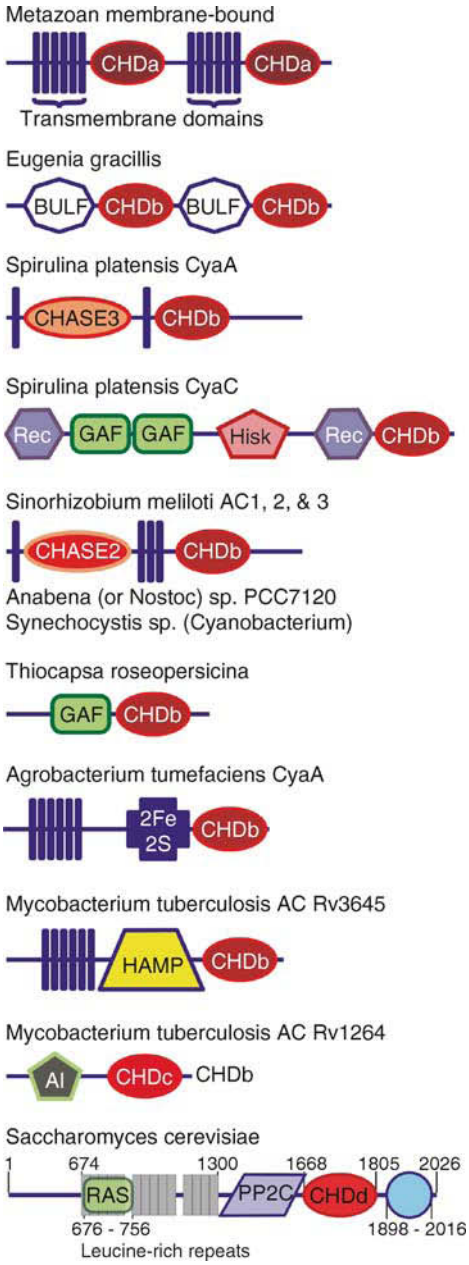


Adenylyl Cyclases. Figure 1 Synthesis, degradation, and actions of cAMP.

Mammalian Adenylyl Cyclases

Of the ten known ►isozymes of mammalian adenylyl cyclase (AC1-AC10; Table 1) all but one are membrane-bound and are regulated via cell-surface receptors linked to heterotrimeric (αβγ) stimulatory (G_s) and inhibitory (G_i) guanine nucleotide-dependent regulatory proteins (►G-proteins) (Fig. 3, Table 2) [1]. These receptors are referred to as G-protein coupled receptors, or GPCRs, and mediate effects of stimulatory and inhibitory hormones and neurotransmitters. $G\alpha_s$ stimulates all of these isozymes save the soluble AC10, and the plant-derived diterpene, ►forskolin, stimulates all isozymes, save AC9 and AC10, by binding within the cleft formed by the enzyme's two cytosolic domains ($\mathbf{C_1}$•$\mathbf{C_2}$; see below). The several isozymes differ more significantly in their responses to $G\alpha_i$ and $G\beta\gamma$ and in the physiological responses they control (Table 2). For example, $G\alpha_i$ inhibits some but not all isozymes and $G\beta\gamma$ inhibits AC1 and AC8, but significantly stimulates AC2, AC4, and AC7, and has reportedly different effects on AC5 and AC6 (Table 2).

Stimulation and inhibition of the enzyme by the GPCR-G-protein cycle occur by analogous mechanisms. Agonists induce hormone receptors to increase a Gα-GDP-GTP exchange and subsequent $G\alpha\beta\gamma$ dissociation ($GDP{\bullet}\alpha_x\beta\gamma + GTP \langle GTP{-}\alpha_x + \beta\gamma + GDP$) (Fig. 4). Consequently, agents that affect either the dissociation of either G_i or G_s, or the association of their respective α_s, α_i, or βγ subunits with adenylyl cyclase could affect rates of cAMP formation in enzyme preparations or in intact cells and tissues. There are several important examples. $G\alpha_s$ is stably activated by poorly hydrolyzable analogs of GTP, e.g. GTPγS



Adenylyl Cyclases. Figure 2 Domain organization of several class III adenylyl cyclases. Domains are abbreviated: CHD: cyclase homology domains for subclasses (IIIa – IIId), predicted transmembrane domains are indicated as vertical blue bars, BLUF: blue light receptor with tightly bound flavin, CHASE: (Cyclase/histidine kinase-associated sensing extracellular) domain, GAF switch domain: cGMP-binding phosphodiesterases, Adenylyl cyclases, and *E. coli* transcription factor FhlA, Rec: receiver domain; HisK: histidine kinase domain, 2Fe-2S: two iron-two sulfur cluster domain, HAMP: tandem amphoteric α-helices present in Histidine kinases, Adenylyl cyclases, Methyl-accepting chemotaxis proteins, and Phosphatases, AI: auto-inhibitory domain, leucine-rich repeats are indicated a vertical grey bars, PP2C: protein phosphatase type 2C catalytic domain, RAS, RAS-associating domain, and CAP: cyclase activating protein. This figure was modified from [2].

or GPP(NH)P, and it activation is hindered by GDPβS. A less obvious example is ▶fluoride. It activates most mammalian adenylyl cyclases, indirectly through its AlF_4^- complex with $G\alpha_s$•GDP. Another example includes the ADP-ribosyltransferase activities of bacterial toxins. The toxin of *Vibrio cholerae* catalyzes the ADP-ribosylation from NAD of GTP•$G\alpha_s$, and that of *Bordetella pertussis* similarly ADP-ribosylates α_i of GDP•$\alpha_i\beta\gamma$, preventing its dissociation. In both cases the effect is elevated adenylyl cyclase activity and contributes to the pathophysiology of these bacteria. Of therapeutic relevance, of course, are agents acting as agonist or antagonist on GPCRs coupled to adenylyl cyclase, with the prominent example being antagonists of β-adrenergic receptors (i.e. β-blockers).

Activities of all isozymes are affected by Ca^{2+}. At higher concentrations (mM) Ca^{2+} is inhibitory through competition with divalent cation required for catalysis (see below). At lower concentrations (<μM), Ca^{2+} regulates activity physiologically. This can be through: (i) a direct effect at the catalytic active site, increasing activity of AC10, or decreasing activity of AC5 and AC6; (ii) as a Ca^{2+}/calmodulin complex, activating ACs 1,3,8; (iii) with calcineurin to inhibit AC9; or (iii) indirectly through activation of PKC. Phosphorylation of adenylyl cyclase varies among the isozymes and is determined by differences in their primary sequences and is catalyzed by cAMP-dependent protein kinase (PKA) or protein kinase C (PKC) (▶Protein Kinase C) (Table 2). Activity of adenylyl cyclases can be indirectly influenced by the specific phosphorylation of hormone receptors or of ▶G-proteins.

Membrane-bound forms of mammalian adenylyl cyclases exhibit a putative topology with twelve membrane-spanning regions and two largely homologous ~40 kDa cytosolic domains ($\mathbf{C_1}$ and $\mathbf{C_2}$) (cf. Fig. 2). Differences in N-terminal and other domains are significant and influence regulation by a variety of agents as noted above (cf. Table 2). AC5($\mathbf{C_1}$) and AC2($\mathbf{C_2}$) domains have been separately expressed, recombined, and the resulting structure was solved in complex with GTP•$G\alpha_s$ (Fig. 5) [3]. α_s•GTP activates the enzyme through interaction with $\mathbf{C_2}$, yielding the active enzyme: Inhibition of adenylyl cyclase may occur by interaction of $G\alpha_i$ with the $\mathbf{C_1}$ domain of adenylyl cyclase, yielding GTP•α_i•$\mathbf{C}$, or by the recombination of βγ with $G\alpha_s$. The structure obtained with β-L-2′,3′-dd-5′-ATP allowed the demonstration that the pseudo symmetric cleft formed by the $\mathbf{C_1}$•$\mathbf{C_2}$ domains binds 5′-ATP, forskolin, and cation at

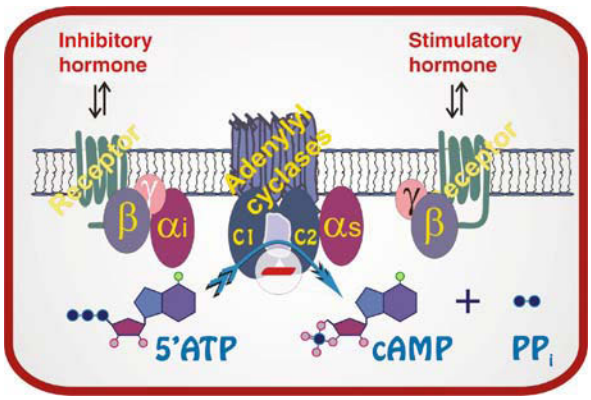
Adenylyl Cyclases. Table 1 Source, accession numbers, size, and gene loci for mammalian adenylyl cyclases

ADCY	Source	Reference accession numbers	Base pairs (mRNA)	Amino acids	Respective species chromosome
1	Bovine	NM_174229	3978	1134	4
	Human	NM_021116	12499	1119	7p13-p12
	Mouse	NM_009622	12259	1118	11 1.25 cM
2	Rat	NM_031007	4008	1090	17p14
	Human	NM_020546	6553	1091	5p15.3
	Mouse	NM_153534	4211	1095	13 41.0 cM
3	Rat	NM_130779	4533	1144	6q14
	Human	NM_004036	4342	1144	2p24-p22
	Mouse	NM_138305	3674	1145	12A-B
4	Rat	NM_019285	3357	1064	15p13
	Human	NM_139247	3320	1077	14q12
	Mouse	NM_080435	3414	1077	14D3
5	Rat	NM_022600	4847	1262	11q22
	Human	NM_183357	3842	1261	3q13.2-3q21
	Mouse	NM_001012765	7069	1262	16B-5
6	Rat	NM_012821	6036	1166	7q36
	Human	NM_015270 (1)	6594	1168	12q12-q13
	Human	NM_020983 (2)	5877	1115	12q12-q13
	Mouse	NM_007405	6038	1168	15F
7	Mouse	NM_007406 (1)	5199	1099	8 40.0 cM
	Mouse	NM_001037723 (2)	4750	1099	8 40.0 cM
	Mouse	NM_001037724 (3)	5938	1099	8 40.0 cM
	Human	NM_001114	6138	1080	16q12-q13
8A	Rat	NM_017142	4601	1248	7q33
	Human	NM_001115	6005	1251	8q24.2
	Mouse	NM_009623	5064	1249	15 37.5 cM
9	Mouse	NM_009624	4457	1353	16 2.0 cM
	Human	NM_001116	7732	1353	16p13.3
10	Human	NM_018417	5061	1610	1q24
Sacy	Mouse	NM_173029	5211	1614	1 H2.3
	Rat	NM_021684	5177	1608	13q23

Notes to Table 1:
(a) Adenylyl cyclases have been numbered in the order in which they were cloned and sequenced. In databases they are referred to as adcy#, with the exception of the soluble AC10, which is referred to variably as Sacy or Sac.
(b) Sources are those for which the database entries are given. The first source listed is the original source from which the isozyme was cloned. In some instances there are variant forms as indicated. For AC8 there are three splice variants; data are provided for variant 8A.
(c) Accession numbers are for the Reference Sequence (*RefSeq*) collection of data from the National Center for Biotechnology Information (NCBI). Values pertinent to the mammalian isozymes of adenylyl cyclases are compiled here. The link is: ►http://www.ncbi.nlm.nih.gov/ These numbers link to a "…comprehensive, integrated, nonredundant set of sequences, including genomic DNA, transcript (RNA), and protein products for several major research organisms. *RefSeq* standards serve as the basis for medical, functional, and diversity studies; they provide a stable reference for gene identification and characterization, mutation analysis, expression studies, polymorphism discovery, and comparative analyses. *RefSeqs* are used as a reagent for the functional annotation of some genome sequencing projects, including those of human and mouse."

two sites [4]. The active site shares topology and reaction mechanism with ►guanylyl cyclases, with which there is considerable homology, and with oligonucleotide polymerases.

Although there is substantial homology among the membrane-bound forms of the mammalian adenylyl cyclases, the striking differences in the character and extent of regulation by a variety of agents imply that primary and secondary structural characteristics are important determinants in the interactions of the enzyme with cell constituents and hence will regulate enzyme activity, the rate of formation of cAMP, and the downstream effects that this will have. All the studies on mammalian adenylyl cyclases



Adenylyl Cyclases. Figure 3 Membrane localization, topology, and regulation of mammalian adenylyl cyclases.

notwithstanding it is uncertain if all forms and variants of the enzyme have been identified, whether all modes of regulation have been determined, when during development, cell life cycles, and cell–cell interactions that specific isozymes are expressed, and how these processes are regulated. Perhaps because of this, the enzyme family continues to be a focus of much research and even as targets for drug discovery.

Catalytic Mechanism

Catalysis by adenylyl cyclases involves cation-mediated attack of the 3′-OH on the α-phosphate of 5′-ATP, with PP_i as leaving group. It is a reversible bireactant sequential mechanism with free cation and cation •5′-ATP as substrates and cAMP, cation•PP_i, and

Adenylyl Cyclases. Table 2 Regulatory characteristics of mammalian adenylyl cyclases

AdCy	Effect of $G\alpha_i$	Effect of Gβγ	Effects of Ca^{2+} and/or calmodulin	Effects of protein kinases	Tissue distribution	Physiological functions
1	↓	↓	↑	No Δ PKA	Brain (neuron), adrenal (medulla)	Neurotransmission, synaptic plasticity, LTP, memory,circadian rhythm
2	↓	↑	No Δ	No Δ PKA ↑ PKC	Brain, lung, skeletal muscle	Synaptic plasticity, arrest of cell proliferation
3	↓	no Δ	↑ (In vitro)		Olfactory epithelium, brain, adrenal, adipose, pancreas	Olfactory response to odorants
4		↑	No Δ		ubiquitous	
5	↓	↑↓	↓ (No CaM)	↓ PKA ↑ PKCα/ζ	Heart, brain (striatum)	Cardiac function, Ca^{2+}-dependent regulation
6	↓	↑↓	↓(No CaM)	↓ PKA ↓ PKC	Heart, kidney, Brain, liver, widespread	Cardiac function, Ca^{2+}-dependent regulation, hormonal regulation of gluconeogenesis, cell proliferation, coincidence detector for NO
7		↑	No Δ	↑ PKC	Brain, platelets, heart, spleen, lung	Ethanol dependency
8		↓	↑		Brain, lung	Neurotransmission, LTP, synaptic plasticity, memory
9			↓ (Ca^{2+}/calcineurin)	↓ PKC	Skeletal muscle, heart, brain, pancreas	Neurotransmission
10	No Δ	No Δ	↑ (no CaM)		Testes (germ cells), widespread	$HC0_3^-$ sensor; defect associated with absorptive hypercalciuria

Notes for Table 2:

(a) Empty cells imply that no information was available. Effects of additions on adenylyl cyclase activity are as indicated: up (↑) arrow: increase, down (↓) arrow: decrease, or "no Δ" (tested, but no effect on activity seen).

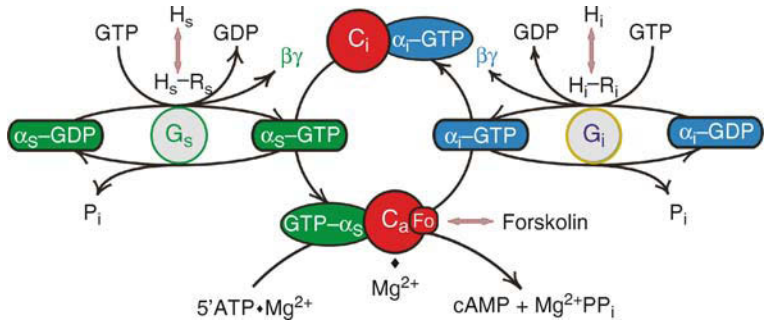
(b) Effects of $G\alpha_i$ or Gβγ are on enzyme stimulated by either $G\alpha_s$ or forskolin. In some instances differences were noted in the effects of different isoforms of β or γ. These are not distinguished here. For AC5 and AC6 both stimulation and inhibition have been reported, the difference being conditionally dependent on stimulation and whether or not full-length enzyme has been expressed.

(c) For effects of Ca^{2+} and/or calmodulin, stimulation of adenylyl cyclase by Ca^{2+} usually requires calmodulin, except in the case of AC10 [Sacy; no calmodulin (CaM)]. All adenylyl cyclases are inhibited by high (mM) concentrations of Ca^{2+}, through competition with divalent cation required for catalysis (cf. Figs. 6 and 7). The inhibition indicated here occurs with low (<μM) concentrations of Ca^{2+}, without calmodulin, but with AC9 Ca^{2+} inhibition is with calcineurin.

(d) Ca^{2+}/calcineurin has been observed to inhibit mouse AC9 but not human AC9.

(e) AC10 (Sacy/Sac), a soluble adenylyl cyclase discovered in testes is widely distributed and functions as a $HC0_3^-$ ion sensor. It is also stimulated by Ca^{2+}, independently of calmodulin.

(f) LTP: long term potentiation in neuronal function, PKA: cAMP-dependent protein kinase, PKC: protein kinase C, CaM: calmodulin.



Adenylyl Cyclases. Figure 4 Regulation of adenylyl cyclases by G-proteins. Abbreviations: H_s, H_i, R_s, and R_i denote hormones and receptors that lead to stimulation or inhibition, respectively, of adenylyl cyclases, Ca and Ci are active and inactive configurations of adenylyl cyclase, Fo: forskolin binding site, G_s and G_i are GTP-dependent regulatory proteins comprising their respective α_s, α_i, and βγ subunits.

free cation as products (Fig. 6; transition state is depicted as **E** ↔ **E***). Cation participation in catalysis through two sites was predicted from enzyme kinetics and was later confirmed in the solved enzyme structure (Fig. 5) [3]. Available data suggest that for some isozymes substrate binding and product release are ordered and for others random. Typically, reaction velocities are considerably greater with Mn^{2+} as cation than with Mg^{2+}. Maximal velocities observed with various ATP analogs follow the order: 2′-d-5′ ATP > ATP > ATPγS > APP(NH)P > APP(CH_2)P. Km values for rat brain cyclase are: K_{MnATP}, ~9μM; K_{Mn}^{2+}, ~4μM; K_{MgATP}, ~60μM; and K_{Mg}^{2+}, ~860μM. Notably, activation of adenylyl cyclases by hormones or by $G\alpha_s$, via the active enzyme configuration GTP•α_s•**C**, causes a reduction in K_{Mg}^{2+} of more than an order of magnitude to ~50μM, without a change in K_{MgATP}.

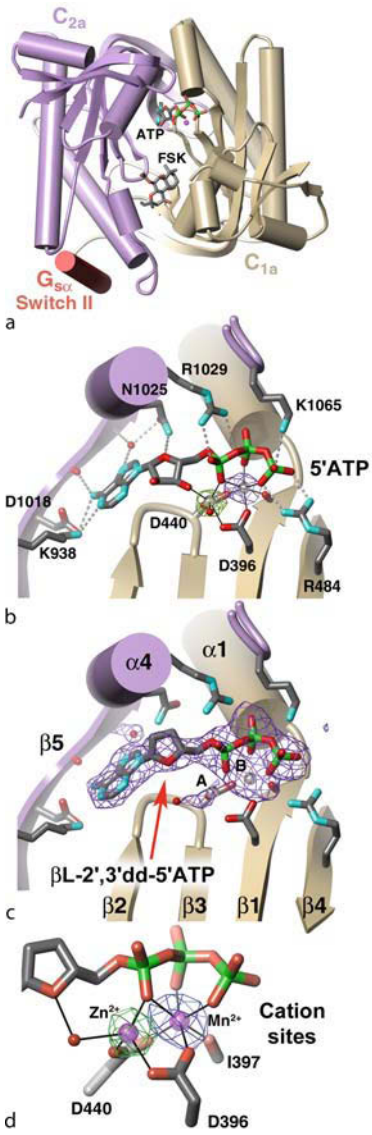
Miscellaneous Observations

Since its first description, adenylyl cyclase has been an intensely investigated enzyme family. Consequently, numerous observations have been made of agents that affect its activity, principally in isolated membranes, but also of purified enzyme. Some of these effects would be of importance for investigators intending to work with the enzyme. First, typical enzyme preparations, whether from native or recombinant sources, are of membranes or membrane extracts that contain enzyme activities that can alter concentrations of substrate or product of adenylyl cyclases. These include activities of cyclic nucleotide phosphodiesterases, ATPases, among others, that must be taken into consideration in assays of adenylyl cyclase activities. In addition, it has been universally observed that the enzyme is protected by thiols, with β-mercaptoethanol, 2,3-dimercaptopropanol, and dithiothreitol being the most commonly used. Conversely, adenylyl cyclases are generally susceptible to oxidants, e.g. H_2O_2, (IC_{50} ~3μM) and benzoquinone (IC_{50} ~3μM), and alkylating agents, e.g. *N*-ethylmaleimide (IC_{50} ~100μM), *p*-aminophenylarsenoxide (IC_{50} ~40μM), *p*-aminophenyldichloroarsine (IC_{50} ~80μM), or *o*-iodosobenzoate (IC_{50} ~10μM for AC1 against calmodulin stimulation). Not surprisingly, the crude membrane-bound enzyme is susceptible to thermal inactivation (e.g. 50% inactivation at 35° in 10 min) and purified enzyme is more labile, but protection is afforded by forskolin, substrate, ►P-site ligands, Ca^{2+}/calmodulin (e.g. with AC1), and by GTPγS•$G\alpha_s$. Proteases also elevate adenylyl cyclase activity. For example, acrosin, trypsin, and thrombin can cause 5–10-fold activation, and these exhibit some isozyme selectivity (AC2 > AC3 >> AC5). The basis for this activation in each case is not clear, though serine proteases are known to cleave $G\alpha_i$, and this could lead to indirect effects on adenylyl cyclase activity.

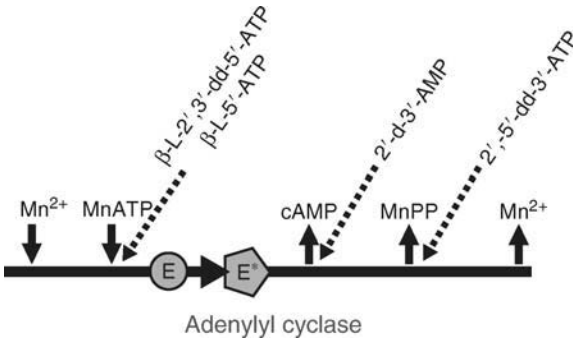
Bacterial and Other Adenylyl Cyclases

Adenylyl cyclases are found throughout the animal kingdom and serve a variety of roles. Structures of enzyme from but a few of these sources have been determined, although amino acid sequences and domain structures have been deduced for an ever increasing number. Available evidence indicates that there is little sequence homology between these adenylyl cyclases and the membrane-bound mammalian form. As is evident from the varied domain structure just of class III adenylyl cyclases (Fig. 2) [2], although the enzyme is principally membrane bound in metazoan species, it may or may not be in lower organisms. Furthermore, those forms that are membrane bound are more often than not regulated by means quite different from that described above for mammalian systems.

A comprehensive summary of these enzyme families is beyond the scope of this chapter, but a few examples are worth emphasis. The Class I adenylyl cyclases of the enterobacteria *Salmonella typhimurium*, *Yersinia pestis*, and *Escherichia coli* are membrane bound yet sequences do not give ready evidence of typical



Adenylyl Cyclases. Figure 5 Catalytic cleft and active site of a chimeric mammalian adenylyl cyclase. The cleft is formed by the pseudosymmetric interaction of enzyme cytosolic domains, C_1 and C_2. Panel A: part of the crystal structure of the chimeric adenylyl cyclase AC5C_1•AC2C_2 with $G\alpha_s$, indicating binding sites for substrate (5′ATP) and forskolin (FSK). The Switch II domain of $G\alpha_s$ interacts with the C_2 domain of adenylyl cyclase. Panel B: the catalytic active site modeled with 5′-ATP and amino acids involved in catalysis; based on structure with β-L-2′,3′-dd-5′-ATP (C). Panel C: structure with β-L-2′,3′-dd-5′-ATP and loci for two metal sites, A and B. Panel D: enlargement of C with Zn^{2+} (metal A) and Mn^{2+} (metal B) used in forming the crystal. Catalysis occurs with the metal catalyzed attack of the ribosyl 3′-OH group of the substrate α-phosphate. Adapted from [3].



Adenylyl Cyclases. Figure 6 Adenylyl cyclase catalytic cycle. Points during the catalytic cycle of adenylyl cyclases at which inhibition by competitive and noncompetitive nucleotides occur; E* represents the catalytic transition state.

transmembrane domains. The enzymes comprise two principal domains, with the catalytic domain being N-terminal to a glucose-sensing regulatory domain; the enzyme is inhibited the presence of glucose. Its regulation is coordinated with that of carbohydrate permeases by the phosphoenolpyruvate:sugar phosphotransferase system. This is important for bacterial responses to changes in nutrient levels. In other bacteria, the enzyme may be regulated in response to nutrients and/or it may constitute a toxic factor in mammals, as with Class II forms of adenylyl cyclase of *Bordetella pertussis*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, or *Yersinia pestis*. These enzymes constitute the 'toxin class' of adenylyl cyclases. The well-studied adenylyl cyclases of *Bordetella pertussis* and *Bacillus anthracis* are both soluble, Ca^{2+}/calmodulin-dependent, but G-protein independent enzymes that are exported from the respective bacteria. (The adenylyl cyclase of *P. aeruginosa* is not calmodulin-dependent.) Because these enzymes are then transported into infected cells, adenylyl cyclase actually constitutes a virulence and toxic factor in mammals. The *B. pertussis* adenylyl cyclase is a large (1706 amino acids) bifunctional enzyme, the N-terminal end constituting the adenylyl cyclase activity fused to a C-terminal end exhibiting hemolytic activity and its capacity for being secreted into external medium. The *B. anthracis* adenylyl cyclase (800 amino acids), also known as 'edema factor' (EF), exhibits four domains, a signal peptide essential for protein secretion, a docking domain allowing binding to the protective antigen (PA), the adenylyl cyclase catalytic site, and a fourth region of unknown function. The *B. anthracis* adenylyl cyclase has been crystallized and its structure determined.

The Class III adenylyl cyclases are sometimes referred to as the ancestral form of the enzyme and

include numerous variants (cf. Fig. 2). Among these there are a couple of noteworthy examples. The adenylyl cyclase of *Saccharomyces cerevisiae* was the first to have been cloned and sequenced and is a prototypical Class III enzyme, with a sequence in the catalytic domain distinct from those of Class I and Class II enzymes and with the catalytic core located at the C-terminal part of the protein (Fig 2). In such yeast/fungi (e.g. *Candida albicans*) the enzyme is membrane bound and is regulated by a G-protein, in these cases Ras. As in mammalian systems it is involved in metabolic control, in mating responses, but also constitutes a virulence factor. In *C. albicans*, for example, which contains only one form of the enzyme, the cAMP signaling pathway is essential for hyphae formation and hence virulence. Sequences have been deduced for a number of enzymes of this family, including *Schizosaccharomyces pombe*, *Saccharomyces kluyveri*, *Trypanosoma brucei*, and *T. equiperdum*, *Neurospora crassa*, and *Dictyostelium discoideum*, where the adenylyl cyclase generates the cAMP that provides the signal for aggregation into a multicellular organism and the development of fruiting bodies.

Given that in many of these systems additional proteins and cofactors participate in the regulation of adenylyl cyclase activity, the full elucidation of the roles in which this enzyme activity participates in their growth, development, and function, is a long way off. This notwithstanding, the fact that the mammalian adenylyl cyclases differ so substantially from those of numerous pathogens in which the enzyme is an essential virulence factor gives motive to the idea that new classes of small molecule inhibitors of the pathogen adenylyl cyclases may be discovered that do not interact with mammalian forms of the enzyme.

Drugs

Although agents which indirectly activate or inhibit mammalian adenylyl cyclases are common and are even used in the treatment of disease, especially drugs targeting G-protein-coupled receptors, drugs acting directly on the enzyme have been less well explored. And for most compounds acting directly on adenylyl cyclases, high selectivity for specific isozymes has not been demonstrated. The main classes of such agents are derivatives of forskolin and of adenine nucleosides. Adenosine and derivatives of it have long been known to inhibit adenylyl cyclases and it became clear early on that certain modifications afforded substantially increased inhibitory potency. Notable are the approximately threefold increase in potency seen with the 2-fluorine substitution on adenine and the increases in potency seen with various modifications to the ribose moiety. The orientation of the ribose (α vs β) and the presence, orientation, or absence of hydroxyl groups clearly contribute to inhibitory potency (Table 3). For example, arabinose and xylose differ from ribose only in the orientation of the 2′- and 3′-OH groups yet exhibit markedly different potencies. Whereas 9-(tetrahydrofuryl)-Ade (►SQ 22,536) and 9-(cyclopentyl)-Ade are without hydroxyl groups and are less potent, they offer metabolic and biochemical stability useful for many types of studies. It is, however, the removal of two of the hydroxyl groups, that elicits the largest improvement in inhibitory potency, in particular the 2′,5′-dideoxy- modification (Table 3). With these improvements in potency, these cell permeable compounds, in particular ►2′,5′-dd-Ado, have become useful research tools and have been used to inhibit adenylyl cyclases and to lower cAMP levels and alter function in numerous studies in isolated cells or intact tissues.

An early observation that 2′-d-3′-AMP was a more potent inhibitor of adenylyl cyclases than 2′-d-Ado suggested that the enzyme would accept substitutions at the 3′-ribose position and that phosphate was particularly well tolerated. This led to the generation of a family of 3′-phosphoryl derivatives of 2′,5′-dideoxyadenosine exhibiting ever greater inhibition with the addition of an increasing number of 3′-phosphoryl groups, the most potent of which is 2′,5′-dideoxyadenosine-3′-tetraphosphate (2′,5′-dd-3′-A4P; Table 4) [5]. These constitute a class of inhibitors historically referred to as ►P-site ligands that caused inhibition of adenylyl

Adenylyl Cyclases. Table 3 ***Nucleoside inhibitors of adenylyl cyclase***. Assays were with a detergent-dispersed adenylyl cyclase from rat brain and were with 100 μM 5′ATП and 5 mM $MnCl_2$ as substrates

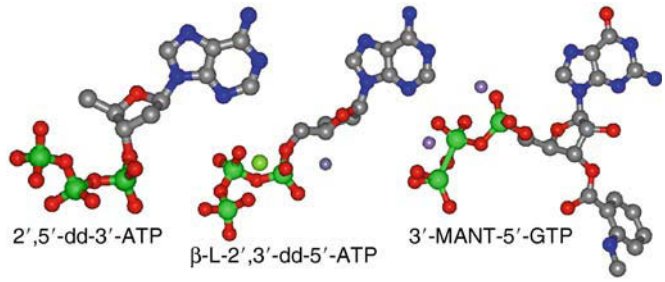
Nucleoside	IC_{50} (μM)
β-Adenosine	82
α-Adenosine	>300
9-(arabinose)-Ade	30
9-(xylose)-Ade	3.2
9-(tetrahydrofuryl)-Ade	20
9-(cyclopentyl)-Ade	100
β-2′-d-Ado	15
β-3′-d-Ado (cordycepin)	13
β-2′-d-Xyl-Ade	15.5
β-2′-d-2-F-Ado	4.6
α-2′-d-2′-F-Ado	>100
β-2′,3′-dd-Ado	9
β-2′,5′-dd-Ado	2.8
β-2′,5′-dd-Xyl-Ade	16.4
β-2′,5′-dd-2-F-Ado	0.89
α-2′,5′-dd-2-F-Ado	>100
β-2′,5′-dd-2,5′-di-F-Ado	0.98
α-2′,5′-dd-2,5′-di-F-Ado	29

Adenylyl Cyclases. Table 4 ***Nucleotide inhibitors of adenylyl cyclase.*** Enzyme source and assay conditions were as for Table 3. Values obtained for 3′-ATP are overestimations due to the formation of 2′:3′-cAMP from 3′-ATP that occurs nonenzymatically in the presence of divalent cation

Adenine nucleoside 3′-phosphates (IC_{50}s μM)			
3′-phosphate	Ado	2′-d-Ado	2′,5′-dd-Ado
None	82	15	2.7
3′ ∼P	8.9	1.2	0.46
3′ ∼PP	3.9	0.14	0.1
3′ ∼PPP	2	0.09	0.04
3′ ∼PPPP	–	0.011	0.0074
3′ ∼PS	–	3.1	0.6
Substrate analogs (IC_{50}s μM)			
β-L-5′-AMP		200	
β-L-2′,3′-dd-5′-AMP		62	
β-D-5′-AP(CH_2)PP		30	
β-L-5′-ATP		3.2	
β-D-2′,3′-dd-5′-ATP		0.76	
β-L-2′,3′-dd-5′-ATP		0.024	
Acyclic 9-substituted-Adenines (IC_{50}s μM)			
PMEA		65	
PMEApp		0.17	
PMEAp(NH)p		0.18	
PMPA		6.3	
PMPApp		0.5	
2′- and 3′-Substituted-5′-NTPs (IC_{50}s μM)			
2′(3′)-MANT-5′-GTPγS		0.02	
2′(3′)-MANT-5′-ITPγS		0.039	
2′(3′)-MANT-5′-ATP		0.064	
3′-MANT-2′-d-5′-ATP		0.14	
3′-d-2′-MANT-5′ATП		0.26	
3′-7M4AMC-2′-d-5′-ATP		0.36	
3′-Dansyl-2′-d-5′-ATP		3.21	
Fluorescent-phoshoryl-derivatives (IC_{50}s μM)			
2′,5′-dd-3′-ATP-(γ-7A4AMC)		0.166	
2′,5′-dd-3′-ATP-(γ-7M4AMC)		0.88	
2′,5′-dd-3′-ADP-(γ-7M4AMC)		1.65	

cyclase that was kinetically either noncompetitive or uncompetitive (cf. Fig. 6). This implied binding of the inhibitor with either a different locus or different configuration than substrate. As it developed, these are configuration selective inhibitors and they provide an exquisite means for inhibition of this signal transduction pathway. We know now that most membrane-bound forms of the mammalian adenylyl cyclase are inhibited by adenine nucleosides and their 3′-polyphosphates derivatives. Inhibition by these ligands is conserved with varying sensitivity in all isozymes, save AC10 and those of bacteria.

Probably all adenylyl cyclases are inhibited competitively by substrate analogs, which bind at the site and to the enzyme configuration with which cation-ATP binds (cf. Fig. 4). One of the best competitive inhibitors is β-L-2′,3′-dideoxyadenosine-5′-triphosphate (β-L-2′,3′-dd-5′-ATP; Table 4) [4], which allowed the identification of the two metal sites within the catalytic active site (cf. Fig. 4) [3]. This ligand has also been labeled with ^{32}P in the β-phosphate and is a useful ligand for reversible, binding displacement assays of adenylyl cyclases [4]. The two inhibitors, ►2′,5′-dd-3′-ATP and β-L-2′,3′-dd-5′-ATP, are comparably potent



Adenylyl Cyclases. Figure 7 Structures of potent inhibitors of adenylyl cyclase. Structures for 2′,5′-dd-3′-ATP (IC_{50} ~40 nM; noncompetitive inhibitor), β-2′,3′-dd-5′-ATP with Mg^{2+} and Zn^{2+} (IC_{50} ~24 nM; competitive inhibitor), and 3′-MANT-GTP with Mn^{2+} (IC_{50} ~90 nM; competitive inhibitor) are from coordinates obtained for these compounds in respective crystal structures with AC5**C$_1$**•AC2**C$_2$**. Divalent cations are indicated: Mn^{2+}: purple, Mg^{2+}: green, Zn^{2+}: blue. The 3′-MANT-group fits into a hydrophobic pocket of the enzyme. Note the difference in contortion of the phosphate chains in these structures relative to positions for divalent cation.

(Table 4), but inhibit adenylyl cyclase by conformationally distinct mechanisms (cf. Fig. 6) by binding within the catalytic cleft in unique structures (Fig. 7).

It has been known for some time that the enzyme tolerated large substitutions to the 3′-ribose position. This was taken advantage of with the development of 2′(3′)-*O*-MANT-derivatives of nucleoside 5′-triphosphates [6] (Table 4). It was surprising, though, that potent inhibition was seen with bases other than adenine, implying that base specificity is less stringent than had been generally assumed. Subsequently, fluorescent derivatives have been made with different fluorophores at 2′- and 3′-positions. 3′-Substitutions showed advantage over corresponding 2′-substitutions and 2′(3′)-*O*-MANT-substitutions were clearly preferable to coumarin and dansyl derivatives, but followed the order of guanosine ≥ inosine > adenosine (Table 4). Fluorescent phosphoryl derivatives were also well tolerated, in particular the 7-amino-coumarin. These fluorescent ligands have opened possibilities for investigations of adenylyl cyclase structure, activity, and interactions with other substances not heretofore possible.

Although the 3′- and 5′-polyphosphate derivatives mentioned above exhibit exquisite inhibitory potency these compounds are not cell permeable. To take advantage of the potency of such derivatives for studies with intact cells and tissues, there are two possibilities. One is chemically to protect the phosphate groups from exonucleotidases that also allows the compound to transit the membrane intact. The other is to provide a precursor molecule that is cell permeable and is then metabolized into an inhibitor by intracellular enzymes. The general term for such a compound is ►prodrug; nucleotide precursors are also referred to as ►pronucleotides. Families of protected monophosphate derivatives were synthesized, based on β-L- and β-D-2′,5′-dd-3′-AMP, β-L-2′,3′-dd-5′-AMP, and the acyclic 9-substituted adenines, ►PMEA and ►PMPA. Protective substituents were: (i) -(*S*-pivaloyl-2-thioethyl)=(*t*-Bu-SATE-); (ii) -*S*-acetyl-2-thioethyl)=(Me-SATE-); (iii) -(*S*-benzyl-2-thioethyl)=(Ph-SATE-); (iv) -cyclosalicyl=(H-Sal-); and (v) -3-methyl-cyclosalicyl=(Me-Sal-). Although triphosphate forms of each of the precursor compounds inhibit isolated adenylyl cyclases with IC_{50}-s in the nanomolar range, only protected forms of 2′,5′-dd-3′-AMP inhibited cAMP formation in intact cells [7]. Of these the SATE-derivatives proved the most effective. None of the pronucleotide forms of 2′,5′-dd-3′-AMP inhibited adenylyl cyclase *per se*, whether isolated from rat brain or OB1771 cells. Nor were identifiable extracellular metabolites of these agents responsible for the drugs' blocking effects on intact cells. These compounds exhibit all the hallmarks of prodrugs. They are taken up, are deprotected, and are converted to extremely potent inhibitors of adenylyl cyclase, *but only by intact cells and tissues*. These prodrugs have been used to block cAMP formation in isolated cells and intact tissue and elicit functional effects (Table 5). For example, pretreatment of isolated rat atria with 1 μM 2′,5′-dd-3′-AMP-bis(t-Bu-SATE) completely blocked the positive chronotropic effects of 1 μM epinephrine. It is likely that pronucleotide inhibitors of adenylyl cyclases

Adenylyl Cyclases. Table 5 ***Prodrug inhibition of [^{3}H] cAMP formation in intact cells.*** Cells were prelabeled for 2 h with [^{3}H]adenine before 50 μM forskolin and pronucleotides were added. After a 15 min incubation the newly formed [^{3}H]cAMP was extracted and quantified as in (7)

Pronucleotide	OB-1771 Preadipocytes	THP1 Monocytes
	IC_{50} (nM)	
2′,5′-dd-3′-AMP-bis(Me-SATE)	6.7	260
2′,5′-dd-2F-Ado-3′-P-bis (Me-SATE)	9.8	110

will find applications in many intact cell systems, as an additional upstream block of the adenylyl cyclase-cAMP-PKA signaling cascade, in biochemical, pharmacological, and potentially even therapeutic contexts.

Abbreviations Used Within the Text

Ado, adenosine
cAMP, adenosine-3′:5′ monophosphate
2′-d-Ado, 2′-deoxyadenosine
2′-d-2-F-Ado, 2′-deoxy-2-fluoro-adenosine
3′-d-Ado, 3′-deoxyadenosine (cordycepin)
2′,5′-dd-Ado, 2′,5′-dideoxyadenosine
2′,3′-dd-Ado, 2′,3′-dideoxyadenosine
2′,5′-dd-2-F-Ado, 2′,5′-dideoxy-2-fluoro-adenosine
2′,5′-dd-2,5′-di-F-Ado, 2′,5′-dideoxy-5′-fluoro-2-fluoro-adenosine
9-CP-Ade, 9-(cyclopentyl)-adenine
9-THF-Ade, 9-(tetrahydrofuryl)-adenine (SQ22,536)
9-Ara-Ade, 9-(arabinofuranosyl)-adenine
9-Xyl-Ade, 9-(xylofuranosyl)-adenine
2′-d-Xyl-Ade, 9-(2-deoxyxylosyl)-adenine
2′,5′-dd-Xyl-Ade, 9-(2,5-dideoxyxylosyl)-adenine
2′-d-3′-AMP, 2′-deoxyadenosine-3′-monophosphate
2′-d-3′-ADP, 2′-deoxyadenosine-3′-diphosphate
2′-d-3′-ATP, 2′-deoxyadenosine-3′-triphosphate
2′-d-3′-AMPS, 3′-(thiophosphoryl)-2′-deoxyadenosine
2′,5′-dd-3′-AMP, 2′,5′-dideoxyadenosine-3′-monophosphate
2′,5′-dd-3′-ADP, 2′,5′-dideoxyadenosine-phos-phate-3′-diphosphate
2′,5′-dd-3′-ATP, 2′,5′-dideoxyadenosine-3′-triphosphate
2′,5′-dd-3′-A4P, 2′,5′-dideoxyadenosine-phos-phate-3′-tetraphosphate
2′,5′-dd-3′-AMPS, 3′-(thiophosphoryl)-2′,5′-dideoxyadenosine
5′-APP(CH2)P, adenosine 5′-(β(-methylene)-triphosphate
β-L-5′-ATP, β-L-adenosine-5′-triphosphate
β-L-2′,3′-dd-5′-ATP, β-L-2′,3′-dideoxyadenosine-5′-triphosphate
PMEA, 9-[(2-phosphonylmethoxy(ethyl)]-adenine
PMEApp, 9-[(2-diphosphorylphosphonylmethoxy (ethyl)]-adenine
PMEAp(NH)p, 9-[(2-iminodiphosphorylphosphonyl-methoxy(ethyl)]-adenine
PMPA, 9-[(2-phosphonylmethoxy)propyl]-adenine
PMPApp, 9-[(2-diphosphorylphosphonylmethoxy (propyl)]-adenine
2′,5′-dd-3′-AMP-bis(Me-SATE), 2′,5′-dideoxyadenosine-3′-(acetyl-2-thioethyl)-phosphate
2′,5′-dd-3′-AMP-bis(*t*-Bu-SATE), 2′,5′-dideoxyadenosine-3′-(pivaloyl-2-thioethyl)-phosphate
2′,5′-dd-3′-AMP-bis(Ph-SATE), 2′,5′-dideoxyadenosine-3′-(phenyl-2-thioethyl)-phosphate
2′,5′-dd-2F-Ado-3′-P-bis(Me-SATE), 2′,5′-dd-2-fluoro-adenosine-3′-(acetyl-2-thioethyl)-phosphate
MANT-5′-GTPγS, 3′-(2′)-O-*N*-methylanthraniloyl-guanosine-5′-[γ-thio]triphosphate
MANT-5′-ITPγS, 3′-(2′)-O-*N*-methylanthraniloyl-inosine-5′[γ-thio]triphosphate
MANT-5′ATP, 3′-(2′)-O-*N*-methylanthraniloyl-5′-ATP
MANT-5′GTP, 3′-(2′)-O-*N*-methylanthraniloyl-5′-GTP
3′-MANT-2′-d-5′-ATP, 3′-O-*N*-methylanthraniloyl-2′-deoxy-5′-ATP
3′-7M4AMC-2′-d-5′-ATP, 3′-(7-methoxy-4-amino-methylcoumarinn)-2′-deoxy-5′-ATP
3′-Dansyl-2′-d-5′-ATP, 3′-(dansyl)-2′-deoxy-5′-ATP
2′,5′-dd-3′-ADP-(β-7M4AMC), 2′,5′-dideoxyadenosine-{β-(7-methoxy-4-aminomethyl-coumarin)}-3′-diphosphate
2′,5′-dd-3′-ATP-(γ-7M4AMC), 2′,5′-dideoxyadenosine-{γ-(7-methoxy-4-aminomethyl-coumarin)}-3′-triphosphate
2′,5′-dd-3′-ATP-(γ-7A4AMC), 2′,5′-dideoxyadenosine-{γ-(7-amino-4-aminomethyl-coumarin)}-3′-triphosphate

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ADH

Antidiuretic Hormone.

►Vasopressin/Oxytocin

ADHD

Attention Deficit Hyperactivity Disorder.

►Psychostimulants

Adhesion Molecules

Adhesion molecules are transmembrane proteins, which through their extracellular part mediate the interaction of cells with other cells or with extracellular components like the extracellular matrix. On the basis of structural and functional similarities, most adhesion molecules can be grouped into families such as cadherins, integrins, selectins, the immunoglobulin superfamily or the syndecans. While some adhesion molecules are passive in their adhesive function, the adhesiveness of other adhesive molecules can be regulated. Some adhesive proteins are very similar to receptors, in that they not only bind other molecules with high selectivity and affinity, but are also able to transduce the binding into an intracellular signal.

►Integrins
►Cadherins
►Table appendix: Adhesion Molecules
►Anti-integrins

Adipocyte

Adipocyte is the term for a fat cell which is used to store energy in the form of triacylglycerols. There are two different types of adipocytes. (i) White adipocytes contain a large lipid droplet and secrete different adipocytokines like adiponectin, and resistin. The lipid may account to 90% of cell mass. (ii) Brown adipocytes contain lipid droplets scattered throughout the single cell and are used to generate heat in a process known as nonshivering thermogenesis.

Fibroblasts, which are undifferentiated pre-adipocytes, can be stimulated and converted into adipocytes.

►Fatty Acid Transport Proteins
►Adipokines

Adipocytokines

►Adipokines

Adipokines

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Synonyms

Adipocytokines

Definition

The term adipokine refers to any protein secreted from adipocytes [1]. Collectively, the various adipokines form the 'adipokinome' which together with the lipid moieties secreted from fat cells (e.g. fatty acids, cholesterol, retinol) constitute what can be referred to as the 'secretome' of adipocytes. Most adipokines are also secreted from other cell types in other organs, but one in particular – adiponectin – is considered to be exclusive to adipocytes.

Basic Characteristics

►White adipose tissue, or white fat, has been traditionally viewed essentially as an organ of fuel storage. Around half of the total cell content of adipose tissue is made up of mature ►adipocytes, the other cell types including fibroblasts, preadipocytes, macrophages and endothelial cells. Lipids are stored in mature white adipocytes as triacylglycerols in the form of a single large droplet. This gives a unilocular appearance under the microscope, and lipid can constitute up to 90% of the cell mass. Triacylglycerols are deposited in the tissue at a time of nutritional excess to be released during periods of negative energy balance – such as fasting and long-term starvation. Lipids allow fuel to be stored at a high energy density since their calorific value is twice that of carbohydrates, and because in contrast to carbohydrate they can be stored with little associated water.

Additional roles traditionally recognised for white adipose tissue include thermal insulation (as in the blubber of sea mammals) and mechanical protection to internal organs. However, in recent years it has become apparent that the function of white adipose tissue is much more extensive. This follows from the discovery that it is a major endocrine organ, secreting a diverse

range of protein hormones and other protein factors – *the* ►adipokines [1–3]. These secreted protein signals and factors were initially called adipocytokines, but the term adipokines is now generally used. This reflects the fact that the name 'adipocytokine' implies that the proteins are cytokines, or cytokine-like. While this is true of some, it is not so for the majority.

The first protein factor to be identified as being secreted from adipocytes was the enzyme lipoprotein lipase, which is responsible for the breakdown of circulating triacylglycerols (largely in the form of lipoproteins) to release free fatty acids and glycerol; however, no special significance was attributed to this particular secretion. The fatty acids are then taken up into the adipocytes and re-esterified to triacylglycerol. The next adipocyte secreted protein to be identified was adipsin. This is a complement-related factor which was initially thought to play an important role in the control of energy balance and body weight as a lipostatic signal, although this was quickly recognised not to be the case. In the early 1990s adipocytes were then found to express and secrete tumour necrosis factor-α (►TNFα), a major pro-inflammatory cytokine. TNFα was immediately linked to the development of insulin resistance in fat cells, but its actions are now considered to be rather more extensive. It plays, for example, a pivotal role in the regulation of the production of a number of other adipokines, particularly those related to ►inflammation.

Leptin and Adiponectin

The protein factor whose identification induced the radical change in our understanding of the physiological role of adipose tissue was the hormone ►leptin (Greek: *leptos* – thin or small). This was discovered in 1994 as the product of the *OB* (*LEP*) gene, mutations in which result in the profound ►obesity of the obese (*ob/ob)* mouse [1–4]. Mutations in the leptin gene have subsequently been identified in humans, and as in mice they are associated with extreme obesity. Leptin is a key satiety factor, providing a signal from adipocytes to the hypothalamus in the neuroendocrine regulation of appetite [3, 4]. In practise, leptin is now recognised to be a pervasive hormone with multiple functions, including as a signal in the maturation of the reproductive system, in immunity and in insulin secretion and glucose utilisation. Although leptin is produced in a number of different cells and organs, the major site of production is the adipocyte, with the amount of body fat being the primary determinant of the circulating level of the hormone. There is, however, acute regulation of leptin production with an important role for insulin and the sympathetic nervous system. Indeed, the sympathetic system provides a negative feedback loop from the brain to adipocytes in the control of leptin production.

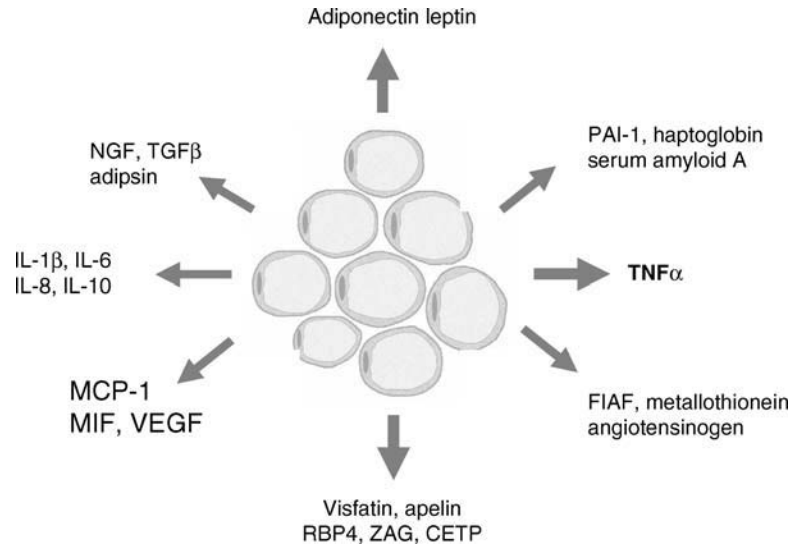
The discovery of leptin resulted in white adipose tissue being recognised as a major endocrine organ. Indeed, in many cases – particularly the obese – it is the largest endocrine organ in the body, amounting to up to 50% or more of total tissue mass. Following the identification of leptin, a rapidly expanding list of other protein hormones and factors secreted from adipocytes has been identified (Fig. 1). The total number of these adipokines is now in excess of 50 distinct molecular entities [1, 2].

The second major hormone, identified to be secreted from adipocytes, was ►adiponectin [3]. This factor was discovered by several groups in the mid 1990s and is also known as Acrp30, AdipoQ, ApM1 and GBP28. Adiponectin (the most widely used name) appears to be secreted exclusively by adipocytes, in contrast to the other adipokines. Adiponectin is involved in a wide range of functions. These include important roles in modulating insulin sensitivity, in inflammation (anti-inflammatory) and atherogenesis. Adiponectin circulates at high concentrations in the blood, occurring in high molecular weight polymeric forms. In marked contrast to other adipokines, its production and circulating levels fall in obesity.

Inflammation

A number of adipokines are linked to inflammation and immunity (Fig. 1). This includes both leptin and adiponectin, and also a number of other key inflammatory proteins, particularly cytokines and chemokines [1]. The cytokines and chemokines encompass interleukin-1β (IL-1β), IL-6, IL-10, TNFα, monocyte chemoattractant protein-1 (MCP-1), and macrophage migration inhibitory factor (MIF). Other major inflammation-related adipokines include nerve growth factor (NGF), and acute phase proteins such as serum amyloid A and haptoglobin. In addition, adipocytes secrete plasminogen activator inhibitor-1 (PAI-1), which is an important thrombotic factor as well as an acute phase protein.

The wide range of inflammation-related factors that adipocytes secrete is linked to the inflammatory response that the tissue exhibits in obesity [1]. Obesity in general, like an increasing number of other diseases, is characterised by a state of mild chronic inflammation, and adipose tissue plays a central role in this. The production of most inflammation-related adipokines increases markedly in obesity and there is an elevated circulating level of a number of these factors as well as of other inflammatory markers such as C-reactive protein (CRP). The increased production of inflammatory adipokines (and decreased production of adiponectin with its anti-inflammatory action) in the obese is considered to play a critical role in the development of the obesity-associated pathologies, particularly type 2 diabetes and the ►metabolic syndrome [1].



Adipokines. Figure 1 Major proteins – adipokines – secreted from white adipocytes. CETP, cholesteryl ester transfer protein; FIAF, fasting-induced adipose factor (angiopoeitin like protein-4) IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol binding protein-4; TGFβ, transforming growth factor-β; TNFα, tumour necrosis factor-α; VEGF, vascular endothelial growth factor; ZAG, zinc α2-glycoprotein.

An important component of the inflammatory state in adipose tissue in obesity comes from the infiltration of the tissue by macrophages. These are likely to be attracted through the secretion by adipocytes of MCP-1 and MIF. The macrophages in turn secrete factors which both directly add to the total production of inflammatory agents by adipose tissue and also catalyse the production of such agents from adipocytes – and perhaps preadipocytes as well.

The wide range of inflammatory and immune factors secreted by adipocytes has led to the view that there are many similarities between these cells and cells of the immune system.

Other Adipokines and Metabolic Processes

In addition to the regulation of energy balance and the mounting of an inflammatory response, adipokines are involved in a number of other physiological and metabolic processes [3, 4]. These include lipid metabolism (e.g. retinol binding protein, cholesteryl ester transfer protein), the control of blood pressure through the renin-angiotensin system (e.g. angiotensinogen), vascular haemostasis (e.g. PAI-1), angiogenesis, glucose homeostasis and stress responses. Several factors are involved in angiogenesis and these not only include the key angiogenic factor vascular endothelial growth factor (VEGF), but also leptin and angiopoietin-like protein 4/fasting-induced adipose factor (Angptl4/FIAF). Leptin and adiponectin – and perhaps IL-6 and MCP-1 – play an important role in glucose homeostasis and insulin sensitivity.

The current view is that through the various adipokines, adipocytes and adipose tissue are implicated in a wide range of physiological functions in a manner that far transcends the original simple paradigm of fuel storage. Indeed, there is extensive cross-talk between adipocytes and other cells and organs, including the brain, skeletal muscle and bone. Within adipose tissue itself, there is cross-talk between adipocytes, macrophages and preadipocytes, particularly in relation to the inflammatory response. The discovery of the adipokines and the endocrine role of adipose tissue has led to parallel developments with skeletal muscle; myocytes are now recognised to secrete protein factors also, in particular IL-6, leading to the concept of 'myokines'.

Drugs

Little attempt has been made to develop drugs targeted specifically to white adipose tissue and the production of adipokines. It is likely, however, that there will be an increasing emphasis on this approach to the pharmacological treatment of obesity-related diseases, given the current views on the centrality of the adipokines to these disorders. It is, of course, the diseases that obesity leads to, rather than obesity itself, that constitute the main medical challenge.

There has been considerable focus on the development of drugs that lead to a reduction in the total amount of adipose tissue. These include agents targeted at limiting fat absorption, the inhibition of appetite, and the stimulation of energy expenditure (thermogenesis)– or a combination thereof. The best example of drugs,

targeted specifically to adipocytes, are the β_3-adrenoceptor agonists such as the first generation compound BRL-37344 [(*RR* + *SS*)-(+)-4-[2-(2-(3-chlorophenyl)-2-hydroxyethyl)amino)propyl] phenoxyacetate]. These stimulate lipolysis and thermogenesis (in brown adipose tissue), but their use in humans has been problematic because the β_3-adrenoceptor appears less important in man than it is in rodents and because of the differences between the human and the rodent (on which early studies were directed) receptors. Such drugs in practise also directly modulate the production of certain adipokines; a potent example is leptin, the expression and production of which is strongly inhibited by β_3-adrenoceptor agonists.

Currently, three drugs are available clinically to treat obesity – Orlistat, Reductil (sibutramine) and Rimonabant. Orlistat inhibits pancreatic lipase, thereby reducing the digestion and subsequent absorption of fatty acids. Sibutramine, which is a serotonin and noradrenergic reuptake inhibitor, suppresses appetite and stimulates energy expenditure. Rimonabant, the most recently introduced anti-obesity drug, is targeted at the endocannabinoid system, acting as an antagonist of the CB1 receptor. Rimonabant inhibits appetite and probably also stimulates expenditure. [5] It is increasingly clear that this drug also interacts with adipose tissue, either indirectly or directly, CB1 receptors being present in the tissue [5]. Adiponectin production appears to be stimulated by Rimonabant and this has raised the possibility that this drug and other CB1 antagonists and agonists might have specific effects on adipokine synthesis, particularly those linked to inflammation and insulin sensitivity. As such, CB1 antagonists may have a potential role in the treatment of the metabolic syndrome.

The established example of current drugs which directly affect adipokine production is the ►thiazolidinediones (such as pioglitazone and rosiglitazone) which were developed as anti-diabetic agents. These compounds, which interact with the ►PPARγ nuclear receptor, have major effects on the production of several adipokines. Thus production of leptin is strongly downregulated, whereas that of adiponectin is upregulated. Importantly, PPARγ activators have an extensive anti-inflammatory action. As a consequence, the production of several inflammation-related adipokines has been shown to be inhibited by treatment with TZDs.

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Adiponectin

A major adipokine, molecular weight 28,000 Da (monomeric form), that is secreted only from adipocytes. It exists at high levels in the plasma and has a number of functions, including an important role in insulin sensitivity, inflammation (anti-anti-inflammatory action) and atherogenesis. Unlike most adipokines, the plasma levels fall in obesity.

►Adipokines
►Arp-activated Protein Kinase

Adipose Tissue

The adipose organ consists of two distinct tissues – brown and white adipose tissue. Brown adipose tissue is specialised for thermoregulatory heat generation (thermogenesis) through the presence of the tissue-specific mitochondrial uncoupling protein-1 (UCP-1); only limited amounts are present in most humans. White adipose tissue is the major fuel storage organ in mammals and birds, the key cell type (~50% of total cell content) being the adipocytes which store large amounts of triacylglycerol; the other cell types are fibroblasts, preadipocytes, macrophages and endothelial cells.

►Adipokines

Adrenal Gland

The adrenal gland is a flattened gland situated above each kidney, consisting of a cortex (outer wall) that

secretes important steroid hormones and a medulla (inner part) that secretes adrenaline (epinephrine) and noradrenaline (norepinephrine).

►Glucocorticoids

Adrenaline

Adrenaline (epinephrine) is a catecholamine, which is released as a neurotransmitter from neurons in the central nervous system and as a hormone from chromaffin cells of the adrenal gland. Adrenaline is required for increased metabolic and cardiovascular demand during stress. Its cellular actions are mediated via plasma membrane bound G-protein-coupled receptors.

►α-Adrenergic System
►β-Adrenergic System

Adrenergic Receptor

Also called adrenoreceptors, are a class of G protein-coupled receptors with a widespread expression in a broad spectrum of different organs and tissues. Adrenergic receptors are activated by their endogenous agonist ligands epinephrine and norepinephrine which belong to the catecholamine transmitters. Adrenergic receptors regulate a range of physiological parameters such as blood pressure and heart rate in a very rapid manner; these rapid changes in parameters such as heart rate are essential elements of physiological defense reactions ("fight and flight").

►α-Adrenergic System
►β-Adrenergic System
►Trace Amines

α-Adrenergic Receptors

►α-Adrenergic System

α-Adrenergic System

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Synonyms

α-Adrenergic receptors; α-Adrenoceptors

Definition

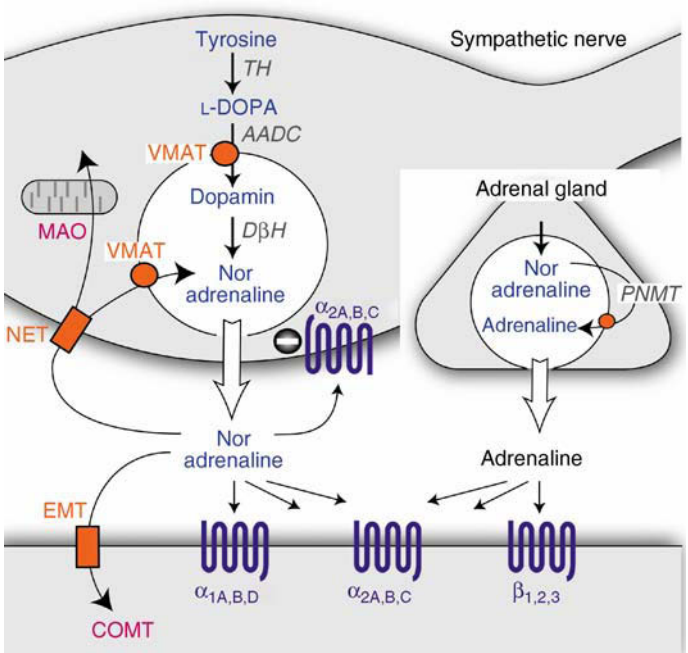
The α-adrenergic system consists of six subtypes of membrane receptors which mediate part of the biological actions of the catecholamines adrenaline and noradrenaline. α-Adrenergic receptors are important regulators of smooth muscle cell contraction (α_1-adrenergic receptors) [1] and presynaptic neurotransmitter release (α_2-adrenergic receptors) [2, 3]. In addition, adrenaline and noradrenaline activate β-adrenergic receptors, which stimulate cardiac contractility and rhythm and inhibit bronchial, vascular, and uterine smooth muscle contraction (►β-adrenergic system).

Basic Characteristics

The adrenergic system is an essential regulator that increases cardiovascular and metabolic capacity during situations of stress, exercise, and disease. Nerve cells in the central and peripheral nervous system synthesize and secrete the neurotransmitters noradrenaline and adrenaline. In the peripheral nervous system, ►noradrenaline and ►adrenaline are released from two different sites: noradrenaline is the principal neurotransmitter of sympathetic neurons that innervate many organs and tissues. In contrast, adrenaline, and to a lesser degree noradrenaline, is produced and secreted from the adrenal gland into the circulation (Fig. 1). Thus, the actions of noradrenaline are mostly restricted to the sites of release from sympathetic nerves, whereas adrenaline acts as a hormone to stimulate many different cells via the blood stream.

Together with dopamine, adrenaline and noradrenaline belong to the endogenous catecholamines that are synthesized from the precursor amino acid tyrosine (Fig. 1). In the first biosynthetic step, tyrosine hydroxylase generates ►L-DOPA which is further converted to dopamine by the aromatic L-amino acid decarboxylase (►Dopa decarboxylase). Dopamine is transported from the cytosol into synaptic vesicles by a vesicular monoamine transporter. In sympathetic nerves, vesicular dopamine β-hydroxylase generates the neurotransmitter noradrenaline. In chromaffin cells of the adrenal medulla, approximately 80% of the noradrenaline is further converted into adrenaline by the enzyme phenylethanolamine-*N*-methyltransferase.

Several mechanisms serve to terminate the biological actions of noradrenaline and adrenaline. From the



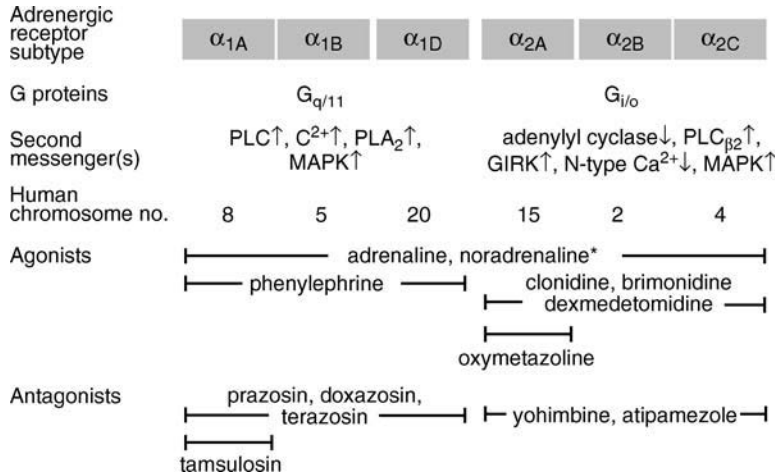
α-Adrenergic System. Figure 1 Synthesis and release of noradrenaline and adrenaline from sympathetic nerve endings (left) and from the adrenal gland (right). Noradrenaline and adrenaline are synthesized from the precursor amino acid tyrosine and are stored at high concentrations in synaptic vesicles. Upon activation of sympathetic nerves or adrenal chromaffin cells, noradrenaline and adrenaline are secreted and can activate adrenergic receptors on surrounding cells (sympathetic nerve), or they enter the blood circulation (adrenaline released from the adrenal gland). Release of noradrenaline from nerve terminals is controlled by presynaptic inhibitory α_2- and activating β_2-adrenergic receptors. Actions of noradrenaline are terminated by uptake into nerve terminals and synaptic vesicles by active transporters (NET, EMT, VMAT) and by uptake into neighboring cells (not shown). Abbreviations: AADC, aromatic L-amino acid decarboxylase; COMT, catechol *O*-methyltransferase; DβH, dopamine β-hydroxylase; EMT, extraneuronal noradrenaline transporter; MAO, monoamine oxidase; NET, noradrenaline transporter; PNMT, phenylethanolamine-*N*-methyltransferase; TH, tyrosine hydroxylase; VMAT, vesicular monoamine transporter.

synaptic cleft, most of the released noradrenaline is recycled by reuptake into the nerve terminals via a specific ►noradrenaline transporter. This transporter is selectively blocked by cocaine, tricyclic antidepressants or selective noradrenaline reuptake inhibitors (SNRIs). After reuptake into the nerve, most of the noradrenaline is transferred into synaptic vesicles. A smaller fraction is destined for degradation by the enzymes ►monoamine oxidase (MAO, in sympathetic nerves) or ►catechol-*O*-methyltransferase (COMT, in neighbouring cells). COMT plays a major role in the metabolism of circulating catecholamines. MAO and COMT are widely distributed, and inhibitors of these enzymes are used for the treatment of mental depression (MAO-A inhibitor, moclobemide) or Parkinson's disease (MAO-B inhibitor, selegiline).

The biological actions of adrenaline and noradrenaline are mediated via nine different ►G-protein-coupled receptors, which are located in the plasma membrane of neuronal and nonneuronal target cells. These receptors are divided into two different groups, α-adrenergic receptors and β-adrenergic receptors (see β-adrenergic system). The distinction between α- and β-adrenergic receptors was first proposed by Ahlquist in 1948 based on experiments with various catecholamine derivatives to produce excitatory (α) or inhibitory (β) responses in isolated smooth muscle systems. Initially, a further subdivision into presynaptic α_2- and postsynaptic α_1-receptors was proposed. However, this anatomical classification of α-adrenergic receptor subtypes was later abandoned.

At present, six α-adrenergic receptors have been identified by molecular cloning: three α_1-adrenergic receptors (α_{1A}, α_{1B}, α_{1D}) and three α_2-subtypes (α_{2A}, α_{2B}, α_{2C}) (Fig. 2). Due to the lack of sufficiently subtype-selective ligands, the unique physiological properties of these α-receptor subtypes, for the most part, have not been fully elucidated. However, recent studies in mice that carry deletions in the genes encoding for individual α-receptor subtypes have greatly advanced the knowledge about the specific functions of these receptors.

α_1-Adrenergic receptors mediate contraction and hypertrophic growth of vascular smooth muscle cells and cardiac myocytes. The three α_1-receptor subtypes share 75% identity in their transmembrane domains,

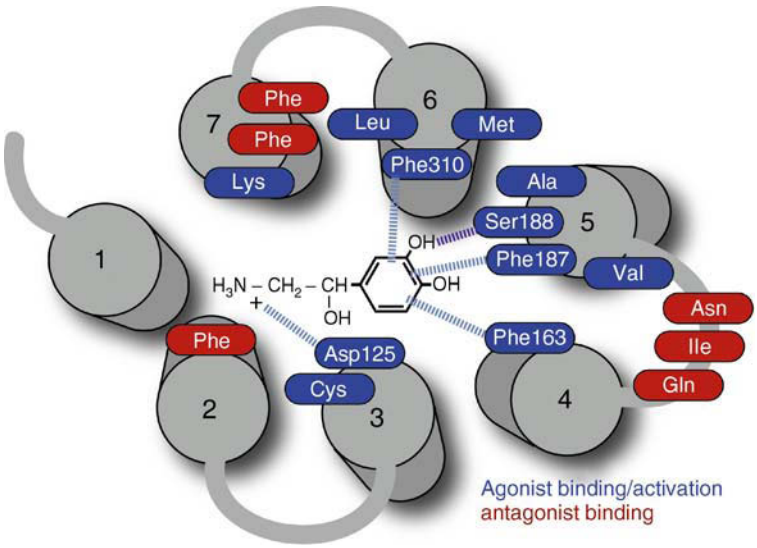


α-Adrenergic System. Figure 2 Subtypes of α-adrenergic receptors, their signaling pathways and agonist and antagonist binding profiles. The proposed topology of α_1- and α_2-adrenergic receptors with 7 transmembrane domains is illustrated. *Adrenaline and noradrenaline can also activate β-adrenergic receptors (see β-adrenergic system). PLA_2, PLC: phospholipases A, C; GIRK: G-protein-activated inwardly rectifying potassium channel, MAPK: mitogen-activated protein kinase.

whereas the degree of homology between α_1- and α_2-receptors is significantly smaller (35–40%). Due to discrepancies between the pharmacological subtype classification, mRNA and protein expression data and experiments with cloned α_1-receptor subtypes, some confusion exists in the literature with respect to the assignment of α_1-receptor subtype nomenclature. In the present terminology, α_{1A} (cloned α_{1c}), α_{1B} (cloned α_{1b}) and α_{1D}-receptors (cloned α_{1d}) can be distinguished. All three subtypes seem to be involved in the regulation of vascular tone, with the α_{1A}-receptor maintaining basal vascular tone and the α_{1B}-receptor mediating the constrictory effects of exogenous α_1-agonists. Cardiac α_1-receptors increase contractile force and mediate antiapoptotic and hypertrophic effects. All α_1-receptor subtypes can activate Gq-proteins, resulting in intracellular stimulation of phospholipases C, A_2, and D, mobilization of Ca^{2+} from intracellular stores and activation of mitogen-activated protein kinase and PI3 kinase pathways. Mutagenesis of receptor subtypes has led to the identification of a number of amino acids involved in agonist binding and receptor activation as well as binding sites for antagonists within the receptor's binding crevice [4, 5] (Fig. 3).

Three genes encoding for α_2-adrenergic receptor subtypes have been identified from several species, termed α_{2A}, α_{2B}, and α_{2C}, respectively (Fig. 2). The pharmacological profile of the α_{2A}-subtype differs significantly between species, thus giving rise to the pharmacological subtypes α_{2A} in humans, rabbits, and pigs and α_{2D} in rats, mice, and guinea pigs. Part of the pharmacological difference between α_{2A}- and α_{2D}-receptors can be explained by a Ser-Ala mutation in the fifth transmembrane helix of the α_{2A}-receptor rendering this receptor less sensitive to the antagonists, rauwolscine and yohimbine. α_2-Adrenergic receptors regulate a wide range of signalling pathways via interaction with multiple heterotrimeric $G_{i/o}$ proteins including inhibition of adenylyl cyclase, stimulation of phospholipase D, stimulation of mitogen-activated protein kinases, stimulation of K^+ currents and inhibition of Ca^{2+} currents. The three α_2-receptor subtypes have unique patterns of tissue distribution in the central nervous system and in peripheral tissues. The α_{2A}-receptor is expressed widely throughout the central nervous system including the locus coeruleus, brain stem nuclei, cerebral cortex, septum, hypothalamus, and hippocampus. In the periphery, α_{2A}-receptors are expressed in kidney, spleen, thymus, lung, and salivary gland. The α_{2B}-receptor primarily shows peripheral expression (kidney, liver, lung, and heart) and only low level expression in thalamic nuclei of the central nervous system. The α_{2C}-receptor appears to be expressed primarily in the central nervous system (striatum, olfactory tubercle, hippocampus, and cerebral cortex), although very low levels of its mRNA are present in the kidney.

α_{2A}-, α_{2B}, and α_{2C}-receptors are located presynaptically in order to inhibit noradrenaline release from sympathetic nerves. Activation of these receptors leads to decreased sympathetic tone, decreased blood pressure and heart rate. Central α_{2A}-receptors mediate sedation and analgesia. α_{2B}-Receptors mediate contraction of vascular smooth muscle, and in the spinal cord they are essential components of the analgesic effect of nitrous oxide. Upon stimulation by agonists, α_1- and α_2-receptor signalling pathways are attenuated by



α-Adrenergic System. Figure 3 Model of agonist and antagonist binding to α_1-adrenergic receptors. The binding pocket of α_1-receptors is depicted from an extracellular viewpoint, transmembrane domains 1–7 are numbered. Amino acids involved in agonist binding and receptor activation are depicted in blue, residues which mediate antagonist binding are shown in red (modified from 5).

several mechanisms at the receptor and postreceptor levels (see β-adrenergic system).

Drugs

Therapeutically, α_1-receptor-mediated vasoconstriction contributes to the beneficial actions of adrenaline applied as an emergency medicine during hypotensive or anaphylactic shock. Addition of adrenaline or noradrenaline to local anaesthetics prevents diffusion of the local anaesthetic from the site of injection and thereby prolongs its action. α_1-Receptor antagonists including prazosin, doxazosin, terazosin, and bunazosin are used to treat patients with hypertension. However, α_1-receptor antagonists are no longer first-line antihypertensive agents since the ALLHAT clinical trial revealed that hypertensive patients taking doxazosin had a higher risk of developing congestive heart failure than patients with diuretic treatment. Tamsulosin is the first α_1-receptor antagonist with selectivity for the α_{1A}-receptor over α_{1B}- and α_{1D}-subtypes. The α_{1A}-selectivity is thought to contribute to the beneficial actions of tamsulosin and alfuzosin in the treatment of benign prostate hypertrophy without lowering bood pressure.

At present, no drugs exist that can selectively activate α_2-receptor subtypes. Clonidine stimulates all three α_2-subtypes with similar potency. Clonidine lowers blood pressure in patients with hypertension and it decreases sympathetic overactivity during opioid withdrawal. In intensive and postoperative care, clonidine is a potent sedative and analgesic and can prevent postoperative shivering. Clonidine and its derivative brimonidine lower intraocular pressure of glaucoma patients when applied locally. Moxonidine may have less sedative side effects than clonidine when used as an antihypertensive. It has been suggested that moxonidine activates "imidazoline receptors" instead of α_2-receptors. The α_2-receptor agonists oxymetazoline and xylometazoline are being used as nasal decongestants. At present, α_2-receptor antagonists are not used in human medicine. However, in veterinary practice the α_2-receptor antagonist atipamezole can rapidly reverse anaesthesia mediated by the α_2-agonist medetomidine. In the future, subtype-selective drugs may greatly improve the therapy of diseases involving α_1- or α_2-adrenergic receptor systems.

►β-Adrenergic System
►Catechol-*O*-Methyltransferase and its Inhibitors
►Neurotransmitter Transporters

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β-Adrenergic System

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Synonyms

β-adrenoceptor system

Definition

The β-adrenergic system defines the effects of the ►sympathetic system mediated via β-adrenergic receptors (synonym: β-adrenoceptors). These are G-protein-coupled receptors primarily causing an activation of adenylyl cyclases. They mediate a plethora of cardiovascular, smooth muscle and metabolic effects. β-Adrenergic receptor antagonists are used to treat various cardiovascular diseases, including hypertension, coronary artery disease, myocardial infarction and heart failure, but also glaucoma and hyperthyroidism. β-Adrenergic receptor agonists are used primarily to treat bronchial asthma and premature labour.

Basic Characteristics

The sympathetic nervous system secretes the ►catecholamines noradrenaline (norepinephrine) from nerve endings and adrenaline (epinephrine) from the adrenal medulla. Noradrenaline is stored in the varicosities (≈nerve endings) of the sympathetic nervous system, often together with ATP or neuropeptide Y. Adrenaline is stored in vesicles of the chromaffin cells of the adrenal medulla together with its precursor noradrenaline at a ratio of about 4:1. Release of noradrenaline and adrenaline is subject to inhibitory control by presynaptic α_{2A}- and α_{2C}-adrenergic receptors, and to – less pronounced – stimulation via presynaptic β_2-adrenergic receptors (see chapter on α-adrenergic system).

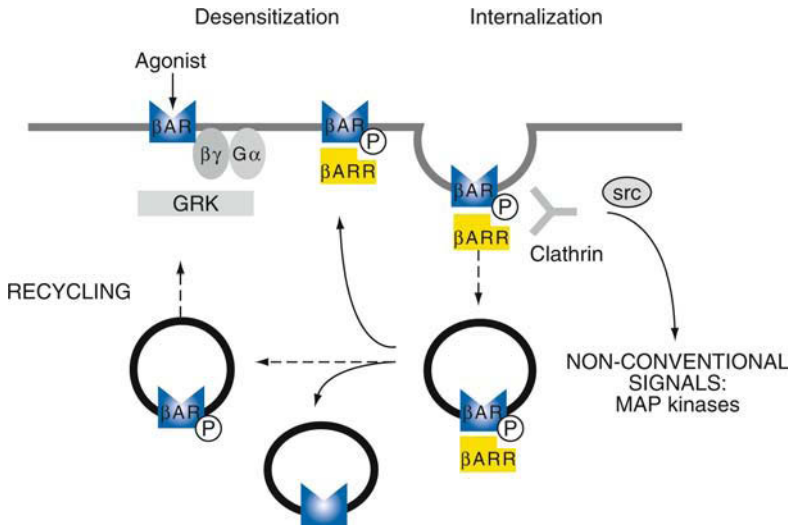
Adrenaline and noradrenaline act on a total of nine adrenergic receptor subtypes, three each of the α_1-, α_2- and β-adrenergic subfamily. The three β-adrenergic receptors are termed β_1, β_2 and β_3. All adrenergic receptors couple to ►G-proteins. G_s is the primary G-protein for all three β-adrenergic receptors and mediates activation of ►adenylyl cyclases, i.e. results in an increase of intracellular cAMP-levels. Activation of ►protein kinase A (PKA) is the main effector pathway of elevated cAMP, but cAMP can also activate cyclic-nucleotide-dependent ion channels, inhibit the metabolism of cGMP by phosphodiesterases and activate the epac proteins (exchange proteins activated by cAMP). Additional signalling pathways have been observed mainly for the β_2-subtype, they include activation of G_i as well as stimulation of ►MAP-kinase pathways (MAP-kinase cascades); activation of these "non-conventional" pathways appears to require the binding of β-arrestins to the receptors (see below). These G-protein-independent signalling pathways have been suggested by a variety of experiments, and it appears that they play a role in long-term signalling regulating cell growth [1–4].

All adrenergic receptors are heptahelical, i.e. they have seven transmembrane helices that form a ligand-binding pocket located in the central transmembrane core of the receptors. Amino acids essential for ligand binding have been mapped extensively for the β_2-adrenergic receptor; they are located in transmembrane helices 3, 5 and 6. Receptor activation involves an agonist-induced intramolecular conformational change that causes a relative movement of the transmembrane helices 3 and 6. This is thought to lead to a rearrangement of the intracellular parts of the receptor, which couple to G-proteins, most notably the part of the third intracellular loop that is adjacent to transmembrane helix 6. These conformational changes can be directly visualized in intact cells with fluorescence techniques and occur over a few hundred milliseconds after agonist binding [5].

Recent studies indicate that – like many other receptors – G-protein-coupled receptors may form dimers, either homodimers or dimers with another type of receptor. The role of dimer formation in the cell surface expression of receptors and in their signalling and the resultant pharmacology are currently under intensive investigation [1].

In addition to agonist-induced activity, many receptors, including the β-adrenergic receptors, display spontaneous or constitutive activity. This means that the unoccupied receptor has some likelihood to adopt an active conformation, couple to G-proteins and generate an intracellular signal. Some compounds classified as antagonists (e.g. propranolol) can suppress constitutive activity and are therefore termed inverse agonists. Constitutive activity is more pronounced for the β_2-than the β_1-subtype, but has not yet been investigated for the β_3-receptor.

The agonist-induced conformational change not only causes receptor activation and generation of an intracellular signal, but also a number of biochemical processes that dampen the signal and cause desensitization of the receptor [2] (Fig. 1). These include (i) phosphorylation of the receptor by members of the G-protein-coupled receptor kinase (GRK) family, followed by binding of β-arrestins, which prevents further activation of G-proteins, (ii) phosphorylation by protein kinases A and C (PKA and PKC), which directly impairs G-protein-coupling, (iii) translocation of the receptors into clathrin-coated pits and internalization into endosomes. Binding of β-arrestins and perhaps also movement of



β-Adrenergic System. Figure 1 Desensitization, internalization and recycling of β-adrenergic receptors. Activation of β-adrenergic receptors causes their phosphorylation by members of the GRKs. Cytosolic β-arrestins then bind to the phosphorylated receptors and prevent further interaction with G-proteins. β-Arrestin-bound receptors assemble in clathrin-coated pits, where the complex appears to interact with other proteins, including dynamin and src-kinase. This leads (i) to the activation of non-conventional signalling pathways (raf-kinases, MAP-kinases, JNK-kinases), and (ii) to the internalization of the receptors to endosomes. Endosomal receptors become either dephosphorylated and recycle back to the cell surface; some endosomal receptors undergo lysosomal degradation.

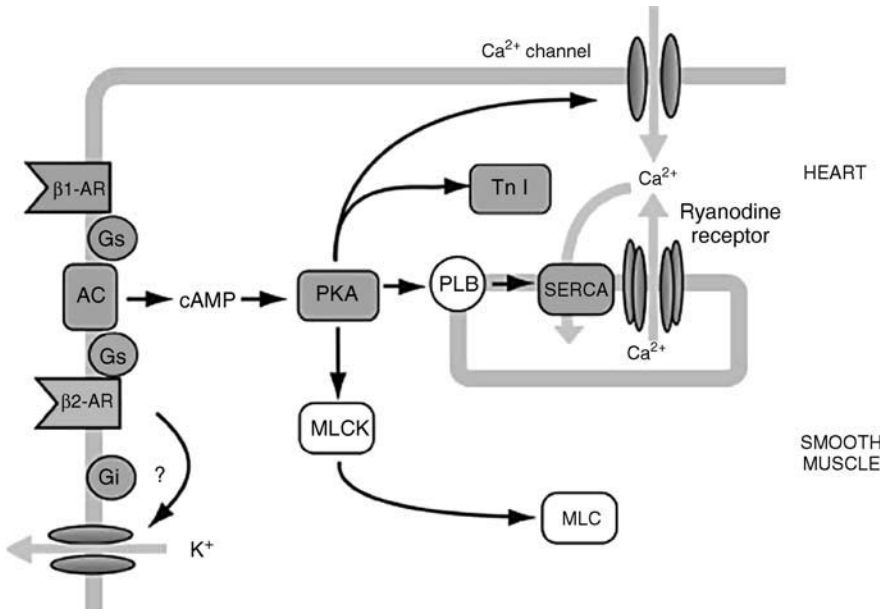
receptors into clathrin-coated pits appear also to be required for a subsequent "wave" of non-conventional signalling, such as activation of MAP-kinases [3]. Most internalized receptors are recycled back to the cell surface, but some are degraded. The regulatory processes described above are most pronounced for the β_2-subtype, and least for the β_3-subtype; they have also been demonstrated for other G-protein-coupled receptors, e.g. the α_2-adrenergic receptors. In addition to these regulatory processes at the level of the receptor protein, the receptor mRNA-levels can be down-regulated, at least in part by destabilization via mRNA-binding proteins; this results in reduced receptor synthesis. Whereas the phosphorylation processes and the subsequent receptor desensitization can occur over a few minutes of agonist exposure, receptor down-regulation takes many hours and may even take days to reach a new steady-state level [2].

β-Adrenergic receptors mediate a plethora of cardiovascular, smooth muscle and metabolic effects (Fig. 2). Cardiac β_1-adrenergic receptors increase the frequency, electrical conduction and force of cardiac contractions as well as cardiac relaxation; they represent the strongest stimulus for the heart. At the same time they increase the generation of ectopic impulse generation and thereby the risk of arrhythmias. These effects are mediated by ►PKA-mediated phosphorylation of calcium channels (resulting in enhanced calcium influx) as well as of phospholamban, a negative regulator of the sarcoplasmic calcium ATPase ►SERCA (resulting in enhanced uptake of calcium into the stores of the sarcoplasmic reticulum). A second important localization of β_1-adrenergic receptors is the cells of the juxtaglomerular apparatus, where they increase the release of renin and thus cause stimulation of the renin–angiotensin system.

β_2-Adrenergic receptors are located primarily on smooth muscle cells and mediate relaxation. This results in bronchodilatation, relaxation of the uterus and vasodilatation (partially mediated by β_1-adrenergic receptors). Liver β_2-adrenergic receptors trigger a protein kinase cascade that results in inhibition of glycogen synthase and activation of phosphorylase and thereby trigger the mobilization of glucose from glycogen stores

β_3-Adrenergic receptors stimulate lipase and cause the breakdown of triglycerides to fatty acids in fat cells. It is still not clear to what extent the β_2-subtype participates in this process. Together with the mobilization of glucose from the liver, lipolysis provides the energy sources for the sympathetic "fight-or-flight" reaction.

Polymorphisms have been described for all β-adrenergic receptors; while initial studies, notably of the β_1-subtype, suggested that these might be predictors of diseases as well as of therapeutic responses, these results have more recently been questioned. Alterations in responsiveness may be limited to certain compounds and a causative role in diseases has not been or remains to be substantiated in larger trials.



β-Adrenergic System. Figure 2 β-Adrenergic signalling in cardiac muscle (predominantly β_1) and smooth muscle (predominantly β_2) cells. Proteins that become more active after activation of β-adrenergic receptors are depicted in grey, those which become less active are depicted in white. Both receptors couple to G_s and lead to activation of adenylyl cyclases, generation of cyclic AMP and activation or protein kinase A (PKA). In *heart muscle cells*, PKA causes phosphorylation of L-type calcium channels (increased Ca^{2+}-influx; relevant site of phosphorylation uncertain), troponin I (TnI; diminishes affinity of troponin C for Ca^{2+} and thus enhances relaxation) and phospholamban (PLB; leads to less inhibition of the sarcoplasmic Ca^{2+} ATPase, SERCA, which pumps Ca^{2+}into the sarcoplasmic stores. This in turn enhances relaxation and enhances Ca^{2+}-release during the next beat) All this leads to more rapid and forceful contraction as well as relaxation. In *smooth muscle cell* the signalling pathways are less clear. PKA-mediated phosphorylation of myosin light chain kinase (MLCK) causes reduced activity of this kinase, which in turn leads to decreased phosphorylation of myosin light chains and, hence, reduced contraction. A second postulated mechanism for relaxation is hyperpolarization via activation of K^+-channels; the signalling pathway is unclear and might involve coupling of β_2-receptors to G_i.

Drugs

β-Adrenergic Receptor Agonists

Agonists as well as antagonists of β-adrenergic receptors are used for the treatment of a variety of conditions. β-Adrenergic receptor antagonists belong to the most frequently used classes of drugs.

The main use of β-adrenergic receptor agonists (β-sympathomimetic drugs) is the symptomatic treatment of bronchial asthma. Stimulation of β_2-adrenergic receptors on smooth muscles produces dilatation of the airways and reduces airway resistance. Although β_2-adrenergic receptor activation inhibits inflammatory mediator release from mast cells and other inflammatory cells, there is no major effect of these drugs on airway inflammation associated with asthma. β_2-Adrenergic receptor agonists are the most effective bronchodilators known. In order to reduce unwanted systemic effects (most notably tachycardia and arrhythmia) inhaled β_2-selective compounds are the drugs of choice; a high first-pass effect helps to reduce systemic effects of the major fraction of the inhaled drug that reaches the gastrointestinal tract. The most frequently used compounds are fenoterol and salbutamol, which have a rapid onset and a short duration of action. Newer lipophilic compounds such a formoterol and salmeterol have a much longer duration of action (up to 12 h), presumably because they are retained in the plasma membrane after dissociation from the receptor, i.e. they remain in the immediate vicinity and can thus re-associate with the receptor.

β_2-Adrenergic receptor agonists are also used to treat premature labour by causing uterine relaxation. Fenoterol and ritodrine are frequently used. The effectiveness of long-term tocolysis is controversial, since both desensitization of the receptors and the symptomatic nature of this treatment may limit their effects to 1–2 days according to one large study.

β_2-Adrenergic receptor agonists, in particular clenbuterol, have been used for their hypertrophic effects on skeletal and also cardiac muscle. They can increase muscle growth in cattle (illegal in many countries) but have also been used by body-builders and athletes as anabolic drugs.

Non-selective β-adrenergic receptor agonists, particularly adrenaline (epinephrine), are used in cardiovascular

emergency situations, most importantly cardiopulmonary resuscitation and anaphylactic shock. They are given to produce stimulation of cardiac electrical activity via β_1-receptors, inhibition of mast cell mediator release via β_2-receptors, and bronchodilatation via β_2-receptors as well as α_1- and α_2-receptor-mediated vasoconstriction.

Relatively selective stimulation of β_1-adrenergic receptors can be achieved with dobutamine. This is a racemic drug of which both isomers activate the β_1-receptor, and in addition the (−) isomer activates α_1-receptors whereas the (+) isomer activates β_2-receptors; the simultaneous activation of α_1- and β_2-receptors results in no major net effect on peripheral resistance, and thus the overall cardiovascular effects are mediated by β_1-stimulation leading to increases in cardiac contractility and output. Dobutamine is used for the short-term treatment of acute cardiac failure and for diagnostic purposes in stress echocardiography.

β-Adrenergic Receptor Antagonists

Clinically used β-adrenergic receptor antagonists ("β-blockers") are either β_1-selective (e.g. bisoprolol, metoprolol, atenolol, betaxolol) or non-selective, i.e. with similar affinity for the β_1- and the β_2-subtype (e.g. propranolol, timolol, celiprolol). Many compounds classified as antagonists are in fact inverse agonists, for example metoprolol, bisoprolol, timolol or propranolol; inverse agonism is more pronounced at β_2- than at β_1-receptors because the latter possess a lower constitutive activity. Some compounds possess a partial agonist activity (PAA, or intrinsic sympathomimetic activity ISA); examples are pindolol or celiprolol. These drugs produce less bradycardia but may be therapeutically less efficient.

Blockade of β-adrenergic receptors are important in the treatment of many cardiovascular diseases. Supraventricular and ventricular tachycardias are treated by reducing pacemaker currents in the SA-node, slowing AV-conduction and decreasing ectopic impulse generation. This is achieved via reductions in pacemaker currents and Ca^{2+}-currents (class II antiarrhythmic drugs). Blockade of cardiac β_1-adrenergic receptors (preferentially with β_1-selective drugs) reduces cardiac frequency, cardiac output, cardiac O_2-consumption and probably prevents β-adrenergically induced cardiac remodelling. Therefore, they are first-line drugs in the treatment of hypertension, angina, myocardial infarction and cardiac failure. In the latter case, treatment must by initiated with very low doses to prevent acute decompensation. While β-adrenergic receptor antagonists have become the most effective means of treatment in heart failure during the past decade, their first-line use in hypertension has recently been questioned.

Non-selective β-adrenergic receptor antagonists (e.g. propranolol) can suppress tachycardia and tremor in patients with hyperthyroidism or tremor caused by stress or nervousness. This use is illegal in certain sports (e.g. shooting).

β-Adrenergic receptor antagonists can reduce aqueous humor production in the eye and thereby reduce intraocular pressure. This is why they represent one of the most frequently used class of drugs in glaucoma. Timolol is the best-established compound, followed by levobunolol and others. High concentrations of these compounds are applied to the eye, and it is, therefore, not really clear whether the effects are indeed mediated via specific interactions with β-adrenergic receptors. In recent years, newer drugs acting on prostaglandin or α_2-adrenergic receptors have become more popular as first-line drugs in glaucoma.

►α-Adrenergic System
►Antiarrhythmic Drugs
►Antihypertensive Drugs
►Catechol-*O*-Methyltransferase and its Inhibitors

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Adrenoceptor

►α-Adrenergic System
►β-Adrenergic System

β-Adrenoceptor System

►β-Adrenergic System

α-Adrenoceptors

►α-Adrenergic System

Adrenomedullin

Human adrenomedullin is a 52-amino acid peptide belonging to the calcitonin/calcitonin gene-related peptide (CGRP)/amylin peptide family. It is synthesized mainly in endothelial cells and elicits vasodilation.

►Calcitonin Gene Related Peptide

Adverse/Unwanted Reactions

All drugs, in addition to their therapeutic effects, have the potential to do harm, i.e. to cause adverse/unwanted reactions (side effects). These may or may not be related to the principal pharmacological action of the drug. Examples of the second category are toxic effects of metabolites of a drug or immunological reactions.

Affective Disorders

Affective (mood) disorders are characterized by changes in mood. The most common manifestation is depression, arranging from mild to severe forms. Psychotic depression is accompanied by hallucinations and illusions. Mania is less common than depression. In bipolar affective disorder, depression alternates with mania.

►Antidepressant Drugs

Affinity

Ligands reside at a point of minimal energy within a binding locus of a protein according to a ratio of the rate the ligand leaves the surface of the protein (k_{off}) and the rate it approaches the protein surface (k_{on}). This ratio is the equilibirum dissociation constant of the ligand–protein complex (denoted $K_{eq}=k_{off}/k_{on}$) and defines the molar concentration of the ligand in the compartment containing the protein that is bound to 50% of the protein at any one instant. The 'affinity' or attraction of the ligand for the protein is the reciprocal of K_{eq}.

►Drug–Receptor Interaction

Age-related Macular Degeneration (AMD)

A disease process characterized by deterioration of the macula of the retina that results in a loss of sharp central vision. AMD is the leading cause of central vision loss in the developed countries today for those over the age of fifty years.

►Tyrosine Kinase Inhibitors

Agonist

Natural or synthethic receptor ligands that induce a conformational change (active conformation) and a signal transduction process upon receptor binding. Agonists may act as typical hormones or neurotransmitters or they may confer paracrine functions, recognize bacterial, viral or other environmental constituents via activating their dedicated receptors.

Ligands that bind to the receptor, but do not exert a maximal cellular reaction when applied at saturating concentrations are referred to as partial agonists. Their remaining activity is termed intrinsic activity ranging between 0% and 100%.

►Drug–Receptor Interaction
►Transmembrane Signaling
►G-protein-coupled Receptors
►Adenosine Receptors
►Chemokine Receptors
►Nuclear Receptors
►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
►Selective Sex Steroid Receptor Modulators

Agouti-related Protein (AgRP)

The agouti gene encodes a paracrine signalling molecule that antagonizes the effect of melanocyte-stimulating hormone (MSH) at the melanocortin-1 receptor. This effect reduces the synthesis of eumelanin, and is responsible for the agouti hair colour in rodents. Agouti-related protein (AgRP) is similar to the agouti protein (25% identical amino acids), and is an endogenous antagonist of αMSH at the melanocortin-3 and melanocortin-4 receptors. AgRP is a potent orexigen, and down-regulation of AgRP is a major mechanism of the anorexigenic effect of leptin.

►Appetite Control

Ah Receptor

►Arylhydrocarbon Receptor

AIDS

AIDS (acquired immunodeficiency syndrome) is the final stage of disease caused by infection with HIV. In this stage, the virus infection has severely affected the immune system, causing a depletion of $CD4^+$ T-helper cells. AIDS is characterized by the manifestation of typical diseases caused by opportunistic infections (Pneumocystis carinii pneumonia, CMV retinitis, candidiasis of the esophagus, cerebral toxoplasmosis), neurological manifestations, cachexia, or certain tumors (Kaposi sarcoma of the skin, B-cell lymphoma).

►Antiviral Drugs

Airway Hyperresponsiveness

Airway hyperresponsiveness is an exaggerated propensity for airways to narrow too easily in response to a wide variety of stimuli. Airway hyperresponsiveness leads to clinical symptoms of wheezing and dyspnea after exposure to allergens, environmental irritants, viral infections, cold air, or exercise.

►Glucocorticoids
►GABAergic System
►Bronchial Asthma

Airway Surface Liquid

Airway surface liquid (ASL) is the very thin fluid layer (<7 μM) maintained at the apical membrane of airway epithelia. ASL thickness is maintained by a tight control of fluid reabsorption and/or secretion, mediated by sodium and/or chloride channels.

►Epithelial Na^+ Channel

AKAPs

AKAPs are cyclic AMP-dependent protein kinase (PKA)-anchoring proteins, a family of about 30 proteins anchoring PKA at subcellular sites in close vicinity to a certain substrate.

►Scaffolding Proteins
►A Kinase Anchoring Proteins (AKAPs)

AKT

Synonyms
PKB

Definition
Akt also known as protein kinase B (PKB) is serine/threonine-specific protein kinase important in mammalian cellular signalling. Akt, originally identified as the oncogene in the transforming retrovirus (AKT8), controls cell survival by inhibiting apoptosis processes. Logically, Akt has been implicated as a major factor in many types of cancer. Akt plays also a crucial role in the insulin signalling pathway.

Akt is activated by binding of plasma membrane phospholipids downstream of insulin receptors, growth and survival factor receptors in a phosphoinositide 3-kinases dependent manner. In humans, there are three genes in the "Akt family": Akt1, Akt2 and Akt3. Their respective functions are still under investigation.

►Phospholipid
►Insulin Receptor
►Tyrosin Kinases
►Growth Factors

Alcohol

►Ethanol

Alcohol Dehydrogenase

Alcohol dehydrogenase is a cytoplasmic enzyme mainly found in the liver, but also in the stomach. The enzyme accomplishes the first step of ethanol metabolism, oxidation to acetaldehyde, which is further metabolized by aldehyde dehydrogenase. Quantitatively, the oxidation of ethanol is more or less independent of the blood concentration and constant with time, i.e. it follows zero-order kinetics (pharmacokinetics). On average, a 70-kg person oxidizes about 10 ml of ethanol per hour.

►Ethanol

Aldehyde Dehydrogenase

Ethanol is almost entirely metabolized in the liver. The first step, oxidation by ►alcohol dehydrogenase, yields acetaldehyde, a reactive and toxic compound. Essentially all of the acetaldehyde is converted to acetate by the liver enzyme aldehyde dehydrogenase. Aldehyde dehydrogenase is inhibited by the drug disulfiram. Given alone, disulfiram is a nontoxic substance. However, ethanol consumption in the presence of disulfiram causes an extremely unpleasant reaction characterized by flushing, hyperventilation, vomiting, sweating, tachycardia, hypotension, vertigo and marked distress. The altered response to alcohol is the rational basis for the use of disulfiram in the treatment of chronic alcoholism.

►Ethanol

Aldosterone

Aldosterone is a small hydrophobic molecule and belongs to the class of steroid hormones. Aldosterone is the major mineralocorticoid in the body. It binds to the mineralocorticoid receptor. This receptor belongs to the superfamily of steroid hormone receptors, which are located intracellularly and, upon binding of the agonist, translocate into the cell nucleus, where they regulate the transcription of those genes, which contain the appropriate hormone responsive regulatory elements. In the intestine and particularly in the distal tubules of the kidney, aldosterone increases the expression of proteins which modulate the activity of the sodium–potassium ATPase and the amylorid-sensitive sodium channel. As a consequence, sodium reabsorption and, secondarily, potassium excretion increase.

►Gluco-Mineralocorticoid Receptors
►Nuclear Receptors
►Renin–Angiotensin–Aldosterone System
►Epithelial Na^+ Channel

Aldosterone Receptor

►Gluco-Mineralocorticoid Receptors

$AlF4^-$

Fluoride forms a tetrahedral ion with aluminum, AlF_4^-, which forms a complex with the GDP ·α β γ form of heterotrimeric G-proteins. In the case of G_s, the complex AlF_4^- ·GDP ·α $_s$β γ behaves much as GTP or the more stable GTP derivatives, GTPγ S or GPP(NH)P,

and causes the dissociation of G_s and the subsequent activation of adenylyl cyclase through the complex AlF_4^- ·GDP ·α_s ·C. AlF_4^- does not activate small, monomeric GTPases.

►G-proteins
►Adenylyl Cyclases

Alkaloid

Alkaloids are heterocyclic basic compounds and widespread in plants. Many of them have specific targets in organisms. For example, the alkaloids atropine and scopolamine of Belladonna are specific antagonists at ►muscarinic receptors.

Alkylating Agents

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Synonyms

Antiproliferative agents; Methylating agents; Chloroethylating agents

Definition

Alkylating agents have the ability to add alkyl groups to many electronegative groups under conditions present in cells. They stop tumor growth by introducing single strand breaks as well as by cross-linking nucleotides in DNA double-helix strands – directly attacking DNA. This makes the strands unable to uncoil and separate. As this is necessary in DNA replication, the cells can no longer divide. Alkylating agents act nonspecifically. Some of them require conversion into active substances *in vivo*.

Mechanism of Action and Clinical Use (Including Side-Effects)

Alkylating agents are DNA-interactive drugs characterized by the formation of covalent DNA adducts. The alkylating agents are the largest class of anticancer agents, comprising five subgroups: nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines and ►triazenes [1].

Several other drugs (e.g., procarbazine, hexamethylmelamine, estramustine, mitomycin C) are thought to act at least in part by alkylation. Alkylating agents are capable of introducing alkyl groups to nucleophilic sites (such as sulfhydryl, amino, phosphate, hydroxyl, carboxyl, and imidazole groups) of other molecules by forming covalent bonds. ►Alkylation damages the structure and/or function of DNA, RNA and various enzymes. The inhibition of DNA synthesis occurs at drug concentrations that are lower than those required to inhibit RNA and protein synthesis. The degree of DNA alkylation correlates well with the cytotoxicity of these drugs. The interaction with DNA also accounts for the mutagenic and carcinogenic potential of these drugs. The 7-nitrogen (N7) and 6-oxygen (O6) of guanine have been shown to be particularly susceptible to attack by ►electrophilic compounds. There are several possible consequences of N7 guanine alkylation:

- Cross-linkage – bifunctional agents may form covalent bonds with each of two adjacent guanine residues and such inter-strand cross-links will lead to inhibition of DNA replication and transcription. Intra-strand and DNA-protein cross-links may also be formed;
- Mis-pairing of bases – alkylating at N7 changes the O6 of guanine to its enoltautomer, which can then form base pairs with thymine. As a consequence gene miscoding may be induced with adenine-thymine pairs replacing guanine-cytosine. Thus defective proteins are produced;

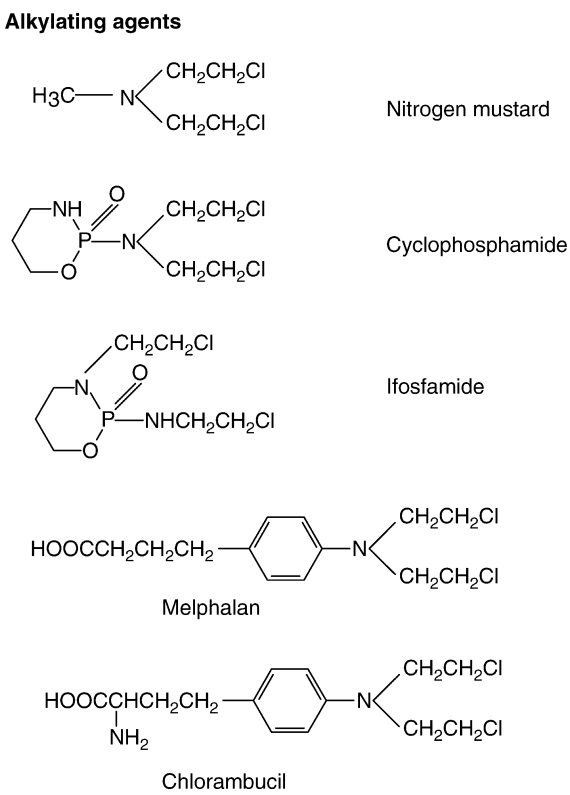
Alkylating agents, subgroups with examples

Alkylating agent	
Classic (nitrogen mustards)	Mechloretamine (nitrogen mustard)
	Cyclophosphamide
	Ifosfamide
	Chlorambucil (leukeran)
	Melphalan (alkeran)
Alkyl sulfonates	Busulfan
Nitrosoureas	Carmustine
	Lomustine
	Streptozocin (streptozotocin, zanosar)
Ethylenimines	Thiotepa
Triazines	Dacarbazine
	Procarbazine
Platinum complexes	Cisplatin
	Carboplatin
	Oxaliplatin
Others	Bendamustine

- Depurination – N7 alkylation may cause cleavage of the imidazole ring and excision of the guanine residue, leading to DNA strand breakage.

Nitrogen Mustards

The first successful chemotherapy of malignant tumors was recorded in 1942 when the first cytostatic drug named mechloretamine (►nitrogen mustard) was used. Later, a large series of nitrogen mustard analogues was synthesized and introduced into the clinical practice (Fig. 1). These analogues are highly reactive compounds that can form covalent bonds with nucleophilic reaction partners in the cell, thus transferring alkyl groups onto nucleic acids and proteins (alkylating reactions). Most of the alkylating agents are bivalent and react by their two chloroethylamine groups. They induce intra-strand and inter-strand cross-links (bridges) within the same DNA strand or between two different DNA strands (so called cross linking). This is the main mode of action of nitrogen mustard and its derivatives. DNA replication is disturbed and eradication or damage of tumor cells is induced. This cytostatic effect is mainly exerted in cells with higher proliferative activity being in the S-phase of the cell cycle when DNA replication (doubling) is performed. Alkylating agents are not phase specific because they act during all phases of the cell cycle. Hence, although to a lower extent, alkylating agents are able to cause cytotoxic reactions in cells outside of the cell cycle (G0 cells, nondividing cells).



Alkylating Agents. Figure 1 Chemical structure of some alkylating agents.

Mechlorethamine (nitrogen mustard): In vivo each chloroethylamine group undergoes intramolecular cyclization with release of a chloride ion. The so formed highly reactive ethylen-immonium derivative alkylates DNA and other biomolecules and causes the cytotoxic effect.

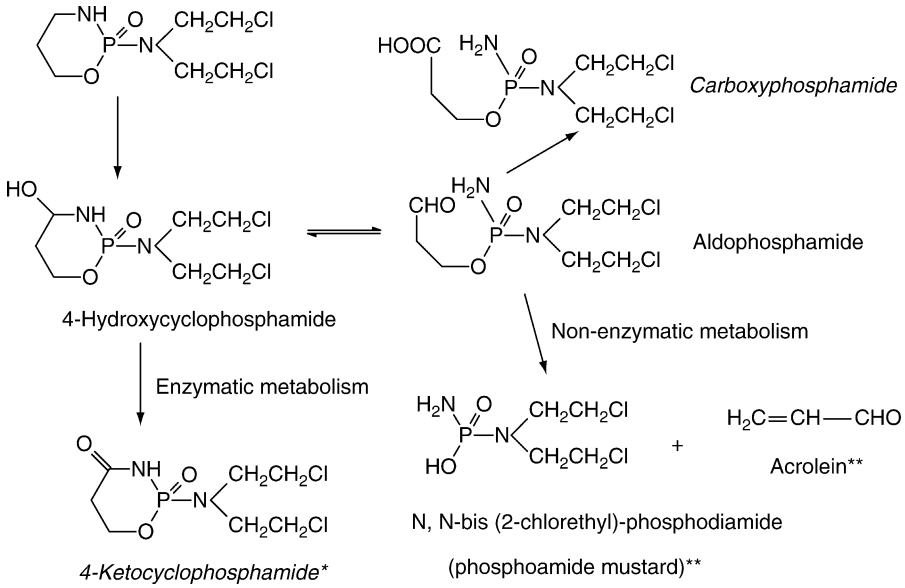
Clinical Use (Including Side-Effects)

Nitrogen mustard is clinically used for the treatment of lymphomas and some forms of lung cancer. The major indication for mechlorethamine is Hodgkin's disease as a part of the MOPP regimen (*m*echlorethamine + vincristine (*o*ncovin) + *p*rocarbazine + *p*rednisone). The usual dose consists of 6 mg/m^2 on days 1 and 8. This drug has pronounced hematological toxicity (myelosuppression).

According to a hypothesis launched by Larionov et al in the 1960s, some new nitrogen mustard derivatives were developed. They contain metabolites and heterocyclic structures as carriers of the cytotoxic chloroethylamine groups. By this way the synthesis of *alkylating metabolites* started: melphalan (sarcolysine) as L- or DL-phenylalanine derivative; prospidine with a tricyclic piperazine moiety and chlorambucil as butyric acid derivative. It was proven that each alkylating metabolite has its own spectrum of selective antitumor activity.

Prospidine influenced human laryngeal tumors. Melphalan showed clinical efficacy in human seminoma and multiple myeloma. It possesses the same general spectrum of antineoplastic activity as other nitrogen mustards do. However, since it does not cause alopecia, melphalan is occasionally substituted for cyclophosphamide in the CMF regimen for breast cancer. Its bone marrow suppression tends to be more prolonged and affects both white cells and platelets. It is widely used in conditioning regimens before stem cell transplantation. Chlorambucil is used for the treatment of chronic lymphocytic leukemia, polycytemia and exerts immunosuppressive activity. It is predominantly used in the treatment of lymphomas and shares teratogenic and carcinogenic properties with the other nitrogen mustards.

German investigators (Brock et al) worked on the creation of alkylating pro-drugs that have cytostatic activity after specific biotransformation in the tumor tissue. Cyclophosphamide (CTX) has well pronounced antitumor activity with the broadest spectrum. It is metabolized to the cytotoxic phosphoamide mustard. In normal tissues with high enzyme level cyclophosphamide is converted to its inactive metabolites (Fig. 2). These differences in biotransformation can explain the relative selectivity of cyclophosphamide towards



Alkylating Agents. Figure 2 Biotransformation of cyclophosphamide – formation of inactive (*) and toxic (**) metabolites.

tumor cells. It is inactive unless metabolized by the liver to 4-hydroxy-cyclophosphamide.

This drug is used for the treatment of breast carcinoma, ovarian tumors, SCLC, multiple myeloma, osteosarcoma and leukemias as a part of combination therapy. High dosages are often curative in Burkitt's lymphoma, a childhood malignancy with very fast growth rate. Oral daily dosages are useful for less aggressive tumors, such as nodular lymphomas, multiple myeloma, and chronic leukemias. Cyclophosphamide can be given orally and as intramuscular, intravenous and intra-arterial injections. Its side effects are typical for alkylating agents and include those caused by damage of cells with high proliferation rate (e.g., hematopoietic cells, gastro-intestinal and epithelial cells). A common toxic effect is the development of hemorrhagic cystitis of the urinary bladder caused by its metabolite acrolein. The risk of developing a carcinoma of the bladder is also increased. Conventional schedules contain therapeutic doses of 100–500 mg/m^2 within 3–14 days.

Ifosfamide is an isomeric form of cyclophosphamide with analogous mode of action.

Clinical trials showed therapeutic efficacy in a broad spectrum of tumors; these include SCLC, testicular tumors, sarcomas, breast cancer, renal cell cancer, pancreatic tumors and lymphomas. Ifosfamide is less myelosuppressive than cyclophosphamide but is more toxic to the bladder. Therefore it is recommended that ifosfamide is coadministered with the thiol compound mesna to avoid hemorrhagic cystitis and to reduce the risk of developing bladder cancer. Other side effects include neurotoxicity and myelosuppression.

Alkyl Sulfonates

British investigators (Haddow and Timmis 1951) synthesized and studied esters of the methanesulfonic acid. The most active derivative was the dimethylsulfonic ester of 1,4-butanedione, known as busulfan. Busulfan interacts with the thiol groups of proteins and amino acids; some of its metabolites can alkylate the thiols of cysteine, peptides and proteins. Busulfan exerts selective cytotoxic activity in hematopoietic bone marrow cells and inhibits the formation of granulocytes and platelets. It slightly affects the lymphoid tissue.

Clinical Use (Including Side-Effects)

Busulfan was used for the treatment of chronic myeloid leukemia and polycytemia vera.

Nitrosourea Derivatives

All members of the group ►nitrosourea derivatives are derivatives of methylnitrosourea and have mutagenic and carcinogenic properties. During the past decades nitrosoureas have been widely used for the treatment of solid tumors. Their cytostatic effects are explained by their influence on the biosynthesis of nucleic acids and proteins. They modify the chemical structure of the nucleotides. Alkylnitrosoureas are highly reactive and alkylate strongly. Bifunctional congeners lead to DNA cross-linking. In addition, they can realize carbamoylation (introduction of the CO-NH_2 group) of biomolecules. Nitrosoureas predominantly affect the synthesis of DNA (replication) rather than synthesis of messenger, ribosomal and transport RNA (transcription). They slightly affect the synthesis of proteins

(translation). Nitrosoureas lead to great disturbances in the cell cycle and proliferation even after single exposure. Nitrosoureas can kill cells in all phases of the cell cycle. These agents undergo hepatic biotransformation to active metabolites such as isocyanates. As lipophilic compounds they easily cross the blood–brain barrier and selectively accumulate in the brain tissue at concentrations 4–5 times higher than that in hepatic and renal tissues. Nitrosoureas have been used for the treatment of gastrointestinal carcinomas. They suppress the growth of lymphoid tumors and metastatic brain tumors. Cross-resistance between nitrosoureas and classic alkylating agents was not observed. They share the feature of causing delayed bone marrow toxicity, which can be cumulative and does appear as long lasting bone marrow suppression.

Streptozotocin is a naturally occurring nitrosourea compound that was isolated from *Streptomyces achromogenes*. Its structure is related to methyl-CCNU. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is not recognized by other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2. Streptozotocin is selectively accumulated in islet cells of the pancreas and is used for treating pancreatic islet cell cancer. It acts through methylation of nucleic acids and proteins. In addition, it produces rapid and severe depletion of the pyridine nucleotides nicotine adenine dinucleotide (NAD) and its reduced form (NADH) in liver and pancreatic islets. Streptozotocin is used for the induction of experimental diabetes in animals.

Clinical Use (Including Side-Effects)

Carmustine is a bicyclohexylnitrosourea (BCNU, Fig. 3) with broad spectrum of antineoplastic activity (e.g., lymphomas, multiple myeloma, sarcomas, brain tumors, gastrointestinal tumors, melanomas). At doses of 80–200 mg/m^2 it is given i.v. at 6 week's intervals.

Lomustine (2-chlorethyl-3cyclohexyl-1-nutrosourea, CCNU, Fig 3) is a nitrosourea for oral application. It is used for the treatment of Hodgkin's lymphomas, brain tumors and bronchial carcinomas at a dose of 3.5 mg/kg (130 mg/m^2) repeated in 6–8 weeks intervals.

Carmustine and lomustine can produce remissions that last 3–6 months in 40–50% of patients with primary brain tumors. Both drugs also are used as secondary treatment of Hodgkin's disease and in clinical trials with combination chemotherapy of various types of lung cancer.

Semustine (methyl-CCNU) is also suited for oral application but has greater toxicity and is therefore rarely used.

Nitrosoureas have been used in the treatment of non-Hodgkin's lymphomas, multiple myeloma, renal cell carcinoma, and colorectal cancer. They produce severe nausea and vomiting in most patients at 4–6 h after administration. The major site of dose-limiting toxicity is the bone marrow (leukopenia and thrombocytopenia). As alkylating agents, these drugs are mutagenic, teratogenic, and carcinogenic.

Usual dose schedules of streptozotocin involve 500 mg/m^2 i.v. during five consecutive days. The major toxicity is renal tubular damage. Treatment of metastatic insulinomas may result in the release of insulin from the tumor and subsequent hypoglycemic coma. Less severe toxicities include diarrhea, anemia, and mild alterations in glucose tolerance or liver function tests.

Ethylenimines

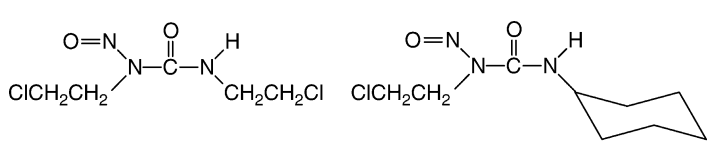
Thiotepa is chemically less reactive than the nitrogen mustards. It has antineoplastic activity against ovarian and breast cancers as well as lymphomas. However, it has been largely supplanted by cyclophosphamide and other nitrogen mustards.

Clinical Use (Including Side-Effects)

It is used by direct instillation into the bladder for multifocal local bladder carcinoma. Nausea and myelosuppression are the major toxicities of thiotepa. It is not a local vesicant and has been safely injected intramuscularly and even intra-thecally.

Triazenes

Dacarbazine is activated by photodecomposition (chemical breakdown caused by radiant energy) and by enzymatic N-demethylation. Formation of a methyl carbonium ion results in methylation of DNA and RNA and inhibition of nucleic acid and protein synthesis. Cells in all phases of the cell cycle are susceptible to dacarbazine. The drug is not appreciably protein bound, and it does not enter the central nervous system.



Carmustine (BCNU) Lomustine (CCNU)

Alkylating Agents. Figure 3 Chemical structure of some nitrosoureas.

Procarbazine is metabolized in the liver and possibly in tumor cells to yield a variety of free radical and alkylating species.

Temozolomide undergoes spontaneous hydrolysis and decarboxylation at physiological pH value and thereafter a methyldiazonium ion is released. This ion forms DNA adducts within guanine rich DNA sequences. Temozolomide has high bioavailability and is metabolized in the liver.

Clinical Use (Including Side-Effects)

Dacarbazine is the most active compound used for treating metastatic melanoma. It is also combined with anthracyclines and other cytostatics in the treatment of different sarcomas and Hodgkin's disease. Dacarbazine may cause severe nausea and vomiting. Myelosuppression results in leukopenia and thrombocytopenia. Alopecia and transient abnormalities in renal and hepatic function also occur.

Procarbazine causes myelosuppression, hypnotic and other effects on the central nervous system, e.g., vivid nightmares. Also, procarbazine causes a disulfiram like syndrome on ingestion of ethanol.

Temozolomide crosses the blood brain barrier and can be used for the treatment of brain tumors (e.g., *glioblastoma multiforme*). The most common side effects are nausea and vomiting.

Platinum Complexes

The chemical structure of the most frequently used platinum drugs (►platinum complexes) is shown in Fig. 4.

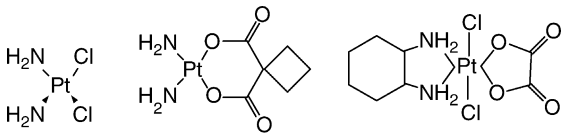
Cisplatin was discovered fortuitously by observing that bacteria present in electrolysis solutions could not divide. It is hypothesized that in the intracellular environment, a chloride is lost and replaced by a water molecule. The resulting species is an efficient bifunctional interactor with DNA, forming platinum-based cross-links similar to that formed by alkylating agents.

Clinical Use (Including Side-Effects)

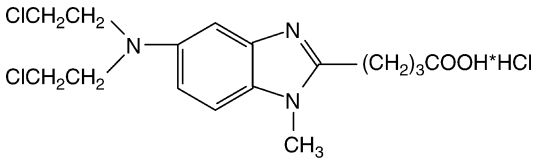
Cisplatin administration requires adequate hydration and forced diuresis to prevent kidney damage. Cisplatin is intensely emetogenic and its use requires adequate antiemetic prophylaxis. Myelosuppression is less evident than with other alkylating agents.

Carboplatin displays less nephro-, oto- and neurotoxicity. However, myelosuppression is more frequent, and as the drug is exclusively cleared through the kidney, adjustment of dose for creatinine clearance must be accomplished.

Oxaliplatin belongs to the group of diaminocyclohexane platinum complexes that can overcome platinum resistance. Its place in the primary and adjuvant treatment of colon cancers is being defined. It is prominently neurotoxic.



Alkylating Agents. Figure 4 Chemical structure of some platinum antineoplastic drugs.



Alkylating Agents. Figure 5 Chemical structure of bendamustine.

Other Alkylating Agents

Bendamustine (Fig. 5) is a representative of the group of bivalent alkylating agents and nitrogen mustard derivatives. It is supposed that bendamustine has a dual mode of action as alkylating agent and antimetabolite due to the presence of a benzimidazole ring and chlorethyl groups in its chemical structure. It is water-soluble and is widely distributed in different tissues. Bendamustine is metabolized in the liver with the formation of monohydroxy and dihydroxy derivatives and some of its metabolites are active antineoplastic compounds. It undergoes renal and biliar excretion.

Clinical Use (Including Side-Effects)

Bendamustine is a useful antineoplastic drug for the treatment of non-Hodgkin's lymphomas, multiple myeloma and as a partner drug in the combination therapy of some solid tumors. The cross-resistance with other alkylating drugs is not complete. Myelosuppression and lymphocytopenia is its main dose-limiting toxicity.

►Antineoplastic Agents

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Alkylation

Alkylation is the transfer of an alkyl group from one molecule to another. The alkyl group may be transferred

as an alkyl carbocation, a free radical or a carbanion (or their equivalents.

►Alkylating Agents

Allele

Allele variant forms of the DNA sequence at a specified locus. For example, alleles at a single-nucleotide polymorphism (SNP) are characterized by the nucleotide that is changing. The combination of two alleles at a locus constitutes a genotype.

►Pharmacogenomics

Allergen

An allergen is usually an inert substance (e.g. pollen, house dust mite faeces) that in some individuals can trigger the generation of an (inappropriate) antigenic response. Mediated by TH2 lymphocytes, it causes B-Lymphocytes to produce lgE. Subsequent exposure of a sensitized individual to the allergen is therefore able to cross-link IgE antibodies on the surface of mast cells and trigger an immune response and histamine release.

►Allergy
►Bronchial Asthma
►Histaminergic System

Allergy

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Synonyms

Hypersensitivity

Definition

The term allergy describes inappropriate immune responses to foreign substances after repeated exposure giving rise to irritant or harmful, and eventually fatal reactions. Its incidence depends on two factors: the occurence and nature of an agent eliciting immune reactions (allergen) and the reactivity of the immune system (►immune defense). In highly industrialized countries allergies may affect more than 30% of the population caused by poorly understood environmental influences. In addition, a genetically determined predisposition exists to develop an allergy.

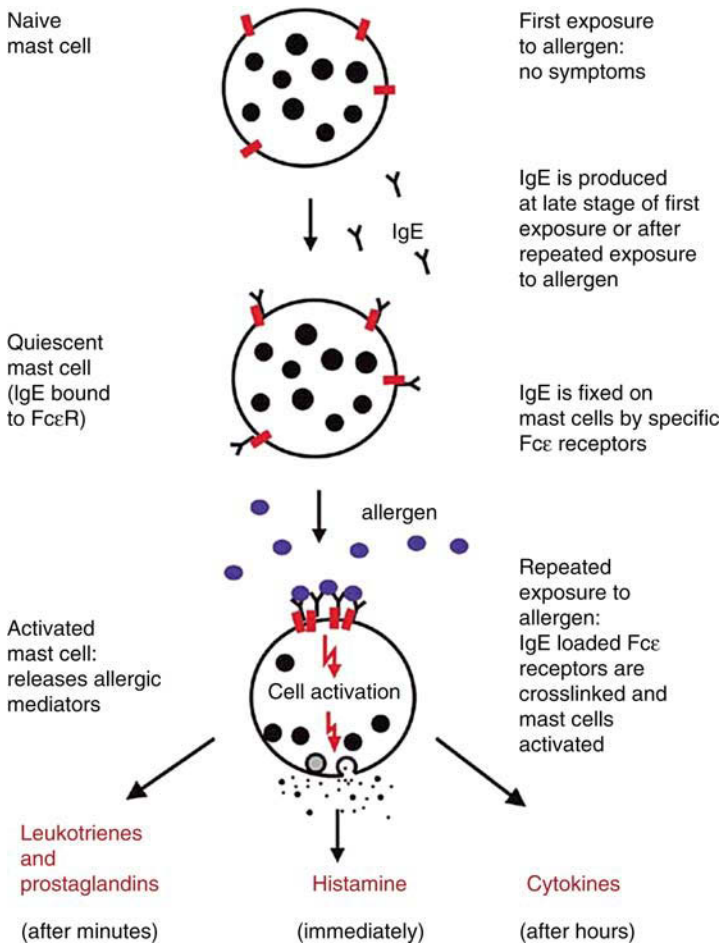
Basic Mechanisms

Currently allergic reactions are classified into four types on the basis of different reaction patterns. Whereas types I–III are dependent on antibodies, the type IV reaction is mediated by cellular immune reactions.

Type I Reactions: Anaphylactic Reaction

This type of allergic reaction is by far the most common one, and may be responsible for more than 80% of all allergies. Often it is used synonymously with allergy.

In some individuals, exposure to an antigen – then termed allergen – leads to the increased production of specific IgE, a subclass of antibodies that physiologically is only synthesized in minute quantities. For this bias of the humoral immune response against an allergen, a subgroup of helper T-lymphocytes, Th-2 cells, plays a key regulatory role by providing the master cytokine interleukin-4 (►immune defense, cytokines). IgE binds with high affinity to receptors (Fcε-receptors) that are present on basophilic granulocytes and most prominently on the closely related mast cells. These cells thus acquire a "borrowed" (as it is, of course, synthesized by B-lymphocytes) allergen specific receptor, which can persist on these cells for long periods, at least several months, perhaps years. If that happens, an individual is "allergic", i.e. sensitized to an allergen without exhibiting any clinical symptoms and without knowing it. Upon reexposure to this specific allergen, the mast cells (and the other IgE-bearing cells) can immediately recognize the allergen with its IgE antibodies. This results in the crosslinking of the Fcε receptors that in turn triggers activation of the mast cells (or basophils). The consequence is rapid degranulation of preformed vesicles and release of a plethora of mediators into their surrounding (see Fig. 1), the most prominent mediator is histamine which is preformed and stored in vesicles and acts immediately upon release. Within minutes, further mediators like leukotrienes are synthesized. These mediators act in concert on cells in their vicinity which bear the appropriate receptors and thereby cause the clinical symptoms of an immediate allergic reaction. These may be itching (urticaria), local swelling (edema), allergic rhinitis



Allergy. Figure 1 Type I Anaphylactic Reaction: IgE-bearing mast cells are activated by allergens to release mediators of acute allergic reactions.

("hay fever"), constriction of bronchi ("asthma"), and when occurring in a generalized form, "anaphylactic" shock and eventual death. Activated mast cells also start synthesizing protein mediators, termed cytokines, in a process that requires some time (a few hours). These cytokines initiate an acute inflammatory response that in its late phase is characterized by the infiltration of leukocytes and especially eosinophilic granulocytes.

Type II Reactions: Cytotoxic Reaction

As a physiological response to an antigen, B-lymphocytes initially always secrete antibodies of the IgM class, only in the late stage of the primary response or upon reexposure to the same antigen, B cells switch immunoglobulin classes and produce IgG, IgA, or IgE (►immune defense). In rare situations the antigen, or a metabolite thereof, either alone or bound to a carrier protein, may bind firmly to surfaces of cells. The antigen on the cell surface is now recognized by specific IgG antibodies, and thus the whole cell is labelled as a "foreign" particle that is consequently – but erroneously – destroyed by the complement system or cellular mechanisms. Type II reactions contribute to autoimmune mechanisms (►autoimmune diseases). They are also responsible for allergic reactions to certain drugs and may induce severe diseases such as drug-induced aplastic anemia or agranulocytosis.

Type III Reactions: Immune Complex Reactions

In the case of the Type III reaction physiologically produced antibodies, predominantly of the IgG subclasses, bind specifically the soluble antigen and form immune complexes. These immune complexes may bind directly to Fcγ receptors or be coated with complement components and thus be opsonized for uptake by phagocytic cells that normally degrade them and thus eliminate them. An "allergic" situation occurs if these immune complexes cannot be ingested appropriately and degraded. Alternatively, and more often, due to a continuous supply of allergen, the phagocytic cell is incapable of coping with the mass of

resulting immune complexes. Thus the phagocytic cells respond to the frustraneous or continuous stimulation of Fcγ receptors by secreting a variety of products into their surrounding. These include catabolic enzymes that degrade unspecifically all available biological macromolecules such as proteins, nucleic acids, carbohydrates, or lipids no matter whether these are foreign or belong to the host, resulting in continuous destruction. It should be noted that this mechanism of damage is identical with that occurring in chronic inflammatory diseases (►inflammation) such as in ►rheumatoid arthritis or nephritis. Typical allergic Type III reactions are pulmonary diseases against inhalative irritants, or "►serum sickness" occurring after administration of high molecular weight proteinacious drugs, originally animal serum applied during passive vaccination, but also murine monoclonal antibodies or other drugs.

Type IV Reactions: Cellular Reactions

At the time when allergic reactions were classified little was known about cellular reactions, thus it appears appropriate today to divide this reaction type in two subgroups.

Type IVa Reactions: Cellular Cytotoxic Reactions

In this type of reaction an antigen elicits the generation of cytotoxic T-lymphocytes (►immune defense). Cytotoxic T-lymphocytes (Tc) destroy antigen bearing cells by inducing apoptosis. This reaction can be viewed as the cellular counterpart to the humoral Type II reactions. They play an important physiological role in the defense of viruses, and can become allergic reactions under the same conditions as described for Type II reactions.

Type IVb Reactions: Delayed Type Hypersensitivity Reactions

Antigens commonly induce the activation of T-lymphocytes of the T helper type (Th). In the case of Type IV b reactions the predominant responding cell is the Th-1 subtype. By secreting many cytokines, including ►interferon γ, Th-1 lymphocytes recruit and activate granulocytes and monocytic cells to mount an inflammatory response. In that respect the Type IVb reaction can also be viewed as a cellular counterpart of a humoral reaction, specifically of a Type III reaction, being of great importance in chronic inflammatory diseases such as rheumatoid arthritis, and glomerulonephritis, or in autoimmune diseases such as systemic lupus erythematodes. In fact, in these situations both Type III and Type IVb contribute to the chronic inflammatory reaction. With respect to allergy, Type IVb reactions are relevant for contact ekzema, i.e. the chronic response of skin to many irritants including chromate, nickel, cosmetics, fabrics, etc.

Pharmacological Intervention

General

The ideal and single curative treatment of an allergy is to strictly avoid exposure to the responsible allergen(s). This requires to elucidate the causative agent. A battery of diagnostic methods is available to achieve this including measurement of IgE in blood (RAST), various methods of eliciting allergic reactions in the skin (skin testing), and provocation of clinical symptoms (e.g. in food allergy). Unfortunately, many allergens are difficult to avoid in daily life as they occur ubiquitously or, in the case of occupational exposure, would require a change in profession. Thus, in many cases pharmacological intervention may be necessary to improve the health of the allergic patient.

Type II, III, and IV allergic reactions are variants of physiologic defense mechanisms only relevant in special situations, which follow a common pathologic pattern. In general, treatment of these forms require anti-inflammatory (►inflammation) or immunosuppressive strategies (►immunosuppression). Therefore, only therapy of Type I reactions will be described here.

Therapy of Type I Reactions

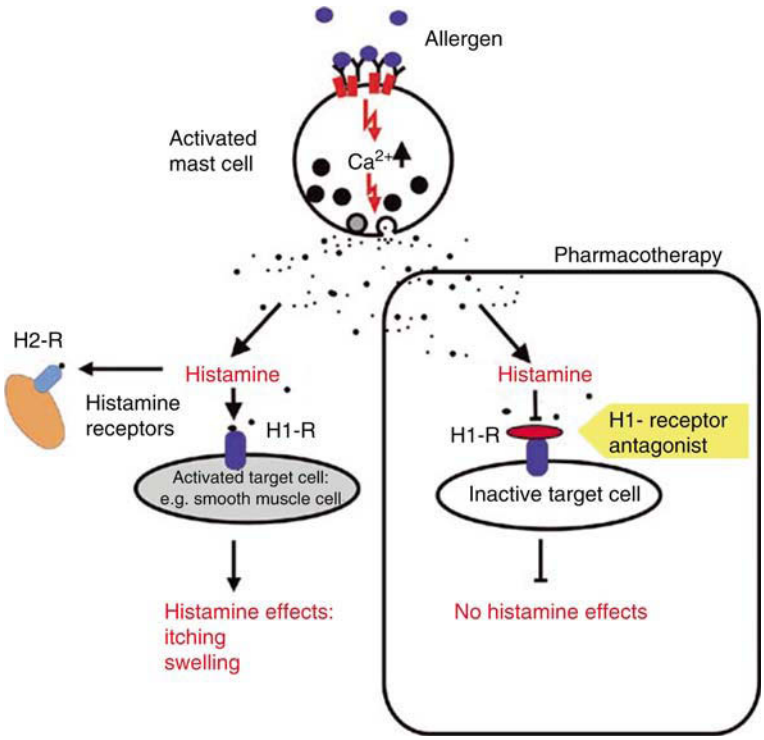
(Rush) Immunotherapy (Hyposensitization)

The inappropriate production of IgE to an allergen is caused by a Th-2 preponderance upon exposure to the allergen. Immunotherapy aims to influence the undesired Th-2 immune response and shift it to a Th-1 answer. It was found empirically and consists of the application of increasing doses of the allergen within a few days, starting with a very low, clinically inapparent, dose, and ending with a dose close to or above the one which is to be expected in a natural situation (e.g. after a bee sting). The high dose usually is applied at monthly intervals for up to three years or even longer. An absolute indication for immunotherapy are allergies to bee or wasp poison, which may result in an anaphylatic shock and may be fatal. At least partial relief may be achieved by immunotherapy in patients with allergies against defined pollen, but mostly fails with complex mixtures of allergens such as proteins of pets (epithelia, hair) or proteins in the faeces of mites (house dust allergy). With the availability of modern molecular biology and the achievements of recombinant DNA technology the identification of the responsible structures of allergens and their production in defined quality and quantities may increase the rate of success also with complex allergens or mixture of allergens in future.

Pharmacotherapy

Histamine H-1 Receptor Antagonists

Histamine H-1 receptor antagonists compete with the binding of histamine to its Type I receptors which are predominantly located in cells of the vasculature, and



Allergy. Figure 2 Histamine H-1 receptor antagonists inhibit response of target cells to histamine and relieve hay fever-like symptoms.

thus block its action (Fig. 2). H-1 receptor antagonists are effective when symptoms occur which involve peripheral blood vessels – around which the majority of mast cells are located – such as urticaria, allergic rhinitis ("hay fever"), or conjunctivitis. As histamine receptors are also present in the brain, their blockade influences the ability to focus and may result in sleepiness. However, modern second generation H-1 receptor antagonists do not cross the blood–brain barrier and thus do not show this undesired sedative side effect (examples: loratadin, fexofenadin, cetirizin).

Leukotriene Antagonists

Leukotrienes are rapidly produced and released during a Type I reaction (Fig. 3). They are responsible for a massive bronchoconstriction in allergic bronchial asthma and attract leukocytes, thus being proinflammatory. Consequently, antagonists of the LTC receptor have been proven useful in the therapy of ►bronchial asthma, often in combination with bronchodilators (example: montelukast).

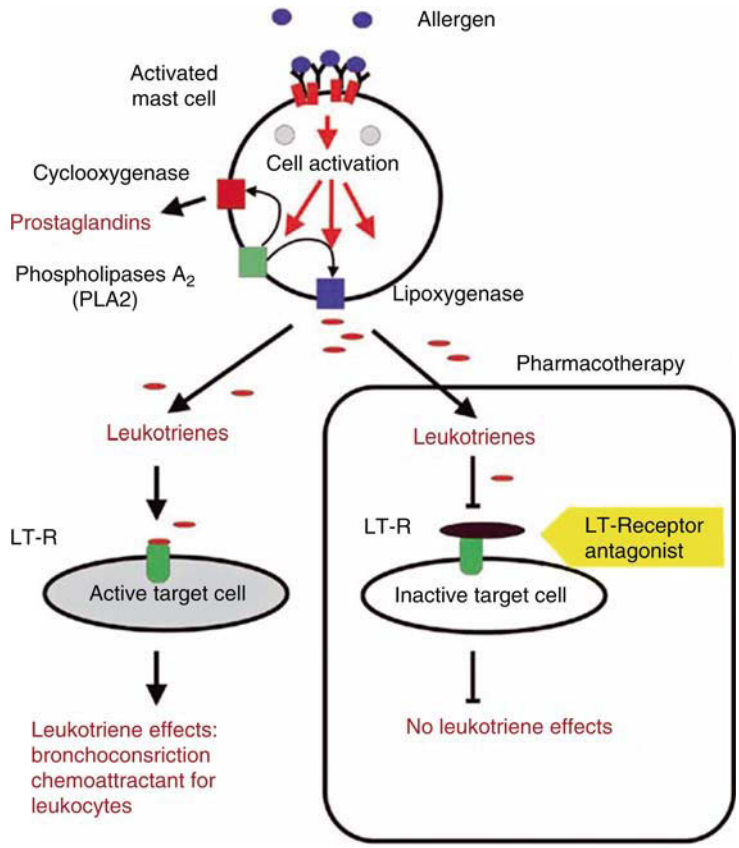
Cromones

Cromones suppress the release of mediators from mast cells by a mechanism that is not known (Fig. 4). In order to achieve the complete suppressive effect, cromones have to be given prophylactically several days to weeks before exposure to seasonal allergens can be expected, emphasizing the importance of warning systems or calendars (e.g. for pollen) as means for initiating therapy. Cromones also are effective in ongoing allergic responses, but then a few days are required to see benefits for the patient. Cromones are practically insoluble and thus are not absorbed beyond the top layers of tissues. This has the advantage that no systemic side effects occur, on the other hand cromones only act locally and must be applied at the site of action wanted. Cromones have beneficial effects in allergic rhinitis (nose drops or spray), conjunctivitis or bronchial asthma (inhalable preparations) (examples: disodium cromoglycate or nedocromil).

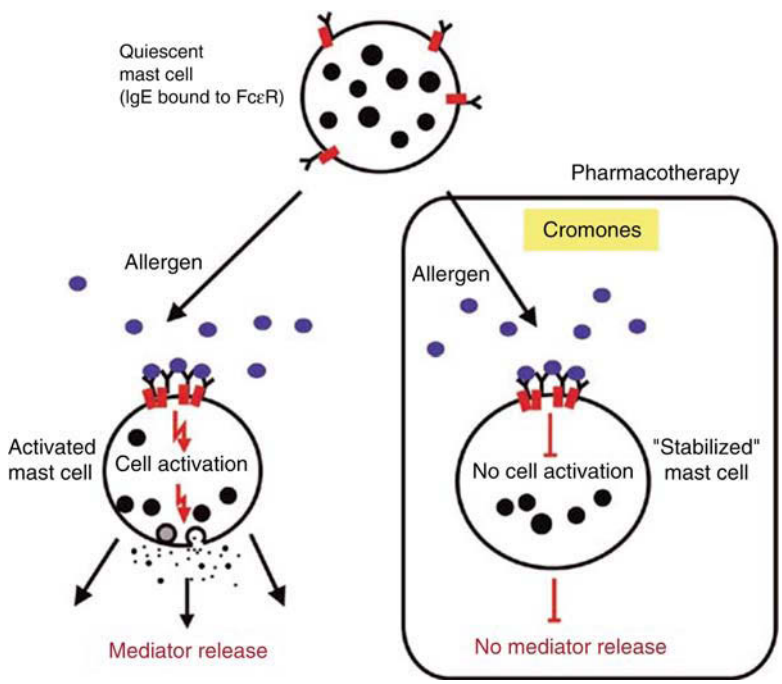
Glucocorticoids

Topically Applied Glucocorticoids – "Inhalable" Glucocorticoids

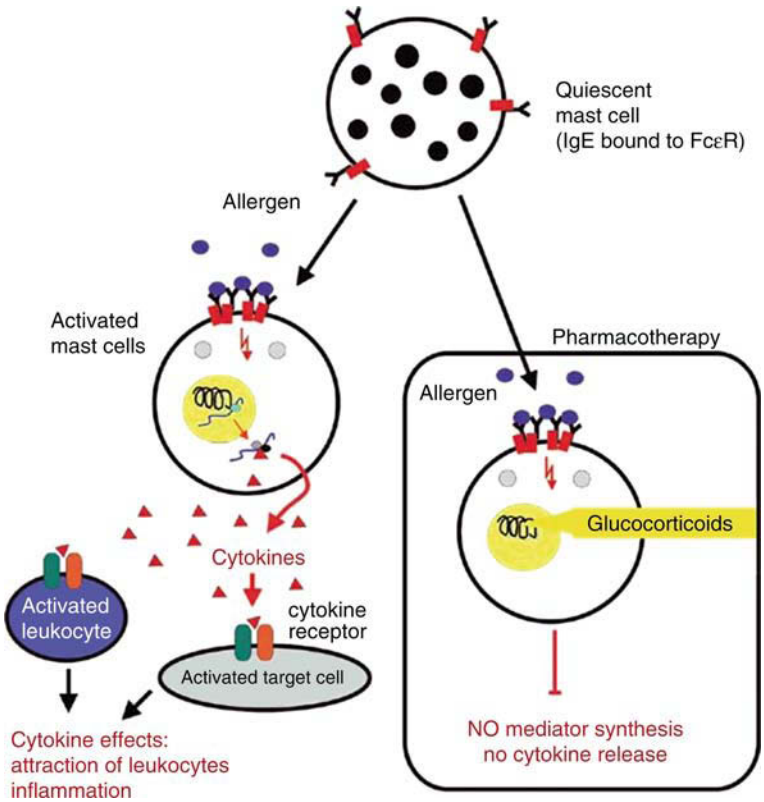
Glucocorticoids are very effective anti-inflammatory drugs. In Type I allergy they affect several different target cells, the most important being the mast cells and infiltrating T-lymphocytes. In general their action is immunosuppressive (►immunosuppression). On mast cells, glucocorticoids mainly affect the synthesis and release of mediators such as the arachidonic acid metabolites and most prominently the cytokines. The molecular mechanism of glucocorticoid action is complex and can be summarized as a regulatory effect on gene induction and expression (Fig. 5).



Allergy. Figure 3 Leukotriene LT receptor antagonists inhibit the response of target cells to leukotrienes and relieve symptoms of allergic asthma bronchiale.



Allergy. Figure 4 Cromones “stabilize” mast cells.



Allergy. Figure 5 Glucocorticoids regulate gene expression, resulting in a decrease of cytokine and mediator release.

Of all glucocorticoids applied to the upper respiratory tract (nose, bronchi) more than 80% may be swallowed and finally absorbed by the gastrointestinal tract. This fraction reaches the circulation after an initial first passage through the liver. "Modern" glucocorticoids for inhalation are chemically modified in a way that they are completely inactviated metabolically by the liver. Thus inhalable glucocorticoids in therapeutic doses are effective in the respiratory tract, but do not give rise to systemic side effects (►glucocorticoids). They play an important role in the long term treatment of ►bronchial asthma. They also have beneficial effects in allergic rhinitis ("hay fever"), especially in seasonal forms (example: beclomethason, budesonid).

Glucocorticoid Ointments

Glucocorticoid ointments are used to treat allergic skin reactions locally. They should be applied only for limited periods to avoid trophic damage to the skin such as thinning (paper skin).

Systematically Applied Glucocorticoids

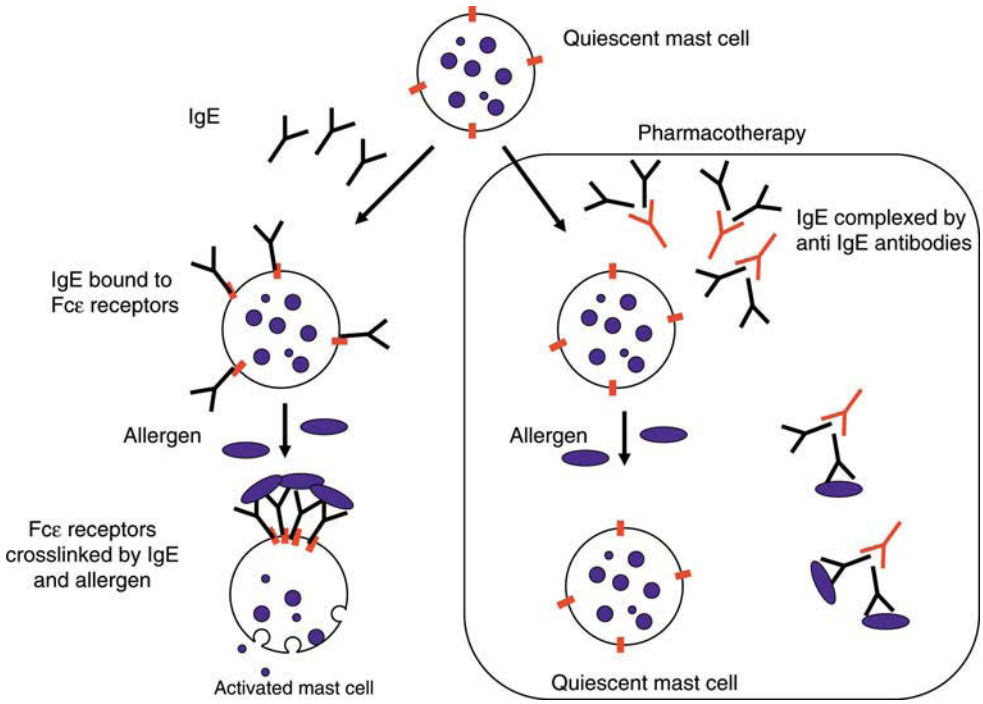
Because of their considerable side effects – which depend on dose and, even more relevant, on the duration of application – systemically applied glucocorticoids are only used in serious allergic diseases. This includes ►bronchial asthma, autoimmune, and chronic inflammatory diseases.

Anti-IgE Antibodies

A modern strategy of pharmacological intervention aims at the neutralization of immunoglobulin E before it binds to the Fcε receptors on the mast cells. This is achieved by applying a monoclonal antibody recognizing the Fc part of human IgE antibodies irrespective of their antigen specificity. By this means IgE cannot bind to the Fce receptor on mast cells, basophils, and dendritic cells. Thus activiation of these cells does not take place even in the presence of allergen, because the allergen will bind to the IgE molecules kept in solution by the neutralizing antibody, and the mast cells remain quiescent (Fig. 6). The humanized monoclonal antibody of the IgG1 type is indicated in patients with moderate and severe asthma if it cannot be managed with glucocorticoids (Omalizumab, Xolair®)

Anaphylactic Shock

The most serious acute Type I reaction is the generalized reaction, the anaphylactic shock. Anaphylactic shock results from a generalized release of mediators from mast cells and basophils. The clinical symptoms are manifested predominantly in



Allergy. Figure 6 Anti IgE antibodies prevent IgE from binding to their receptors on mast cells, and thus from releasing allergic mediators.

1. Circulation: Leakage of fluid from the vasculature into the surrounding tissue causes edema, drop in blood pressure and finally hemodynamic shock.
2. Heart: Histamine (and also other mediators) induce arrhythmias which can be fatal.
3. Respiratory tract: all symptoms associated with allergy can occur, starting from profuse rhinitis to severe asthma and suffocation.
4. Gastrointestinal system: cramps and diarrhea.
5. Skin: generalized urticaria and erythema.

Treatment

The fate of the patient largely depends on the first 30 min of an anaphylactic shock reaction. Thus persons with a known history of hypersensitivity reactions towards bee or wasp poison should always carry an emergency set during the insect season (see below).

1. Intravenous infusion of epinephrin: 0.1–0.5 mg epinephrin dissolved in plasma replacement, this can be repeated after 5 min.
2. Plasma replacement (any): this should also be used as a continuous access to the intravenous blood.
3. Glucocorticoids: 300 mg to 1 g as bolus
4. Histamine H-1 receptor antagonist

Emergency Set Contains:

1. a ready to use epinephrine solution in a special syringe allowing sequential application in two doses
2. readily resorptive glucocorticoid solution (orally)
3. readily resorptive histamine H-1 receptor antagonist (**cave:** sedation!!)
4. (if available: inhalable epinephrine)

►Immune Defense
►Inflammation
►Immunosuppressive Agents
►Humanized Monoclonal Antibodies

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Allodynia

The sensation of pain, following injury or disease, in response to a previously non-noxious stimulus is termed ‘allodynia’. Tactile allodynia is caused by

recruitment of low-threshold (non-nociceptive) sensory fibres (Aβ) in nociceptive pathways.

►Pain and Nociception
►Galanin Receptors

Alloimmunity

Alloimmunity is the immune response mounted by a host on the basis of differences in major histocompatibility antigens expressed on the surface of a donor cell from the same species as the host.

►Immune Defense

Allostatic State

A state of chronic deviation of a regulatory system from its normal (homeostatic) operating level is defined as an allostatic state. In the context of drug addiction this term has been introduced by George Koob and Michel Le Moal and represents a chronic deviation of reward set point by dysregulation of reward circuits and brain stress systems that provide a negative motivational state that drives addictive behavior.

►Drug Addiction/Dependence

Allosteric Modulators

Unlike competitive antagonists that bind to the same domain on the receptor as the agonist, allosteric modulators bind to their own site on the receptor and produce an effect on agonism through a protein conformational change. Allosteric modulators can affect the affinity of the receptor for the agonist or simply the responsiveness of the receptor to the agonist. A hallmark of allosteric interaction is that the effect reaches a maximal asymptote corresponding to saturation of the allosteric sites on the receptor. For example, an allosteric modulator may produce a maximal 10-fold decrease in the affinity of the receptor for the agonist upon saturation of the allosteric sites on the receptor.

►Drug–Receptor Interaction
►Adenosine Receptors
►Metabotropic Glutamate Receptors
►Benzodiazepines

Allylamines

►Antifungal Drugs

ALS

►Amyotrophic Lateral Sclerosis

Alternative Splicing

Alternative splicing is the process occurring when eukaryotic pre-mRNA, which includes several introns and exons, is transcribed from one gene to undergo distinct cutting and pasting processes yielding different mature mRNAs. It is an important cellular mechanism that leads to temporal and tissue-specific expression of unique mRNA products from a single gene. It thereby increases protein diversity by allowing multiple, sometimes functionally distinct, proteins to be encoded by the same gene.

►Ca^{2+} Channel Blockers
►Cholinesterases

Alzheimer's Disease

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Synonyms

Morbus Alzheimer (most common form of dementia)

Definition

Alzheimer's disease (AD) is a progressive, degenerative and irreversible neurodegenerative disorder of the brain. The greatest risk factor for developing AD is increasing age. The likelihood of developing AD doubles almost every five years after age 65, reaching nearly 50 percent risk after age 85. ►Familial Alzheimer's disease (FAD) is a hereditary form of the disease that occurs earlier in life with an onset before the age of 65 and accounting for 5–10% of total AD cases. The majority of cases is sporadic without a clear inheritance pattern and occurs after age 65. So far, there is no cure for the impaired memory and the loss of mental abilities affecting normal activities of daily living. Cognitive tests and exclusion of other diseases are used to diagnose AD with a restricted accuracy in living patients. A confirmation of the diagnosis is only possible by autopsy. The key pathological hallmarks of AD are the extracellular ►amyloid plaques and intracellular neurofibrillary tangles and its constituent, the tau microtubule-associated protein. The principle component of plaques is the amyloid beta peptide (Aβ). The Aβ peptide is derived from the amyloid precursor protein (APP) by two consecutive proteolytic cleavage events. Aggregates of the Aβ peptide are ultimately believed to cause the neurodegeneration that leads to AD.

Basic Mechanisms

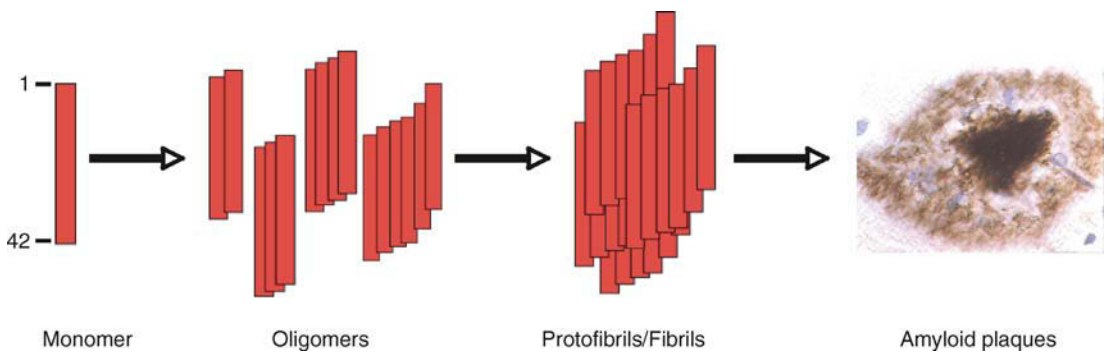
The Amyloid Hypothesis

According to the "amyloid hypothesis," the Aβ peptide plays a critical role in the pathogenesis of Alzheimer's disease [1]. Major forms of Aβ produced encompass 38, 40 or 42 residues. Aβ42 is more prone to aggregation than Aβ40 and in animal models an increased Aβ42/Aβ40 ratio results in amyloid plaque pathology even when total Aβ levels are reduced [4]. The generation of Aβ is a normal process and Aβ is present in the brains and body fluids of humans throughout life. Neuronal injury appears to result from an ordered self-association of Aβ monomers or dimers before plaque formation can be observed. A loss of neurons was observed in brains of transgenic mice expressing human mutant amyloid precursor protein at sites of Aβ aggregation but, most importantly, was also clearly observed in brain areas distant from plaques. This finding together with the correlation found between levels of soluble Aβ oligomers and the severity of synaptic loss, led to the conclusion that aggregation of Aβ into oligomers is essential for toxicity. Aβ monomers can spontaneously aggregate into oligomers or protofibrils on the pathway to fibril formation (Fig. 1). It seems that oligomerization is an intermediate step in the formation of protofibrils or the end point of an alternative non-fibrillar assembly process prior to plaques or tangle pathology [3].

Oligomeric forms of Aβ either derived from synthetic Aβ or purified from Aβ-containing cell culture medium could be shown to trigger hippocampal synapse loss and are thus suspected to be the effectors of synaptic dysfunction in AD. Based on these findings and findings obtained from APP transgenic mice and human brain tissue the amyloid hypothesis of AD pathogenesis has been modified. Rather than extracellular plaque Aβ causing toxicity, neuronal Aβ42 accumulation may play a direct role in causing neuronal dysfunction, neuronal death and dementia caused by oligomer formation of Aβ42 inside of the cells.

Aβ is Derived form the Amyloid Precursor Protein (APP)

APP is a type-I transmembrane protein that is part of an evolutionarily conserved protein family, including the amyloid precursor-like proteins 1 (APLP1) and 2 (APLP2). APP and APLPs are functionally redundant and form homo- and hetero-oligomers. The absence of the Aβ sequence in the APLPs underlines the importance of APP that can only give rise to the Aβ species. Decreasing the formation of soluble Aβ



Alzheimer's Disease. Figure 1 Aβ monomers can self-associate to form dimers, trimers and higher oligomers. Globular structures of synthetic Aβ42 are known as Aβ-derived diffusible ligands (ADDLs) (3–12-mers of Aβ). These structures are similar to the smallest protofibrils and represent the earliest macromolecular assembly of synthetic Aβ. The characteristic amyloid fiber exhibits a high beta-sheet content and is derived in vitro by a nucleation-dependent self-association and an associated conformational transition from random to beta-sheet conformation of the Aβ molecule. Intermediate protofibrils in turn self-associate to form mature fibers.

monomers and dimers to prevent the generation of cytotoxic oligomers should prove an effective strategy against AD.

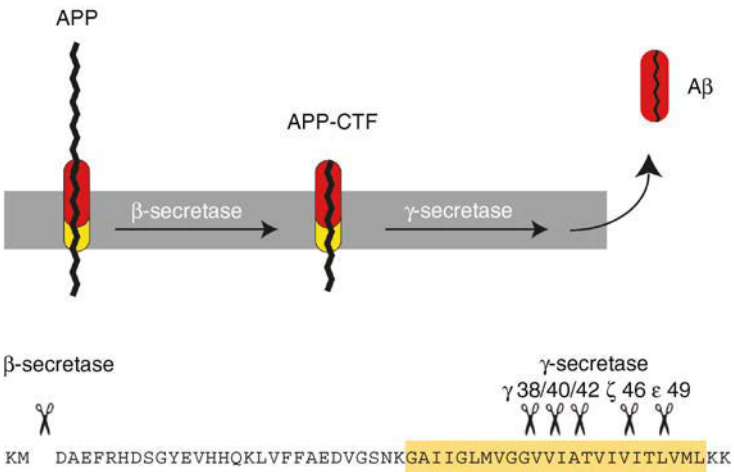
APP undergoes proteolytic processing by several ►secretases. First, the bulk of the ectodomain needs to be removed by membrane-bound α- or β-secretases leading to secreted forms of APP and membrane-bound C-terminal fragments α-CTF or β-CTF, respectively. Regulated intramembrane proteolysis (RIP) of the β-CTF by γ-secretase occurs only after ectodomain shedding and releases the Aβ peptide from the membrane (Fig. 2).

The γ-secretase has been reported to cleave at variable sites thus generating Aβ peptides of varying lengths with mainly 37–43 residues. An explanation for the multiple cleavages of γ-secretase was provided indicating a sequential proteolytic cleavage mechanism to release Aβ [2]. Accordingly, the first cut occurs at the cytoplasmic edge of the transmembrane sequence (TMS) at the ε-site, i.e. residue 49 or 48 of β-CTF. The products Aβ49/Aβ48 remain membrane-bound and are further processed in a sequential action mode into Aβ46/Aβ45, i.e. the ζ-site. Aβ46 is further processed into Aβ43 and finally Aβ40 whereas Aβ45 is the direct precursor of Aβ42. The underlying mechanism for the regulation is uncertain but alterations in Aβ production may be reflected by the dynamic nature of the substrate and/or altered enzyme/substrate interactions. The current view is that the generation of Aβ42 and Aβ38 levels can be regulated by the strength of homophilic interactions within the APP TMS itself leading to the generation of the shorter species Aβ37, Aβ35 and Aβ34 if the interaction is attenuated. Any events stabilizing dimerization would increase the production of Aβ42 and vice versa. It could be considered that AD risk factors like high cholesterol levels or disturbed lipid homeostasis, oxidative stress, disturbed metal homeostasis or familial AD mutations affect the dimer stability of β-CTF and thus influences Aβ production. Compounds targeting the dimerization of the APP TMS and interfering with the interaction motif of two γ-secretase substrate molecules may be useful as future therapeutics to prevent generation of Aβ42.

Pharmacological Intervention

The current treatments cannot cure the disease but are suspected to offer patients modest improvement in some symptoms. For treating the cognitive symptoms of AD, a class of drugs known as cholinesterase inhibitors, such as Galantamin, Rivastigmin, Donepezil and Tacrin, act in delaying the breakdown of acetylcholine that facilitates normal functioning of nerve cells. Another approved agent, Memantine, is an uncompetitive NMDA antagonist which prevents the excessive binding of glutamate to the receptor. Other examples used to treat behavioral and psychiatric symptoms of AD include antidepressant medications for low mood and irritability as well as anxiolytics and antipsychotic medications.

Drugs in clinical development that directly target the Aβ pathway are at an early stage. Inhibitors of β- and γ-secretases that can lower the Aβ production have entered clinical phase trials with β-secretase inhibitors being years behind the development of γ-secretase inhibitors. Functional γ-secretase inhibitors have been shown to reduce the rate of Aβ formation *in vitro* and *in vivo*. The reduction of Aβ monomer levels could prevent oligomer formation and subsequent synaptotoxicity. Numerous anti-amyloid approaches to



Alzheimer's Disease. Figure 2 Aβ is derived from the APP by the sequential action of proteolytic activities exerted by β- and γ-secretases. APP-CTF is (C99) produced after cleavage of the APP by β-secretase and represents the substrate of the γ-secretase. The yellow box marks membrane embedded amino acid residues of Aβ peptide. Scissors represent the main cleavage sites of β- and γ-secretase, e.g. the ζ–, ε–, and γ–cleavages at positions 49, 46, 42, 40 and 38.

disassemble, neutralize or degrade Aβ oligomers are under development. For the latter, an up-regulation of enzymes involved in Aβ degradation, such as over-expression of neprilysin (NEP) or the insulin degrading enzyme (IDE) led to the desired effects. Aβ levels in APP transgenic animals were found decreased and plaque burden was reduced. Alternatively, inhibitors of Aβ aggregation have been tested *in vitro* for two different purposes: first, to stabilize the monomer and then to shift these molecules into the normal degradation pathway and second, to bind to Aβ oligomers and to disrupt these structures. The compound tramiprosate is a sulfated glycosaminoglycan mimetic, which is suggested to preferentially bind to soluble Aβ and can inhibit plaque formation in APP transgenic mice. Other compounds such as AZD-103 (scyllo-cyclohexanehexol) were shown to disassemble large Aβ oligomers and to neutralize Aβ dimers and trimers. Similarly, it has been shown that anti-Aβ antibodies hold a great promise for elucidating the Aβ clearance by redistributing Aβ from the brain to the systemic circulation. New therapeutic approaches based on the assumption that the APP pathway underlies the cause of the disease will have more and more to consider structural and functional information on the biological roles of APP and APLPs that may prove useful for novel strategies against this devastating disorder. Thus, compounds targeting the oligomerization mediated by the APP TMS and interfering with the interaction of two γ-secretase substrate molecules may be useful as therapeutics to prevent generation of Aβ42.

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Ames Test

The Ames test measures the reversion from mutant to wild type form (back-mutation) in a culture of Salmonella. The test is used to screen large numbers of compounds for their potential mutagenicity.

Amiloride

Prototypic inhibitor of the family of Na/H exchangers and of epithelial Na+ channels.

►Epithelial Na^+ Channel
►Na^+/H^+ Exchangers

Amiloride-sensitive Na^+ Channel

The amiloride-sensitive Na^+ channel (ENaC) is a cell membrane glycoprotein selective for sodium ions, which is composed of three subunits (α, β and γ). Gating of sodium is inhibited by the diuretic amiloride.

►Epithelial Na^+ Channels
►Na^+/H^+ Exchangers
►Endothelins

γ-Aminobutyric Acid (GABA)

►GABAergic System

Aminoglycosides

►Ribosomal Protein Synthesis Inhibitors

Aminopeptidase

An exopeptidase that sequentially releases an amino acid from the N-terminus of a protein or peptide. Examples include cystinyl aminopeptidase (MEROPS M01.011), which removes a terminal cysteine from the biologically important peptides oxytocin and vasopressin, and methionyl aminopeptidase (M24.001), which removes the initiating methionine from cytosolic

proteins as they are being synthesized. Aminopeptidases are included in Enzyme Nomenclature subsubclass 3.4.11.

►Non-viral Peptidases

AMP, Cyclic

►Cyclic Adenosine Monophosphate
►Adenylyl Cyclases

AMP-activated Protein Kinase

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Synonyms

AMP-activated protein kinase, AMPK; SNF1 complex (fungi); SNF1-related kinase-1 (higher plants)

Definition

The AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that acts as a sensor of cellular energy status [1, 2, 3]. It is activated, via mechanisms described below, by an increase in the cellular AMP:ATP ratio. Because all eukaryotic cells express a very active adenylate kinase, which maintains the reaction 2ADP ↔ ATP + AMP close to equilibrium at all times, any increase in the ADP:ATP is amplified into a much larger increase in the AMP:ATP ratio. This can happen due to a metabolic stress that interferes with ATP production (e.g. hypoxia, glucose deprivation), or that increases ATP consumption (e.g. muscle contraction). Once activated by a fall in cellular energy status or other stimulus, AMPK switches on ATP-generating catabolic pathways, while switching off ATP-consuming processes to restore energy status. The system is the primary target of the ►biguanide drugs (e.g. ►metformin), and is both a direct and indirect target of the ►thiazolidinediones (e.g. ►rosiglitazone, ►pioglitazone), all of which are used as insulin-sensitizing drugs in the treatment of type 2 diabetes.

Basic Characteristics

AMP-activated protein kinases are heterotrimeric complexes comprised of catalytic α subunits and regulatory β and γ subunits (Table 1). Each subunit is encoded by at least two genes, some of which can also be subject to alternate splicing, leading to a diverse array of possible heterotrimeric combinations.

The α subunits, for which two isoforms exist in mammals (α1, α2), contain conventional protein serine/threonine kinase domains at the N-terminus, with a threonine residue in the activation loop (Thr-172) that must be phosphorylated by upstream kinases (see below) before the kinase is active. The kinase domain is followed by an autoinhibitory domain, whose effect is somehow relieved by interaction with the other subunits. The C-terminal domain of the α subunit is required for the formation of a complex with the C-terminal domain of the β subunit, which in turn mediates binding to the γ subunit. The α1 and α2 catalytic subunit isoforms are widely distributed, although α2 is most abundant in muscle and may be absent in cells of the endothelial/hemopoietic lineage.

The β subunits, for which two isoforms exist in mammals (β1, β2), contain a central glycogen-binding domain. This domain, related to noncatalytic domains found in enzymes that metabolize the α1→6 branch points in α1→4-linked glucans, such as starch and glycogen, causes the AMPK complex to bind to glycogen, although the physiological role of this remains unclear. The β subunits also contain a C-terminal domain that is responsible for the association with the α and γ subunits. The two β subunit isoforms (β1 and β2) are widely distributed, although β2 is most abundant in muscle and may be low or absent in some cell types.

The γ subunits, for which three isoforms exist in mammals (γ1, γ2, γ3), contain variable N-terminal regions followed by four tandem repeats of a 60 residue sequence known as a CBS motif. CBS motifs, named after the enzyme cystathionine β-synthase in which they are also found, occur in around 20 proteins in the human genome and always occur in tandem pairs. A pair of CBS motifs form a stable domain known as a Bateman domain, the function of which appears to be the binding of regulatory adenosine-containing ligands such as AMP, ATP, or S-adenosylmethionine (the latter being an activating ligand for cystathionine β-synthase). Bateman domains have central clefts formed by two hydrophobic β-sheets, with the adenosine moiety of the ligand binding within this cleft. The three AMPK γ subunits are unusual, in that they contain two Bateman domains (i.e. four CBS motifs), each of which binds one molecule of the activating nucleotide, AMP, or the inhibitory nucleotide, ATP. Thus, two molecules of nucleotide bind to each γ subunit, with binding being highly cooperative. The γ subunits also contain a short

AMP-activated Protein Kinase. Table 1 Information about subunit isoforms of AMP-activated protein kinase. Data refer to the full-length forms of the human isoforms. The γ2 and γ3 isoforms also exist as splice variants that are N-terminal truncations, with lower molecular mass and number of amino acids (38 kDa and 328 amino acids for the short form of γ2, 52 kDa and 464 amino acids for the short form of γ3). Other splice variants may also exist

Isoform	Mass (kDa)	Gene name	Amino acids	Domains	Domain location (approx.)	Domain function	Site of major expression	Chromosome location
α1	63	PRKAA1	550	Kinase domain	1–270	Catalytic	Ubiquitous?	5p11–p14
				Autoinhibitory domain	290–335	Regulatory		
				C-terminal domain	393–550	β subunit binding		
α2	62	PRKAA2	552	Kinase domain	1–268	Catalytic	Muscle, liver	1p31
				Autoinhibitory domain	288–333	Regulatory		
				C-terminal domain	397–552	β subunit binding		
β1	30	PRKAB1	270	Glycogen binding domain	72–151	Glycogen binding	Ubiquitous?	12q24.1–24.3
				C-terminal domain	179–270	αγ binding		
β2	30	PRKAB2	272	Glycogen binding domain	72–154	Glycogen binding	Muscle	1q21.1
				C-terminal domain	181–272	αγ binding		
γ1	38	PRKAG1	331	Association domain	17–42	β subunit binding	Ubiquitous?	12q12–q14
				Bateman domain 1	43–178	AMP/ATP binding		
				Bateman domain 2	199–331	AMP/ATP binding		
γ2	63	PRKAG2	569	N-terminal domain	1–247	Targetting?	Muscle?	7q36
				association domain	248–274	β subunit binding		
				Bateman domain 1	275–410	AMP/ATP binding		
				Bateman domain 2	431–556	AMP/ATP binding		
γ3	54	PRKAG3	328	N-terminal domain	1–171	Targetting?	Muscle	2q35
				Association domain	172–197	β subunit binding		
				Bateman domain 1	198–333	AMP/ATP binding		
				Bateman domain 2	358–454	AMP/ATP binding		

conserved sequence of about 25 residues immediately N-terminal to the first CBS motif, which appears to be involved in the interaction with the β subunit. The function of the variable N-terminal domains present in the γ2 and γ3 isoforms (some or all of which can be removed by RNA splicing) are not known, although one possibility is that they are involved in subcellular targeting of the complex. The γ1 isoform is the major isoform in most cell types and appears to be universally distributed. The γ2 isoform is most abundant in skeletal

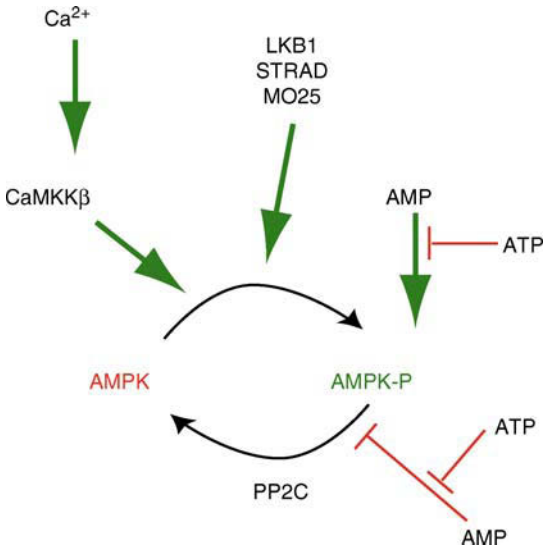
and cardiac muscle but probably also occurs elsewhere. The γ3 isoform appears to be almost restricted to skeletal muscle.

Upstream kinases can phosphorylate the AMPK α subunits at Thr-172, thus activating the kinase complex by >100-fold. The major upstream kinase in most mammalian cells is a complex between the tumor suppressor, LKB1, and two accessory subunits, STRAD and MO25. LKB1, which does not require to be phosphorylated for activity, is completely inactive unless STRAD is bound to it, while MO25, a protein containing helical repeats distantly related to armadillo proteins, appears to stabilize the LKB1:STRAD complex. STRAD is a "pseudokinase" that has a domain related to a protein kinase domain. This domain has no catalytic activity but still binds ATP, although this is not required for its ability to activate LKB1.

In humans, the LKB1 complex is now known to act as an upstream kinase for the whole subfamily of AMPK-related protein kinases, including twelve other kinases with functions distinct from AMPK, as well as the two catalytic subunit isoforms of AMPK (α1 and α2). The LKB1 complex appears to be constitutively active, thus constantly phosphorylating Thr-172 on α1 and α2. However, the phosphate is normally rapidly removed by dephosphorylation by protein phosphatases, with the physiological phosphatase probably being a form of protein phosphatase-2C. Binding of AMP to the γ subunit of the AMPK complex markedly inhibits dephosphorylation by protein phosphatase-2C, thus providing a sensitive switch mechanism that converts AMPK to the active phosphorylated form (Fig. 1). AMP binding also allosterically activates AMPK up to tenfold, so that the system will remain hyper-activated until AMP dissociates (the combined effect of phosphorylation and allosteric activation is at least 1000-fold). The second CBS motif (in the N-terminal Bateman domain) of all γ subunits contains a sequence that resembles the sequence of the target sites on downstream substrates for AMPK, except that it contains a nonphosphorylatable residue in place of serine or threonine. An attractive hypothesis is that this "pseudosubstrate" sequence binds to the substrate-binding groove on the α subunit in the absence of AMP. Some of the residues within this pseudosubstrate sequence also appear to be directly involved with AMP binding, suggesting an obvious mechanism whereby AMP binding relieves this pseudosubstrate interaction and causes allosteric activation.

Both effects of AMP (inhibition of dephosphorylation and activation) are antagonized by high concentrations of ATP, which compete with AMP for binding at the Bateman domains. Thus, the AMPK system can monitor changes in the cellular AMP:ATP ratio.

As well as the activation of AMPK by increases in cellular AMP:ATP, due to phosphorylation by LKB1,



AMP-activated Protein Kinase. Figure 1 Mechanism of activation of AMPK by AMP and Ca^{2+}. In resting cells, the catalytic α subunit of the AMPK complex is continually phosphorylated at Thr-172 by the LKB1:STRAD:MO25 complex, but the phosphate is at the same time rapidly removed by protein phosphatases (probably a form of protein phosphatase-2C, PP2C). Binding of AMP due to the AMPK γ subunit, due to elevation of AMP in response to metabolic stress, inhibits dephosphorylation and thus tends to switch the AMPK complex to the active, phosphorylated form, where it is further activated allosterically by AMP. High concentrations of ATP antagonize both effects of AMP, by competing with AMP for binding at the γ subunit. In some cell types that express CaMKKβ, elevation of cytosolic Ca^{2+} due to an external stimulus can cause increased phosphorylation of AMPK. This is not dependent on an increase in AMP, although it may be further accentuated by it.

AMPK can also be activated by a Ca^{2+}-mediated pathway involving phosphorylation at Thr-172 by the Ca^{2+}/calmodulin-dependent protein kinase, CaMKKβ. CaMKKα and CaMKKβ were discovered as the upstream kinase for the calmodulin-dependent protein kinases-1 and -IV; they both activate AMPK in a Ca^{2+}/calmodulin-dependent manner in cell-free assays, although CaMKKβ appears to much more active against AMPK in intact cells. Expression of CaMKKα and CaMKKβ primarily occurs in neural tissues, but CaMKKβ is also expressed in some other cell types. Thus, the Ca^{2+}-mediated pathway for AMPK activation has now been shown to occur in response to depolarization in rat neuronal tissue, in response to thrombin (acting via a Gq-coupled receptor) in endothelial cells, and in response to activation of the T cell receptor in T cells.

AMPK is also regulated by a number of cytokines, including ►adipokines secreted from adipocytes that

are thought to play a key role in the regulation of whole body energy balance. Thus, it is activated by the adipokine ►leptin in skeletal muscle, where it stimulates energy expenditure by activating fatty acid oxidation whereas, conversely, it is inhibited by leptin in the hypothalamus. The latter appears to be involved in the ability of leptin to inhibit food intake, because other agents that stimulate AMPK in the hypothalamus, such as the gut hormone ghrelin, cannabinoids, and hypoglycemia, all stimulate food intake in rodents. AMPK is also activated in muscle and liver by the ►adipokine ►adiponectin, with activation of AMPK by ►adiponectin in the liver being required for its hypoglycemic effects, and by the proposed "►myokine" interleukin-6. The molecular mechanism(s) by which these cytokines regulate AMPK remain unclear at present.

Once activated, the AMPK system switches on catabolic pathways that generate ATP (upper entries in Table 2), such as the uptake and oxidation of fatty acids and glucose, while at the same time inhibiting ATP-consuming processes (lower entries in Table 2) such as biosynthetic pathways involved in energy storage, and in cell growth and proliferation. AMPK achieves this both by direct phosphorylation of metabolic enzymes, and by phosphorylation of transcription factors or coactivators that regulate gene expression.

Drugs

The first pharmacological agent shown to activate AMPK was 5-aminoimidazole-4-carboxamide (AICA) riboside, also known as ►acadesine. This adenosine analogue is taken up into cells by adenosine transporters and phosphorylated by adenosine kinase to the monophosphorylated form, AICA ribotide or ZMP. ZMP accumulates inside cells to higher concentrations than the concentration of AICA riboside present in the medium, and it mimics both effects of AMP on AMPK system (allosteric activation and inhibition of

AMP-activated Protein Kinase. Table 2 Metabolic effects of AMPK activation. In cases marked with an *asterisk, there is evidence that AMPK mediates its effects by modulating the target named, although it is not yet clear whether the protein is directly phosphorylated by AMPK

Metabolic process	Effect	Immediate target	Immediate effects	Tissue
Glucose uptake	↑	AS160?	↑ GLUT4 translocation	Muscle
Glucose uptake	↑	*Myocyte enhancer factor-2	↑ GLUT4 expression	Muscle
Glucose uptake	↑	?	↑ GLUT1 activity	Many cells
Glycolysis	↑	6-phosphofructo-2-kinase (cardiac isoform)	↑ Activity	Cardiac myocytes
			↑ Fructose-2,6-bisphosphate	
Glycolysis	↑	6-phosphofructo-2-kinase (inducible isoform)	↑ Activity	Monocytes, macrophages
			↑ Fructose-2,6-bisphosphate	
Fatty acid oxidation	↑	Acetyl-CoA carboxylase-2	↓ Activity, ↓ malonyl-CoA	Liver, muscle, others
			↑ CPT1 activity	
Mitochondrial biogenesis	↑	*PGC-1α?	↑ PGC-1α expression	Muscle, others?
Fatty acid synthesis	↓	Acetyl-CoA carboxylase-1	↓ Activity	All cells?
Fatty acid synthesis	↓	*SREBP-1c, *HNF-4α	↓ Expression ACC1, fatty acid synthase	Liver
Cholesterol synthesis	↓	HMG-CoA reductase	↓ Activity	Liver
Glycogen synthesis	↓	Muscle glycogen synthase	↓ Activity	Muscle
Gluconeogenesis	↓	TORC2, others?	↓ Expression PEP carboxykinase, glucose-6-phosphatase	Liver
Protein synthesis	↓	*Elongation factor-2 kinase	↓ Translation elongation	All cells?
Protein synthesis	↓	TSC2	↓ TOR, ↓ S6K1	All cells?
			↓ Translation initiation	
Autophagy	↑	TSC2?	↓ TOR	All cells?
Glucose uptake	↓	?	↓ Insulin stimulation	Adipocytes
Lipolysis	↓	Hormone sensitive lipase (HSL)	↓ Adrenergic stimulation of lipolysis	Adipocytes

dephosphorylation), although it is less potent that AMP itself. Thus, AICA riboside can be used to activate AMPK in intact cells and in vivo. It has been used in animal models of obesity and insulin resistance such as the ob/ob mouse, the fa/fa rat and the fat-fed rat, where it improves glucose tolerance, increases insulin sensitivity and lowered blood pressure. Acadesine has also undergone clinical trials in humans, particularly as an acute treatment during coronary artery bypass graft surgery. These studies were initiated before it was realized that the drug was a potential AMPK activator, and the rationale was that it could be used to replenish adenine nucleotides lost from the cardiac muscle during periods of ischemia. These trials appear to have yielded inconclusive results regarding the efficacy of acadesine in such circumstances.

In 2001 it was reported that AMPK was activated by the ►biguanide metformin, and the following year it was reported that it was activated by the ►thiazolidinediones, ►rosiglitazone, and ►pioglitazone. ►Metformin is currently the most widely prescribed drug used to treat type 2 diabetes (current estimates are that it is used to treat 120 million worldwide), while the ►thiazolidinediones are another major drug class used to treat insulin resistance and type 2 diabetes. ►Metformin (and its sister drug ►phenformin) have no known target other than AMPK, and recent evidence involving mice in which the LKB1 gene was knocked down in liver (thus preventing activation of AMPK by the ►biguanides) supports the idea that the primary target of the drug is liver AMPK. Activation of liver AMPK decreases hepatic glucose production by downregulating expression of enzymes of gluconeogenesis, e.g. phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. ►Metformin and ►phenformin do not activate AMPK directly in cell-free assays but, surprisingly, appear to work indirectly by inhibiting complex I of the respiratory chain, thus increasing the cellular AMP:ATP ratio. This mechanism of action explains the main side effect of ►phenformin, i.e. lactic acidosis, which led to the withdrawal of the drug. There appears to be a much lower risk of lactic acidosis in the case of ►metformin, where gastrointestinal disturbance is the major side effect. However, there is evidence from animal studies that the major source of lactic acid produced in response to ►biguanides is the gut, and it is possible that inhibition of the respiratory chain in intestinal epithelial cells may explain the gastrointestinal side effects commonly reported during metformin use.

The thiazolidinediones have also been reported to act as inhibitors of the respiratory chain at high concentrations, and this appears to account for their ability to activate AMPK in cultured cells. However, the primary target of the thiazolidinediones appears to be the peroxisome proliferator-activated receptor-γ (►PPAR-γ), a member of the nuclear receptor superfamily expressed in adipocytes. One of the major effects of stimulation of ►PPAR-γ in adipocytes is the release of the ►adipokine adiponectin, and studies in ►adiponectin-deficient mice suggest that this accounts for many, although not all, of the effects of ►thiazolidinediones. AMPK is in any case a major target for ►adiponectin action, with knockdown of liver AMPK by expression of a dominant negative mutant preventing the hypoglycemic effects of ►adiponectin.

►Diabetes Mellitus
►Antidiabetic Drug Other than Insulin

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AMPA Receptors

The ionotropic glutamate receptors have been classified based upon their pharmacology and form three distinct subgroups. These are the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors, and kainate (KA) receptors. AMPA receptors are responsible for the majority of fast excitatory synaptic transmission, and their over-activation is potently excitotoxic. AMPA receptors conduct Na^+, K^+ and sometimes Ca^{2+} in response to ligand binding. The specific conducting and kinetic properties depend on the receptor subunit composition as each mature AMPA receptor is assembled from four individual subunits. The influx of ions causes a fast excitatory postsynaptic response, supports NMDA receptor activation, and the Ca^{2+} component can also activate second messenger pathways, including many protein kinases.

►Neurotransmitter Transporters
►Ionotropic Glutamate Receptors

Amphetamine

Amphetamine and related compounds are indirect acting sympathomimetic agents that are frequently abused due to their stimulant properties on the central nervous system. Amphetamines act by inducing the

biogenic amine transporters to reverse or efflux neurotransmitter into the synapse. This drug-induced nonvesicular release of dopamine, norepinephrine and serotonin is thought to be the major action associated with the amphetamines. Clinically, the amphetamines are effective in the treatment of narcolepsy and Attention Deficit Hyperactivity Disorder (ADHD).

►Psychostimulants
►Neurotensin/Neuromedin N

Amphipathic

Possessing both hydrophilic and hydrophobic properties.

►Bile Acids

AMPK

►AMP-activated Protein Kinase

Amyloid

This is an extracellular deposition of an "insoluble" protein, which has adopted a β-sheet structure due to an unknown event that induced misfolding of unstable proteins. The name "amyloid" has been given according to the amyloid staining properties, which are similar to carbohydrate deposits, e.g., amyloid can be identified with congo red and seen under polarized light (birefringence test).

►Alzheimer's Disease

Aβ Amyloid

Aβ amyloid is a 4 kD peptide which is the principle constituent of the Alzheimer amyloid found extracellularly in the brains of Alzheimer patients. Aβ amyloid is cleaved from a larger precursor protein, amyloid precursor protein (APP). APP is a member of a family that includes various proteins. It is present in the dendrites, cell bodies and axons of neurons. Neuronal APP is probably the source of most of the Aβ amyloid deposited in the central nervous system of Alzheimer patients. APP is cleaved by various proteases, α-, β- and γ-secretase. The endopeptidase α-secretase cleaves within the Aβ region of APP, resulting in the secretion of the extracellular domain of APP; hence, the cleavage does not produce the Aβ peptide. In contrast, the β-secretase and γ-secretase cleavages do result in production of the Aβ peptide.

►Alzheimer's Disease

Amyloid Precursor Protein

Amyloid precursor protein (APP) is the precursor of β-amyloid, the main component of senile plaques found in the brain of Alzheimer patients. The production of β-amyloid from APP to the cells from abnormal proteolytic cleavage of the amyloid precursor protein. Enzymes involved in this cleavage may be suitable targets for the therapy of Alzheimer's disease.

►Alzheimer's Disease

Amyotrophic Lateral Sclerosis (ALS)

ALS is a disorder of the motor neurons and the cortical neurons that provide their input. The disorder is characterized by rapidly progressive weakness and muscle atrophy. Most affected patients die of respiratory compromise and pneumonia after 2 to 3 years. There is prominent loss of motor neurons in the spinal cord and brainstem although the oculomotor neurons are spared. Large pyramidal motor neurons in layer V of motor cortex, which are the origin of the descending corticospinal tracts, are also lost.

It has been suggested that excitotoxic neurotransmitters such as glutamate participate in the death of motor neurons in ALS. This may be a consequence of diminished uptake of synaptic glutamate by an astroglial glutamate transporter, GLT1 (EAAT2) because overexpression of GLT1 in animals can delay the onset and the natural course of the disease.

ALS can run in families (10% of the cases) and superoxide dismutase 1 (SOD1) is an enzyme that has been found mutated in affected families; the majority of ALS patients, however, have normal SOD1. The importance of the SOD1 mutation is that transgenic mice expressing mutant human SOD1 develop a progressive degeneration of motor neurons that closely mimic the human disease. Disease causing mutations of SOD1, however, do not reduce the capacity of the enzyme to perform the catabolism of superoxide radicals.

►Neurotransmitter Transporters
►Neurotrophic Factors

Anabolic Steroids

Anabolic steroids increase muscle mass and strength. They are used by some athletes to enhance performance.

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Anaesthetics

►General Anaesthetics
►Local Anaesthetics

Analeptics

The term analeptics refers to convulsants and respiratory stimulants (i.e. central nervous system stimulants). They comprise a reverse group of agents (for example amphifinazole and doxapram (respiratory stimulants) and strychnine, biculline and picrotoxin). Analeptics are mainly experimental drugs. Only amphifinazole and doxapram are occasionally used for the treatment of acute ventilatory failure.

Analgesia

►Analgesics

Analgesics

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Synonyms

Pain medication

Definition

Analgesics interfere with the generation and/or transmission of impulses following noxious stimulation (►nociception) in the nervous system. This can occur at peripheral and/or central levels of the ►neuraxis. The therapeutic aim is to diminish the perception of ►pain.

Mechanisms of Action

Analgesics aim at modulating the generation of noxious chemicals (e.g., prostaglandins) or the activation of neuronal receptors/ion channels transducing/transmitting noxious stimuli (e.g., peptide-, kinin-, monoamine-receptors, Na^+ channels) [5]. Clinically used drugs include opioids, nonsteroidal antiinflammatory drugs (NSAIDs), serotoninergic compounds, antiepileptics, and antidepressants (Table 1) [1]. Adrenergic agonists, excitatory amino acid (e.g., *N*-methyl-D-aspartate; NMDA) receptor antagonists, neurotrophin antagonists, peptide antagonists, kinin receptor antagonists, cannabinoids, and ion channel (e.g., ►TRP, ►P2X) blockers are currently under investigation but are not used routinely yet. ►Local anaesthetics are used for local and regional anesthetic techniques. Mixed drugs (e.g., tramadol) combine various mechanisms [3].

Opioids

Opioids act on heptahelical ►G-protein-coupled receptors. Three types of opioid receptors (μ, δ, κ) have been cloned. Additional subtypes (e.g., μ_1, μ_2, δ_1, δ_2), possibly resulting from gene polymorphisms, splice variants or alternative processing have been proposed. Opioid receptors are localized and can be activated

Analgesics. Table 1 Analgesics

Drugs	Targets	Mechanisms	Functional consequences	Side effects
Opioids	G-protein coupled μ-, δ-, κ-receptors	↓ cAMP ↓ Ca^{2+} currents ↑ K^+ currents	↓ Excitability of peripheral and central neurons ↓ Release of excitatory neurotransmitters	μ, δ: sedation, nausea, euphoria/reward, respiratory depression, constipation κ: dysphoria/aversion, diuresis, sedation
NSAIDs	Cyclooxygenases (COX-1, COX-2)	↓ Prostaglandins ↓ Thromboxanes	↓ Sensitization of sensory neurons ↑ Inhibition of spinal neurons	Nonselective: gastrointestinal ulcers, perforation, bleeding, renal impairment COX-2: thrombosis, myocardial infarction, stroke
Serotonin agonists	G-protein coupled 5-HT receptors 5-HT_3: ion channels	↓ cAMP (5-HT_1) ↑ cAMP ($5\text{-HT}_{4–7}$) ↑ PLC (5-HT_2)	↓ Release of excitatory neuropeptides ↓ Neurogenic inflammation ↑ vasoconstriction	Myocardial infarction, stroke, peripheral vascular occlusion
Antiepileptics	Na^+, Ca^{2+} channels GABA receptors	↓ Na^+currents ↓ Ca^{2+} currents ↑ GABA receptor activity	↓ Excitability of peripheral and central neurons ↓ Release of excitatory neurotransmitters	Sedation, dizziness, cognitive impairment, ataxia, hepatotoxicity, thrombocytopenia
Antidepressants	Noradrenaline/5-HT transporters Na^+, K^+ channels	↓ Noradrenaline/5-HT reuptake ↓ Na^+ currents ↑ K^+ currents	↓ Excitability of peripheral and central neurons	Cardiac arrhythmia, myocardial infarction, sedation, nausea, dry mouth, constipation, dizziness, sleep disturbance, blurred vision

along all levels of the neuraxis including peripheral and central processes of primary sensory neurons (►nociceptors), spinal cord (interneurons, projection neurons), brainstem, midbrain, and cortex. All opioid receptors couple to G-proteins (mainly G_i/G_o) and subsequently inhibit adenylyl cyclase, decrease the conductance of voltage-gated ►Ca^{2+} channels and/or open rectifying ►potassium channels. These effects ultimately result in decreased neuronal activity. The prevention of Ca^{2+} influx inhibits the release of excitatory (pronociceptive) neurotransmitters. A prominent example is the suppression of ►tachykinin (substance P) release from primary sensory neurons both within the spinal cord and from their peripheral terminals within injured tissue. At the postsynaptic membrane, opioids produce hyperpolarization by opening K^+ channels, thereby preventing excitation or propagation of action potentials in second order projection neurons. In addition, opioids inhibit sensory neuron-specific tetrodotoxin-resistant ►Na^+-channels, ►TRPV1 channels and excitatory postsynaptic currents evoked by ►glutamate receptors (e.g., NMDA) in the spinal cord. The result is decreased transmission of nociceptive stimuli at all levels of the neuraxis and profoundly reduced perception of pain [2, 3]. Endogenous opioid receptor ligands are derived from the precursors proopiomelanocortin (encoding β-endorphin), proenkephalin (encoding Met-enkephalin and Leu-enkephalin) and prodynorphin (encoding dynorphins). These peptides contain the common Tyr-Gly-Gly-Phe-[Met/Leu] sequence at their amino terminals, known as the opioid motif. β-Endorphin and the enkephalins are potent antinociceptive agents acting at μ and δ receptors. Dynorphins can elicit both pro- and antinociceptive effects via κ-opioid and/or NMDA receptors. A fourth group of tetrapeptides (endomorphins) with yet unknown precursors do not contain the pan-opioid motif but bind to μ-receptors with high selectivity. Opioid peptides and receptors are expressed throughout the central and peripheral nervous system, in neuroendocrine tissues and in immune cells [3, 4].

Nonsteroidal Antiinflammatory Drugs

NSAIDs inhibit ►cyclooxygenases (COX), the enzymes that catalyze the transformation of arachidonic acid (a ubiquitous cell component generated from phospholipids) to prostaglandins and thromboxanes. Two isoforms, COX-1 and COX-2, are constitutively expressed in peripheral tissues and in the central nervous

system. In response to injury and inflammatory mediators, (e.g., ►cytokines, growth factors) both isoforms can be upregulated, resulting in increased concentrations of prostaglandins. In the periphery, prostaglandins (mainly PGE_2) sensitize nociceptors by phosphorylation of ion channels (e.g., Na^+, TRPV1) via ►EP receptor activation. As a result, nociceptors become more responsive to noxious mechanical (e.g., pressure, hollow organ distension), chemical (e.g., acidosis, ►bradykinin, ►neurotrophic factors) or thermal stimuli. In the spinal cord PGE_2 blocks glycinergic neuronal inhibition, enhances excitatory amino acid release, and depolarizes ascending neurons. These mechanisms facilitate the generation of impulses within nociceptors and their transmission through the spinal cord to higher brain areas [5]. By blocking one (selective COX-2 inhibitors) or both enzymes (nonselective NSAIDs) prostaglandin formation diminishes. Subsequently nociceptors become less responsive to noxious stimuli and spinal neurotransmission is attenuated [3].

Serotoninergic Drugs

►Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter found in the sympathetic nervous system, in the gastrointestinal tract, and in platelets. It acts on 5-HT receptors expressed at all levels of the neuraxis and on blood vessels. Within the dorsal horn of the spinal cord serotoninergic neurons contribute to endogenous pain inhibition. With the exception of $5\text{-}HT_3$ (a ligand-gated ion channel), 5-HT receptors are G-protein coupled receptors. $5\text{-}HT_{1B/1D}$ agonists (triptans) have been studied extensively and are effective against neurovascular (migraine, cluster) headaches. Migraine is thought to be related to the release of neuropeptides (e.g., ►calcitonin gene related peptide) from trigeminal sensory neurons innervating meningeal and intracranial blood vessels. This leads to vasodilation, an inflammatory reaction, and subsequent pain. Triptans inhibit neurogenic inflammation via $5\text{-}HT_{1D}$ receptors on trigeminal afferents, with possible additional sites of action on thalamic neurons and in the periaqueductal grey. The activation of vascular $5\text{-}HT_{1B}$ receptors constricts meningeal (and coronary) vessels. The latter effects have stimulated a search for nonvasoconstrictor approaches such as highly selective $5HT_{1D}$ and $5HT_{1F}$ agonists. However, none of them demonstrated clinical antimigraine effects so far [3].

Antiepileptic Drugs

Antiepileptics are used in ►neuropathic pain resulting from lesions to the peripheral (e.g., diabetes, herpes) or central nervous system (e.g., stroke). Such syndromes have been attributed to ectopic activity in sensitized nociceptors from regenerating nerve sprouts, recruitment of previously "silent" nociceptors, and/or spontaneous neuronal activity. This may result in sensitization of primary afferents and subsequent sensitization of second- and third order ascending neurons. Among the best studied mechanisms are the increased expression and trafficking of ion channels (e.g., Na^+, Ca^{2+}, TRP) and increased activity at ►glutamate (NMDA) receptor sites. The mechanisms of action of antiepileptics include neuronal membrane stabilization by blockage of pathologically active voltage-sensitive Na^+ channels (carbamazepine, phenytoin, lamotrigine, topiramate), blockage of voltage-dependent Ca^{2+} channels (gabapentin, pregabalin), inhibition of presynaptic release of excitatory neurotransmitters (gabapentin, lamotrigine) and enhancing the activity of γ-aminobutyric acid ►(GABA) receptors (topiramate) [3].

Antidepressants

Antidepressants are used in the treatment of neuropathic pain and headache. They include the classic tricyclic compounds and are divided into nonselective noradrenaline/5-HT reuptake inhibitors (e.g., amitriptyline, imipramine, clomipramine, venlafaxine), preferential noradrenaline reuptake inhibitors (e.g., desipramine, nortriptyline) and selective 5-HT reuptake inhibitors (e.g., citalopram, paroxetine, fluoxetine). The reuptake block leads to a stimulation of endogenous monoaminergic pain inhibition in the spinal cord and brain. In addition, tricyclics have NMDA receptor antagonist, endogenous opioid enhancing, Na^+ channel blocking, and K^+ channel opening effects which can suppress peripheral and central sensitization. Block of cardiac ion channels by tricyclics can lead to life-threatening arrhythmias. The selective 5-HT transporter inhibitors have a different side effect profile and are safer in cases of overdose [3].

Clinical Use and Side Effects

Analgesics are used in both acute and chronic pain. Whereas acute (e.g., postoperative, posttraumatic) pain is generally amenable to drug therapy, chronic pain is a complex disease in its own right and needs to be differentiated into malignant (cancer-related) and nonmalignant (e.g., musculoskeletal, ►neuropathic, inflammatory) pain. Acute and cancer-related pain is commonly treatable with opioids, NSAIDs, and/or local anesthetic blocks. Chronic nonmalignant pain requires a multidisciplinary approach encompassing various pharmacological and nonpharmacological (e.g., psychological, physiotherapeutic) treatment strategies. Various routes of drug administration (e.g., oral, intravenous, subcutaneous, intrathecal, ►epidural, topical, intraarticular, transnasal) are used, depending on the clinical circumstances. Local anesthetics are used topically and in regional (e.g., epidural) anesthetic techniques for the treatment of acute (e.g., associated with surgery, child birth) and some selected chronic pain syndromes.

Opioids

Opioids are the most effective drugs for severe acute and cancer-related chronic pain. They do not improve quality of life in chronic noncancer pain. The commonly available agents (e.g., morphine, codeine, methadone, fentanyl and its derivatives) are μ-agonists. Naloxone is a nonselective antagonist at all three receptors. Partial agonists must occupy a greater fraction of the available pool of functional receptors than full agonists to induce a response (e.g., analgesia) of equivalent magnitude. Mixed agonist/antagonists (e.g., buprenorphine, butorphanol, nalbuphine, pentazocine) may act as agonists at low doses and as antagonists (at the same or a different receptor) at higher doses. Such compounds typically exhibit ceiling effects for analgesia and they may elicit an acute ►withdrawal syndrome when administered together with a pure agonist. All three receptors (μ, δ, κ) mediate analgesia but differing side effects. μ-Receptors mediate respiratory depression, sedation, reward/euphoria, nausea, urinary retention, biliary spasm, and constipation. κ-Receptors mediate dysphoric, aversive, sedative, and diuretic effects, but do not mediate constipation. δ-Receptors mediate reward/euphoria and, to a lesser degree, respiratory depression and constipation. ►Tolerance and physical ►dependence occur with prolonged administration of pure agonists, and abrupt discontinuation or antagonist administration can result in a withdrawal syndrome [3]. Opioids are effective in the periphery (e.g., topical or intraarticular administration, particularly in inflamed tissue), at the spinal cord (intrathecal or epidural administration), and systemically (e.g., intravenous or oral administration). The clinical choice of a particular compound is based on pharmacokinetic considerations (route of administration, desired onset or duration, lipophilicity) and on side effects associated with the respective route of drug delivery. Dosages are dependent on patient characteristics, type of pain and route of administration. Systemically and spinally administered μ-opioids can produce similar side effects, depending on dosage and rostral/systemic redistribution. For intrathecal application lipophilic drugs are preferred because they are trapped in the spinal cord and less likely to migrate to the brain within the cerebrospinal fluid. Adverse side effects can be minimized by careful dose titration and close patient monitoring, or can be treated by comedication (e.g., laxatives) or naloxone. The peripheral application of small, systemically inactive doses is devoid of side effects. Current research aims at the development of opioids with restricted access to the brain [2, 4].

Nonsteroidal Antiinflammatory Drugs

Less severe pain states (e.g., arthritis, menstruation, headache, minor surgery) are commonly treated with nonselective NSAIDs (e.g., aspirin, ibuprofen, indomethacin, diclofenac). NSAIDs are mostly used orally. Some agents are available for parenteral, rectal, or topical application. Over-the-counter availability and self medication have led to frequent abuse and toxicity. Side effects have been attributed to COX-1 induced blockade of thromboxane production and impairment of platelet function (gastrointestinal and other bleeding disorders), decrease of tissue-protective prostaglandins (gastrointestinal ulcers, perforation), and decrease of renal vasodilatory prostaglandins (nephrotoxicity). The development of selective COX-2 inhibitors was driven by the assumption that COX-2 expression is selectively induced in inflamed tissue and that the constitutive tissue-protective COX-1 would be spared. It has now become clear that COX-2 expression is constitutive in many tissues (e.g., gastrointestinal epithelium, vascular endothelium, spinal cord) and COX-2 inhibition may exacerbate inflammation, impair ulcer healing, and decrease formation of vasoprotective prostacyclin. Selective COX-2 inhibitors confer an increased risk of thrombosis, myocardial infarction, hypertension, and stroke. Both classes of COX inhibitors can cause rare anaphylactic reactions. Acetaminophen (paracetamol) has relatively weak anti-inflammatory and antiplatelet activity. It is used for osteoarthritis, headache, and fever [1].

Serotoninergic Drugs

Triptans can be applied orally, subcutaneously, or transnasally and have been used in the treatment of migraine. All triptans narrow coronary arteries via 5-HT_{1B} receptors by up to 20% at clinical doses and should not be administered to patients with risk factors or manifest coronary, cerebrovascular, or peripheral vascular disease. Some triptans have the potential for significant drug–drug interactions (e.g., with monoamine oxidase inhibitors, propranolol, cimetidine, hepatic P450-metabolized medications, P-glycoprotein pump inhibitors). Rational use of triptans should be restricted to patients with disability associated with migraine [1].

Antiepileptic Drugs

Antiepileptics have been used for neuropathic pain and for migraine prophylaxis. They are frequently coadministered with antidepressants. The most common adverse effects are impaired mental (somnolence, dizziness, cognitive impairment, fatigue) and motor function (ataxia) which limits clinical use, particularly in elderly patients. Serious side effects have been reported, including hepatotoxicity, thrombocytopenia and life-threatening dermatologic and hematologic reactions. Plasma drug concentrations should be monitored [1].

Antidepressants

Antidepressants are used in neuropathic pain and migraine prophylaxis. Tricyclics require monitoring of plasma drug concentrations to achieve optimal effect

and avoid toxicity, unless sufficient pain relief is obtained with low doses (up to 75 mg/day of imipramine or amitriptyline). In patients with ischemic heart disease there may be increased mortality from sudden arrythmia, and in patients with recent myocardial infarction, arrythmia, or cardiac decompensation tricyclics should not be used at all. Tricyclics also block histamine, cholinergic and adrenergic receptor sites. Adverse events include sedation, nausea, dry mouth, constipation, dizziness, sleep disturbance, and blurred vision [1].

►Non-steroidal Anti-inflammatory Drugs
►Opioid Systems
►Local Anaesthetics
►Voltage-dependent Na^+ Channels

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Analogous Proteins

Two proteins with related folds but unrelated sequences are called analogous. During evolution, analogous proteins independently developed the same fold.

►Bioinformatics

Anandamide

A natural sythesised cannabinoid interacting with the cannabinoid receptor I and II. In addition, anandamide blocks receptor-independent all LVA-calcium channels.

►Voltage-dependent Ca^{2+} Channels
►Endocannabinoids

Anaphase Promoting Complex (APC)

The APC is a complex, which is activated during mitosis and initiates anaphase by targeting key cell cycle regulators for proteasomal degradation. As proteolysis is irreversible cell cycle progression cannot be reverted. Targets are mitotic cyclins and securin. Proteolysis of securin activates separase, which cleaves cohesins thereby allowing detachment of sister chromatids.

►Ubiquitin/Proteasome

Anaphylactic Shock

The term anaphylactic shock describes a severe generalized type I allergic reaction associated with cardiovascular shock, airway constriction and heart arrhythmias, which, if left untreated, may cause death.

►Allergy

Anchoring Protein

►Adaptor Proteins
►A Kinase Anchoring Proteins (AKAPs)

Andersen's Syndrome

Andersen's syndrome is a rare disorder characterized by periodic paralysis, cardiac arrhythmias, and dysmorphic features.

►K^+-Channels

Androgen

Androgens, represented by testosterone, are male sex hormones involved in reproduction, behavior, and bone and muscle growth.

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
►Selective Sex Steroid Receptor Modulators

Androgen Receptor

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Anemia, Macrocytic Hyperchromic

Macrocytic or magaloblastic anemia is caused by disturbances of DNA synthesis. It occurs, for example, in both folic acid and vitamin B12 deficiencies. Hematopoesis is slowed down due to reduced DNA synthesis and a reduced number of abnormally large (macrocytic) and hemaglobin-rich (hyperchromic) erythrocytes is released.

►Vitamin B12

Angel Dust

►Psychotomimetic Drugs

Angina Pectoris

A reversible attack of chest discomfort, usually caused by an imbalance between the oxygen demand of the working heart muscle and the insufficient supply through narrow, atherosclerotic coronary arteries.

►Atherosclerosis

Angiogenesis and Vascular Morphogenesis

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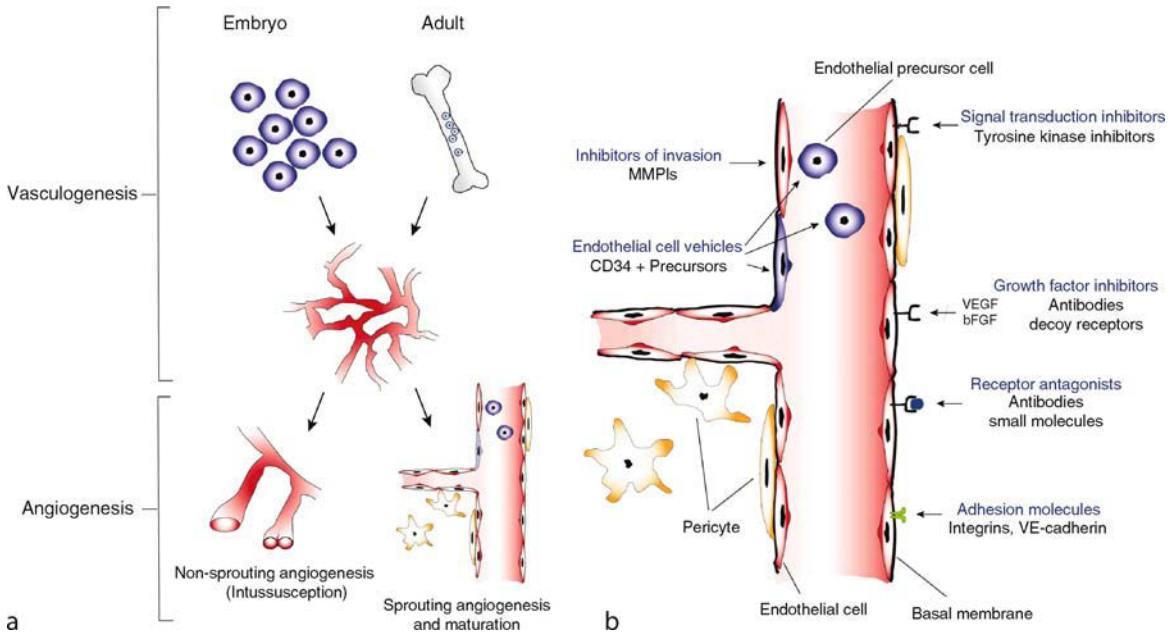
Definition

Angiogenesis and vascular morphogenesis comprise all mechanisms and processes that lead to the development of new blood vessels. These include vasculogenesis, sprouting and non-sprouting angiogenesis, vessel assembly and maturation, and vascular remodeling. The formation of new blood and lymphatic vessels takes place primarily during embryonic development and blood neo-vessel formation is physiologically limited to few organs in the healthy adult such as the female reproductive system. Pathologic angiogenesis is widespread and is associated with diseases as diverse as tumors, wound healing, inflammatory diseases, skin diseases, eye diseases, and joint diseases.

Basic Mechanisms

Vasculogenesis and Angiogenesis

Blood vessels arise during embryogenesis by vasculogenesis and angiogenesis (Fig. 1a). These two processes are tightly regulated and display common stimulatory and inhibitory pathways [1]. During vasculogenesis, endothelial cells differentiate from mesodermal origin while during angiogenesis endothelial cells are formed from preexistent ones. Establishment of the vascular system involves formation of the vitelline (yolk sac) vasculature, the allantois (precursor of the umbilical vasculature of the placenta) and the cardiovascular system that ultimately merge to form a continuous vascular system. Differentiation of vascular lineages is synchronized and organized in parallel to other embryonic lineages. Contribution from the other germ layers (ectoderm and endoderm) to vasculogenesis has not been reported even though recent evidence has demonstrated the contribution of ectodermal tissue as a source of vascular differentiating factors as well as a support to form vascular tubes. For instance, the contribution of the peripheral neural system to vascular development has recently been demonstrated. Nerve-derived ►vascular endothelial growth factor (VEGF) is necessary for arteriogenesis from a primitive capillary plexus. In a similar but inverse manner, the endothelium also appears to exert an active organ differentiation-inducing function, e.g., endothelial precursor cells have



Angiogenesis and Vascular Morphogenesis. Figure 1 Molecular mechanisms of vasculogenesis and angiogenesis (a) and the corresponding targets for therapeutic intervention. The primary formation of blood vessels occurs through mechanisms of vasculogenesis. Vasculogenesis refers to the formation of a vascular network from precursor cells (angioblasts) as it occurs developmentally by *in situ* differentiation or in the adult by distal recruitment of angioblastic stem cells from the bone marrow. The secondary level of vascular morphogenesis describes the angiogenic formation of blood vessels. Angiogenesis refers to the formation of vessels and vascular networks from preexisting vascular structures. This can occur through classical sprouting angiogenesis or through mechanisms of non-sprouting angiogenesis. The growing vascular network assembles and matures, eventually allowing directional blood flow. Pharmacological intervention of angiogenesis is based on different strategies interfering directly or indirectly with the endothelium as indicated in (b).

been shown to have an inducing effect on organ development in the pancreas and the liver.

The first stages of blood vessel formation involve the differentiation of mesodermal precursor cells into a plexus of angioblasts followed by their organization into angioblast cords taking the appearance of a hollow tube [2]. This first primitive vascular plexus expands by angiogenesis, the sprouting of new capillaries from preexisting vessels, and intussusception, a process during which interstitial tissue columns are inserted into the lumen of preexisting vessels (also termed non-sprouting angiogenesis; Fig. 1a). Sprouting and non-sprouting angiogenesis contribute to an increasing complexity of the growing vascular network. The network assembles and matures by the recruitment of pericytes and smooth muscle cells, eventually allowing the directional flow of blood. The morphogenic events leading to a mature vascular network involve several additional steps including vessel assembly, maturation, and acquisition of vessel identity and organotypic differentiation.

Vessel identity represents one of the major differentiation processes during blood vessel formation. Arteries and veins are structurally and functionally distinct. It was long assumed that arteries and veins differentiate in response to differing blood flow rates and pressure gradients. Yet, the recent identification of molecules with an arteriovenous asymmetric expression pattern (arterial markers: ▶ephrinB2, Notch 1, Delta-like 4 [Dll4], Connexin-40, Neuropilin-1, Bmx; venous markers: EphB4, Neuropilin-2) has paved the way for the genetic understanding of arteriovenous differentiation. Emerging is a concept of distinct transcriptional programs driving the differentiation of different endothelial lineages (arterial differentiation: sonic hedgehog → VEGF → VEGFR → FoxC1/FoxC2 → Notch → Notch downstream transcription factors [Hey1/Hey2 {mammals}; Gridlock {zebrafish}]; venous differentiation: transcription factor COUP-TFII [maintains venous differentiation by inhibiting Notch pathway; lymphatic differentiation: transcription factor Prox1).

Molecular Regulators of the Angiogenic Cascade

The supply of oxygen and nutrients is a critical determinant for mammalian cell survival. Therefore, cells are located within a distance of 100 μm to maximally 150 μm of blood vessels. Multicellular

organisms growing beyond this size must recruit new blood vessels in order to achieve growth by mechanisms of vasculogenesis and angiogenesis. The regulation of this process is tightly controlled by a balance of pro- and antiangiogenic molecules. To date, more than 20 stimulators and 20 inhibitors have been identified (Table 1) and the composition of the "angiogenic cocktail" has not been well defined for most situations involving angiogenesis. Of the many angioregulatory molecules, well-defined families of molecules, i.e., the

Angiogenesis and Vascular Morphogenesis. Table 1 Positive and negative endogenous regulators of angiogenesis (noncomprehensive list)

Stimulators	Inhibitors
Peptide growth factors	***Proteolytic peptides***
VEGF-A, -B, -C, -D, -E	Angiostatin (plasminogen fragment)
PlGF	Vasostatin (calreticulin fragment)
Ang-1, Ang-2, ANGPTL4	α6 IV NC1 domain (collagen α1 IV fragment)
FGF-1, -2	Endostatin (collagen α1 XVIII fragment)
PDGF-BB	Tumstatin (collagen α3 IV fragment)
EGF	Canstatin (collagen α2 IV fragment)
TGF-α, TGF-β	Arresten (collagen α1 IV fragment)
HGF	Restin (α1 Collagen IV NC1 fragment)
IGF-1, -2	Endorepellin (Perlecan fragment)
	Anastallin (fibronectin fragment)
	Antithrombin III fragment
Platelet-derived regulators	***Inhibitors of enzymatic activity***
PD-ECGF, TP	TIMP-1, -2, -3, -4
NPY	PAI-1, -2
Multifunctional cytokines/immune mediators	***Multifunctional cytokines/immune mediators***
TNF-α (low dose)	TNF-α (high dose)
Chemokines	***Chemokines***
MCP-1 (CCL2)	PF-4
IL-8 (CXCL8)	IP-10
Gro-α, -β, -γ, (CXCL1, 2, 3)	Gro-β
ENA-78 (CXCL5)	
NAP-2 (CXCL7)	
GCP-2 (CXCL2)	
Enzymes	***Extracellular matrix molecules***
Angiogenin (ribonuclease A homolog)	Thrombospondins
Hormones	Fibulin-5
Estrogens	***Hormones/metabolites***
Prostaglandin-E_1, -E_2	2-ME
Follistatin	Proliferin-related protein
Hyalorunan oligosaccharides	
Gangliosides	***Oligosaccharides***
Hematopoietic growth factors	Hyaluronan, HMW species
Erythropoietin	
G-CSF	
GM-CSF	

Abbreviations: VEGF, vascular endothelial growth factor; -A, -B, -C, -D, -E; PlGF, placenta growth factor; Ang, angiopoietin, ANGPTL, angiopoietin like FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; TNF, tumor necrosis factor; MCP, monocyte chemoattractant protein; IL, interleukin; PD-ECGF, platelet-derived endothelial cell growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; TIMP, tissue metalloproteinase inhibitor; PAI, plasminogen activator inhibitor; PF, platelet factor; IP-10, interferon-γ-inducible protein-10; 2-ME, 2-Methoxyestradiol; HMW, high molecular weight, TP, Thymidine Phosphorylase, NPY, neuropeptide Y.

VEGFs, the ►angiopoietins, the Ephrins and Notch molecules stand out as they act specifically or preferentially on the vascular system and have, thus, to be considered as key regulatory molecules of the angiogenic cascade [3].

The best characterized angiogenic signaling pathway is VEGF. This has been unambiguously demonstrated through gene targeting experiments in mice which have shown that disruption of just one VEGF allele is not compatible with life and leads to early embryonic lethality. VEGF, now designated VEGF-A, is a member of a family of growth factors comprising VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E [4]. VEGF molecules exert their vascular specificity through the limited expression of their corresponding receptors, VEGFR-1, VEGFR-2, and VEGFR-3 which are differentially expressed by blood and lymphatic endothelial cells, respectively. Additionally, ►placenta growth factor (PlGF) is a VEGF related molecule (PlGF-1 and PlGF-2) which exclusively binds to VEGFR-1. PlGF is dispensable for embryonic and reproductive angiogenesis but plays important roles in pathological angiogenesis as it relates to tumor growth or cardiac ischemia. Despite its requisite role in angiogenesis, VEGF must act in concert with other growth factors. The angiopoietins (Ang-1 and Ang-2) have been identified as ligands for the endothelial cell receptor tyrosine kinase Tie-2. Gene ablation experiments in mice suggested a role of the Ang/Tie-2 system in vessel remodeling and stabilization of the growing neo-blood and -lymphatic vasculature. There are now four members of the angiopoietin (Ang) family, although Ang-3 and Ang-4 may represent widely diverged counterparts of the same gene locus in mouse and man. All of the known angiopoietins bind to Tie-2 and no ligand has yet been solidly established for the second Tie receptor, Tie-1.

More recently, the role of axon-guidance receptors and ligands during developmental angiogenesis has emerged as a focus in the field of angiogenesis research. These families of molecules involve Semaphorin/Neuropilin/Plexin, ephrin/Eph, Slit/robo, and Netrin/UNC. The ►Eph receptor tyrosine kinases comprise the largest known family of receptor tyrosine kinase receptors and interact in a specific, yet somewhat promiscuous manner with their corresponding ephrin ligands. Although initially characterized as regulators of axonal outgrowth in the nervous system, gene inactivation experiments in mice have revealed key roles for ephrinB2 and its EphB2, EphB3, and EphB4 receptors during vascular development. Mouse embryos lacking ephrinB2 or EphB4 (or the combination of EphB2 and EphB3) suffer dramatic defects in early angiogenic remodeling that are similar to those seen in mice lacking Ang-1 or Tie-2. Moreover, ephrinB2 and EphB4 display remarkable asymmetric arterio-venous expression patterns, with ephrinB2 selectively marking arterial vessels and EphB4 preferentially being expressed by venous endothelial cells. More recently, several reports have associated class A Eph receptor signaling with tumor-associated angiogenesis. Another family of molecules, Notch receptors and their ligands Delta-like and jagged, have been associated to venous and arterial specification during development and to also play critical roles during tumor angiogenesis. Dll4-deficient mice display the same vascular haploinsufficiency as VEGF with embryonic lethality of heterozygously targeted mice. Dll4 binds to Notch 1 and 4 receptors inducing a cascade of events leading to the up-regulation of the transcriptional repressors, Hey1 and Hey2. Dll4 expression is strongly up-regulated during angiogenesis. Its expression is induced by VEGF and it acts as a negative feedback regulator that restrains vascular sprouting and branching. Consistent with this role, the genetic deletion or manipulatory inhibition of Dll4 results in excessive, nonproductive angiogenesis and is therefore considered and explored as an attractive tumor angiogenesis target.

Angiogenic activation induces a specific gene expression program in endothelial cells enabling the cells to execute the complex molecular tasks required to grow new blood vessels. The invasive ingrowth of angiogenic endothelial cells involves a set of adhesion molecules including the integrin heterodimers $\alpha_v\beta_3$ and $\alpha_v\beta_5$ as well as the homotypic endothelial cell-specific adhesion molecule VE-cadherin. Likewise, sprouting endothelial cells display a shift of their proteolytic balance toward a proinvasive phenotype, involving the plasminogen activator (tPA and uPA) and plasminogen activator inhibitor system (PAI). Angiogenic endothelial cells deposit their own extracellular matrix, rearrange their cytoskeleton, and activate their proliferative machinery. All of these molecular systems are extensively being explored to therapeutically interfere with the angiogenic process.

Angiogenesis in Pathological Conditions

Abnormal vessel growth is involved in numerous pathological conditions. Pioneering work more than 35 years ago showed that the growth of solid tumors is critically dependent on the supply with new blood vessels. While some of this supply may be provided by mechanisms of vessel cooption, the process whereby a growing tumor is preying on the preexistent vasculature, the primary mechanism of tumor vascularization appears to be the angiogenic growth of blood vessels from the tumor neighboring blood vessels. Tumor cells release proangiogenic growth factors, such as VEGF, which diffuse into nearby tissues and bind and activate receptors on endothelial cells of preexisting blood vessels. Secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs) results in the

degradation of basement membrane and extracellular matrix components, allowing endothelial cells to invade and proliferate, and form new lumen containing vessels. Tumor angiogenesis involves the specific molecular regulators of the angiogenic cascade as they have been identified during developmental or reproductive angiogenesis. However, tumor angiogenesis also involves additional mechanisms that include the pleiotrophic angiogenic growth factors, for example as a consequence of the inflammatory response usually associated with tumor growth.

In addition to tumor angiogenesis, an increasing list of diseases is now recognized to critically depend on either increased or reduced angiogenesis. For example, increased angiogenesis occurs during diabetic retinopathy, macula degeneration, arthritic joint diseases, and hyperproliferative skin diseases. In turn, reduced angiogenesis may have a negative impact on wound healing and the regenerative processes associated with ischemic diseases as they occur in the heart or during peripheral limb ischemia.

Pharmacological Intervention

Both angio-inhibitory as well as angio-stimulatory therapies are presently being explored extensively for a number of indications [5]. Angio-inhibitory therapies are being developed most intensely to therapeutically interfere with tumor angiogenesis and tumor growth (Fig. 1b). Other major antiangiogenic therapies that are currently in clinical development target retinal diseases (diabetic retinopathy, macular degeneration), joint diseases (arthritis), as well as hyperproliferative skin diseases (psoriasis). In February 2004, the US Food and Drug Administration approved Bevacizumab, a humanized anti-VEGF-A monoclonal antibody for the treatment of metastatic colorectal cancer in combination with 5-fluorouracil-based chemotherapy. In December 2004, the FDA approved pegaptinib, an aptamer that blocks VEGF-A_{165}, for the treatment of the neovascular form of age-related macular degeneration. In turn, angio-stimulatory therapies are being developed to stimulate wound healing angiogenesis, peripheral limb ischemia, and arteriogenic growth of collateral vessels during cardiac ischemia.

Antiangiogenic Tumor Targeting

Antiangiogenic tumor targeting is conceptually a particularly attractive therapeutic avenue for a number of reasons: (i) As an oncofetal mechanism that is mostly downregulated in the healthy adult, targeting of angiogenesis should lead to minimal side effects even after prolonged treatment, (ii) tumor-associated angiogenesis is a physiological host mechanism and its pharmacological inhibition should, consequently, not lead to the development of resistance, (iii) each tumor capillary potentially supplies hundreds of tumor cells and the targeting of the tumor vasculature should, thus, lead to a potentiation of the antitumorigenic effect, and (iv) in contrast to the interstitial location of tumor cells, direct contact of the vasculature to the circulation allows efficient access of therapeutic agents.

A number of approaches have been taken to inhibit tumor angiogenesis and other diseases involving angiogenesis (Table 2). Pharmacological inhibition of angiogenesis is aimed at interfering with the angiogenic cascade or the immature neovasculature. Pharmacological agents may be synthetic or semisynthetic substances, endogenous inhibitors of angiogenesis, or biological antagonists of the angiogenic cascade. In contrast, vascular targeting is aimed at utilizing specific molecular determinants of the neovasculature for the delivery of a biological, chemical, or physical activity that will then locally act angiocidal or tumoricidal. Moreover, in addition to their role on tumor cells, chemotherapeutic agents contribute may exert an antiangiogenic effect by targeting bone-marrow-derived proangiogenic cells, dividing endothelial cells from sprouting vessels, and endothelial cell progenitor incorporation to the lumen of growing vessels.

A comprehensive website summarizing the status of tumor antiangiogenic compounds in various stages of clinical trial is maintained by the National Cancer Institute at: ►www.cancer.gov/clinicaltrials/developments/anti-angio-table. Following is a survey of the most important substances currently in clinical development.

Specific Synthetic and Biological Antagonists of the Angiogenic Cascade

Specific inhibition of any of the key regulators of the angiogenic cascade is one of the most specific and selective ways to interfere with angiogenesis. A number of experimental strategies have been taken to interfere with the interaction of VEGF with its receptors. These include antisenses, ribozymes (i.e., Angiozyme in clinical trial Phase I) and antibody approaches to inhibit VEGF, the development of small molecular weight antagonists to the VEGF receptors, as well as the use of soluble VEGF receptors. A number of new generation small molecular weight VEGF receptor antagonists are rapidly proceeding in various stages of clinical trials (Table 3).

One of the most important steps to block angiogenesis has been made in targeting VEGF-A using the humanized antibody, Bevacizumab (Avastin) or a chimeric, soluble VEGF receptor ("VEGF-trap") which is also undergoing clinical development as an anticancer agent. Bevacizumab is a nonimmunogenic, 93% humanized murine monoclonal antibody that binds all VEGF-A isoforms. Bevacizumab has been clinically approved in 2004 in combination with chemotherapy

Angiogenesis and Vascular Morphogenesis. Table 2 Antiangiogenic therapeutic strategies (noncomprehensive list)

Substance	Mechanism
Biological antagonists	
VEGF inhibitors	Humanized neutralizing antibodies, antisense oligonucleotides, siRNA, aptamers
VEGF receptor blockers	Small receptor tyrosine kinase antagonists
VEGF-trap	Inhibition with soluble forms of VEGF-R1, VEGF-R2
$\alpha_v\beta_3$ integrin antagonists	Induce angiogenic endothelial cell apoptosis
Endogenous inhibitors	
Angiostatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Endostatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Vasostatin	Inhibits EC proliferation, adhesion to laminin and induces EC apoptosis
Tumstatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Canstatin	Inhibits EC proliferation, migration, and induces EC apoptosis
Arresten	Inhibits EC proliferation, migration, and induces EC apoptosis
Restin (α1 Collagen IV NC1 fragment)	Inhibits EC proliferation and induces EC apoptosis
Maspin (Mammary serine protease inhibitor)	Induces EC apoptosis
Vascular Endothelial Growth Inhibitor (Protein sharing homology with TNF-α)	Inhibits EC proliferation
Derivative of prolactin	Inhibits EC proliferation and induces EC apoptosis
Proliferin-related protein (PRP) (16 kDa fragment of the prolactin)	Inhibits EC proliferation and induces EC apoptosis
Pigment epithelial-derived factor	Induces EC apoptosis
2-methoxyestradiol (2ME2)	Induces EC apoptosis
IL-12	Modulates angiogenic factors
IL-18	Modulates angiogenic factors
IL-24	Inhibits EC differentiation and migration induced by VEGF and bFGF
Interferons	Inhibits EC proliferation and induces EC apoptosis
NK4 (4-kringle domains of HGF)	Inhibits EC differentiation and migration induced by VEGF
Thrombospondins	Inhibits EC proliferation, migration and induces EC apoptosis by binding to endothelial CD36
Synthetic/semisynthetic inhibitors	
Carboxyamidotriazole	Calcium channel blocker
CM101	Analog of group B streptococcus toxin, binds to tumor endothelium, induces inflammation
Marimastat	Metalloproteinase inhibitor, inhibits endothelial cell invasion
Pentosan polysulfate	Inhibits heparin-binding growth factors
TNP470	Analog of fumagillin, inhibits cell migration and proliferation
Thalidomide	Polycyclic teratogen, antiangiogenic mechanism unknown
Vascular targeting	
Regional TNF-α therapy	Isolated limb perfusion to target in transit metastases
Antibody targeting	Use of mono-and bispecific antibodies to target components of angiogenic blood vessels (e.g., VEGF receptors, endoglin, L19 antigen) to deliver specific angio- and/or tumoricidal activity
Vascular gene therapy	Transfer of dominant-negative receptors or suicide genes under the control of angiogenic endothelial cell specific promoters

for the first line treatment of advanced colorectal tumors. Phase III clinical trials have since been successfully completed for other tumors, including mammary tumors and lung tumors.

VEGF-Trap is a protein-based product candidate designed to bind all forms of VEGF and the related PlGF, and prevents their interaction with cell surface receptors. VEGF Trap is being pursued in phase II

Angiogenesis and Vascular Morphogenesis. Table 3 Antiangiogenic drugs (noncomprehensive list)

Target	Drug
Extracellular matrix	Dalteparin
Endothelial cells	ABT-510
	CNGRC peptide TNF alpha conjugate (NGR-TNF)
	Combretastatin A4 Phosphate
	Dimethylxanthenone Acetic Acide
	Lenalidomide
	LY317615 (Enzastaurin)
	PPI-2458
	soy isoflavone (Genistein; soy protein isolate)
	Tamoxifen Citrate
Activators of angiogenesis	ADH-1 (Exherin™)
	Cetuximab (Erbitux™)
	AG-013736
	AMG-706
	Neovastat (AE-941)
	Anti-VEGF Antibody (Bevacizumab; Avastin™; Lucentis™)
	VEGF-Trap
	AZD2171
	Bay 43-9006 (Sorafenib tosylate; Nexavar™)
	BMS-582664
	CHIR-265
	GW786034 (Pazopanib)
	PI-88
	PTK787/ZK 222584 (Vatalanib)
	RAD001 (Everolimus)
	Suramin
	SU11248 (Sunitinib malate; Sutent™)
	SU6668
	XL184
	ZD6474
	ZD1839 (Gefitinib; Iressa)
Endothelial-specific integrin/survival signaling	ATN-161
	EMD 121974 (Cilengitide)
Nonspecific mechanism of action	Celecoxib
	Thalidomide

The table lists drugs with antiangiogenic activity that are in clinical development. Some of the compounds are already clinically approved as specific antiangiogenic drugs (e.g., Bevacizumab [Avastin] or Sunitinib [Sutent]. Others are approved as antiangiogenic as well as antitumorigenic drugs (e.g., Sorafenib [Nexavar]. A third group of drugs is approved as tumorigenic drugs, but is also well established to have antiangiogenic activity (e.g., Cetiximab [Erbitus] or Tamoxifen). Most of the listed compounds are in various phases of clinical trials. Given the rapid pace of clinical development, the reader is encouraged to confirm the actual status of a listed compound through an internet search.

studies including advanced ovarian cancer and non-small cell lung adenocarcinoma. In addition, five phase II single-agent studies have been started for relapsed/refractory multiple myeloma, metastatic colorectal cancer, recurrent or metastatic cancer of the urothelium, locally advanced or metastatic gynecological soft tissue sarcomas and recurrent malignant gliomas.

Paralleling the clinical implementation of large molecular weight VEGF inhibitors (antibodies, ligand traps), the first small molecular weight VEGF receptor antagonists have received clinical approval. Sunitinib (Sutent) is a combined VEGF receptor and PDGF receptor inhibitor and thereby blocks angiogenesis and vessel maturation. Sorafenib (Nexavar) targets the VEGF receptors as well as the tumor target c-raf

and acts thereby as a combined antiangiogenic and antitumorigenic drug.

The different VEGF/VEGFR targeting drugs have somewhat varying clinical efficacy. Yet, hitherto approved antiangiogenic drugs in combination with chemotherapy lead on average to an approximately 25% extension of mean life expectancy compared with chemotherapy alone. Depending on the tumor type, this translates in absolute figures to "only" 4.5 to only 9 months. Yet, it needs to be considered that the clinical data are not parametrically distributed, i.e., while some patients respond very beneficially to antiangiogenic treatments, many show little or no clinical response. This reflects the lack of stratifying diagnostic and prognostic procedures that would be necessary to identify those patients that would benefit most from an antiangiogenic intervention (note: "*a targeted therapy requires targeted diagnostic and prognostic procedures!*")

VEGF and VEGFR blockers specifically interfere with VEGF/VEGFR signaling. Yet, their functional mechanism of action is not well understood. VEGF/VEGFR inhibition blocks angiogenesis and is thereby supposed to starve tumors to death. Yet, more recent work rather suggests that VEGF/VEGFR blockade prunes the immature tumor neovasculature leaving a network of more mature vessels that actually facilitates a more regular blood flow. This "normalization" effect supposedly facilitates better access of chemotherapeutic drugs to the tumor which would explain the empirically determined good synergy of antiangiogenic and chemotherapeutic treatments and the lack of efficacy of antiangiogenic monotherapies. However, the mechanistic dissociation of antiangiogenic tumor starvation versus tumor vessel normalization is subject of intense controversy in the field and focus of much ongoing experimental work.

Inhibitors of the Angiopoietin/Tie-2 and Ephrin/Eph systems are in preclinical development which parallels the biological target validation of these molecules. As vascular assembly, maturation, and homeostasis regulating molecules, therapeutic interference with these molecular systems may hold promise for a number of vascular indications.

Interference with specific cell–cell and cell–matrix adhesion mechanisms is another rapidly advancing approach to therapeutically interfere with angiogenesis. Antagonistic antibodies (Vitaxin) to the integrin heterodimer $\alpha_v\beta_3$ have been shown to act on the blood vessels of tumors but not on the resting organ vasculature. Vitaxin demonstrated some promise in ►Phase II clinical trials.

The growing list of endogenous inhibitors of angiogenesis holds great promise for therapeutic applications (Table 2). Substances most advanced in clinical development include Endostatin, Angiostatin, Interleukin-12, Thrombospondin, and Tumstatin. As endogenous substances, these molecules have a long half life in the plasma and are, thus, particularly attractive for long-term treatments. Some of them, like Endostatin and Angiostatin, are the proteolytically generated fragments of larger molecules. Endostatin is a 20 kDa C-terminal fragment of collagen XVIII and specifically inhibits endothelial cell proliferation, angiogenesis, and tumor growth. Primary tumors may regress to dormant microscopic lesions. Furthermore, the concept of a dormancy therapy is being extended to Endostatin using cycles of therapy. Angiostatin, a 38 kDa internal domain of plasminogen, is a circulating endogenous protein that supposedly binds ATP synthase on the surface of endothelial cells. It thereby induces endothelial and tumor cell apoptosis, and inhibits endothelial cell migration and tubule formation. Yet, it does not appear to affect growth-factor-induced signal transduction. Angiostatin inhibits matrix-enhanced plasminogen activation, which, in part accounts for its angio-suppressive and anti-invasive properties. Of great interest, Angiostatin is generated by free sulfhydryl donors (e.g., d-penicillamine and captopril) that may partially explain their angio-suppressive properties. Taken together, the clinical development of endogenous angiogenesis inhibitors is proceeding slowly and the results of hitherto pursued clinical trials were not particularly encouraging. It may well emerge that endogenous inhibitors of angiogenesis have little short term interventional therapeutic potential. Yet, they may be very effective preventive drugs which may conceptually actually reflect much better their physiological roles.

Nonspecific Synthetic and Biological Angiogenesis Inhibitors

Systematic screening experiments have identified more than 100 synthetic compounds with potent antiangiogenic activity. The mode of action for most of these molecules is not well understood, but some of the 40 compounds are well advanced in clinical trials (Table 3). The first substance to have entered clinical trials was the Fumagillin-derivative AGM 1470. Fumagillin is an antibiotic which inhibits bFGF- and PDGF-induced endothelial cell proliferation. The mechanism of action of AGM 1470 is poorly understood, but it was shown that it binds and inhibits the metalloprotease methionine aminopeptidase (MetAp-2).

Other antibiotics with antiangiogenic activity are minocycline and herbimycin A. Carboxyamidotriazole (CAI) inhibits the calcium influx into cells and suppresses the proliferation of endothelial cells. It inhibits angiogenesis and metastasis, but it is not an endothelial cell-specific substance. Similarly, the metalloproteinase have long been considered as promising antiangiogenic drugs. Yet, the field is moving slowly following the failure of some MMP inhibitors in advanced clinical trials.

Some natural compounds have also demonstrated antiangiogenic activities. Neovastat (Æ-941) is a naturally occurring (shark cartilage extract), oral agent that shows angio-suppressive and anti-MMP activities in vitro and in the chorioallantoic membrane (CAM) assay. Squalamine (MSI 1256 F), originally derived from the liver of a dogfish shark, is a novel noncytotoxic aminosterol with potent antiangiogenic properties in vivo and in vitro. Squalamine prevents the neovascularization of tumors by suppressing endothelial cell migration and proliferation. Combretastatins are small organic molecules found in the bark of the African bush willow, the *Combretum caffrum*. Combretastatins not only suppress proliferating endothelium, but also specifically target tumor endothelium. The combretastatin A-4 prodrug is a derivative of combretastatin, which is activated by a phosphatase selectively amplified in proliferating endothelial cells. Combretastatin A-4 induces apoptosis in human endothelial cells. In tumor-bearing mice, combretastatin A-4 significantly enhanced the antitumor effects of radiation therapy.

Vascular Targeting

The goal of vascular targeting is to utilize specific molecular determinants of angiogenic endothelium to deliver substances or activities that destroy the vasculature. Unlike antiangiogenic drugs that inhibit the formation of neo-vessels, vascular targeting agents (VTAs) occlude the preexisting blood vessels of tumors to cause tumor cell death from ischemia and extensive hemorrhagic necrosis. VTAs can indirectly kill tumor cells that are resistant to conventional antiproliferative cancer therapies, i.e., cells in areas distant from blood vessels where drug penetration is poor, and hypoxia can lead to radiation and drug resistance. There are broadly two types of VTAs, small molecules and ligand-based, which are grouped together, because they both cause acute vascular shutdown in tumors leading to massive necrosis. The small molecules include tubulin-destabilizing drugs, combretastatin A-4 disodium-phosphate, ZD6126, AVE8062, and Oxi 4503, and the flavonoid, DMXAA. Ligand-based VTAs use antibodies, peptides, or growth factors that bind selectively to tumor versus normal vessels to target tumors with agents that occlude blood vessels. The ligand-based VTAs include fusion proteins (e.g., vascular endothelial growth factor linked to the plant toxin gelonin), immunotoxins (e.g., monoclonal antibodies to endoglin conjugated to ricin A), antibodies linked to cytokines, liposomally encapsulated drugs, and gene therapy approaches.

Proangiogenic Therapies

Antiangiogenesis research has driven the field. Yet, there are a number of indications which may benefit from an induction of angiogenesis, including wound healing, cardiac ischemia, and peripheral limb ischemia. Various approaches have been taken to therapeutically deliver angiogenic cytokines such as VEGF and FGF-2. These include the local administration of recombinant proteins and gene therapeutic delivery of angiogenic cytokines. Individual cytokine therapy may have limitations as it may be capable of inducing a neovascular response, but it may not induce the growth of a patent neovascular network that is stable for prolonged periods of time. This notion has led to alternative strategies aimed at inducing the complex endogenous angiogenic program and not just a single cytokine. For example, experiments are underway to locally induce hypoxia-inducible factor-1 (HIF-1), a key regulator of the hypoxia response program which is able to control the complex endogenous program of angiogenesis induction. Several angiogenic factors (VEGF-A, VEGF-C, FGF-1, FGF-2 and FGF-4) have been tested in patients with myocardial or limb ischemia.

Direct laser-assisted myocardial revascularization (DMR) is an approved technique in the US, Europe, and parts of Asia to create numerous myocardial channels. This results in the induction of a massive inflammatory reaction, which in turn induces angiogenesis. The other FDA-approved pro-angiogenic therapy is the use of recombinant human platelet-derived growth factor (Regranex) for use in the treatment of diabetic neuropathic foot ulcers.

►Vascular Indothelial Growth Factor
►Matrix Metalloproteinases

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Angioplasty

A percutaneous catheter procedure that inflates a balloon in areas of narrowing (stenosis) in arteries.

►Atherosclerosis

Angiopoietins

Angiopoietins are growth factor ligands of the receptor tyrosine kinase Tie-2 which are critical regulators of vascular assembly and differentiation.

►Angiogenesis and Vascular Morphogenesis

Angiotensin Converting Enzyme

Synonyms

ACE

Definition

Angiotensin converting enzyme (ACE) is identical to kininase II. It is an essential component of both the renin–angiotensin system and the kallikrein–kinin system. Three different forms of ACE are known. It exists as a membrane bound protein with a molecular weight of 150–180 kD, anchored to the cytoplasmatic membrane of endothelial cells and as a circulating protein of similar size. The enzyme consists of two highly homologous lobes with an active site in each lobe. Interestingly, although still of uncertain consequence, the active sites exhibit different catalytic profiles and different affinities for ACE inhibitors. A third, smaller form of ACE (90 kD) with only one active site is expressed in mature germ cells.

The substrate specificity of ACE is low. ACE cleaves a variety of pairs of amino acids from the carboxy-terminal part of several peptide substrates. The conversion of ANG I to ANG II and the degradation of bradykinin to inactive fragments are considered the most important functions of ACE. Both peptides have profound impact on the cardiovascular system and beyond. ACE is thus an important target for ACE inhibitors. These compounds are frequently and efficiently used in the treatment of hypertension and cardiac failure.

►ACE Inhibitors

Angiotensin Converting Enzyme-2 (ACE2)

ACE2 is the closest human homologue of angiotensin converting enzyme is a type I integral membrane protein that hydrolyses dynorphin A (1–13), apelin-13, apelin-36 and desArg(9) bradykinin.

►Apelins

Angiotensin II Receptor-like 1

►Apelins

Angiotensin Receptors

Angiotensin receptors mediate the effects of angiotensin (ANG) II, the effector peptide of the renin–angiotensin system. Two receptors termed AT1 and AT2 have been characterized in detail. Both receptors belong to the super family of G-protein coupled receptors. Most of the cardiovascular functions of ANG II are mediated through the AT1 receptor. This receptor is usually Gq/11-coupled and its activation leads to intracellular calcium surges and protein kinase C activation. Downstream of the G-protein activation, small GTP-binding proteins such as RAS and RHOA and tyrosine kinase cascades are activated. These include members of the MAP-Kinase and JAK/STAT pathways. Finally transcription factors, such as AP-1, NF-κ-B, and the STATs (signal transducer and activators of transcription) are activated, which initiates the expression of growth-related genes and/or is involved in inflammatory processes. This explains the effects of ANG II on growth, proliferation, and its assumed role in inflammation. The functions of the AT2 receptor are still a matter of debate. The AT2 receptor is probably involved in differentiation processes, inhibits proliferation, and induces apoptosis, and thus may partially counteract some effects of AT1 receptor activation. It is expressed during embryonic development in a tightly controlled manner. In adults it is expressed in adrenal gland and ovary and its expression is induced during inflammatory processes and tissue damage. At present, there are no drugs available, which specifically inhibit or stimulate the AT2 receptor.

►Renin–Angiotensin–Aldosterone System
►ACE Inhibitors
►Nuclear Factor-κB

Angiotensinogen

►Renin–Angiotensin–Aldosterone System
►ACE Inhibitors

Anion Exchange Resin

Anion exchange resins are basic polymers with a high affinity for anions. Because different anions compete for binding to them, they can be used to sequester anions. Clinically used anion exchange resins such as cholestyramine are used to sequester bile acids in the intestine, thereby preventing their reabsorption. As a consequence, the absorption of exogenous cholesterol is decreased. The accompanying increase in low density lipoprotein (LDL)-receptors leads to the removal of LDL from the blood and, thereby, to a reduction of LDL cholesterol. This effect underlies the use of cholestyramine in the treatment of hyperlipidaemia.

►HMG-CoA-reductase-inhibitors

Ankyrin Repeat

The ankyrin repeat motif is one of the most common protein–protein interaction domains. Ankyrin repeats are modules of about 33 amino acids repeated in tandem. They are found in a large number of proteins with diverse cellular functions such as transcriptional regulators, signal transducers, cell-cycle regulators, and cytoskeletal proteins.

►Nuclear Factor Kappa B
►Adaptor Proteins

Annexins

Annexins form an evolutionary conserved family of Ca^{2+} and phospholipid binding proteins implicated in membrane trafficking and the regulation of Ca^{2+} currents across membranes.

►Ca^{2+}-Binding Proteins

Anomalous Rectifiers

►Inward Rectifier K^+ Channels

Anorexigenic

Appetite-suppressing. Neuropeptide modulators and gut hormones with anorexigenic effects are α-melanocortin-stimulating hormone (α-MSH), cocaine- and amphetamine-regulated transcript (CART), glucagon-like peptide-1 (GLP-1), leptin, insulin, oxyntomodulin, pancreatic peptide PP, peptide YY and $PYY_{3–36}$, and others.

►Appetite Control
►Anti-Obesity Drugs

Antacids

Antacids are neutralizing agents. Examples are magnesium hydroxide, magnesium trisylicate and aluminium hydroxide. Prior to the introduction of histamine-H_2 receptor antagonists and proton pump inhibitors, they were the standard drugs for the treatment of duodenal/peptic ulcers. Today their clinical use is limited to the treatment of dyspepsia and the symptomatic relieve for patients with peptic ulcers.

►Histaminergic System
►Proton Pump Inhibitors and Acid Pump Antagonists

Antagonist

Antagonists are natural or synthetic ligands that bind to a receptor but do not cause a downstream signal trasduction process. Antagonists that bind to the same ligand-binding site than the respective agonist act as competitive antagonists. Antagonists (or blockers) that bind to another binding site and exert their pathway-disrupting activity by allosteric effects are termed

noncompetitive antagonists. Antagonists are commonly synthetic or semisynthetic compounds and can be used as drugs in clinical or experimental settings.

►Drug–Receptor Interaction
►Transmembrane Signaling
►G-protein–coupled Receptors
►Dopamine System
►Adenosine Receptors
►Chemokine Receptors
►Endocannabinoids
►Nuclear Receptors
►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
►Selective Sex Steroid Receptor Modulators

Anterograde Amnesia

Often referred to as "short-term memory loss", this form of amnesia results in the inability to transfer new events to long term memory. The sufferer will still be able to recall older memories but will not be able to remember recent events once attention has been switched to something else.

►Sleep

Anthelminthic Drugs

Anthelminthic drugs are used for the treatment of worm infections. They represent a small but diverse group of drugs with regard to both their chemical structure and their mechanism of action. Like antimicrobial agents they are effective against certain types of worm and ineffective against others. The benzimidazoles (mebendazole, thiabendazole and albendazole) are broad-spectrum agents and the main group of anthelminthics used in the clinic. They induce multiple biochemical changes. However, their main mechanism of action appears to be inhibition of microtubule formation by binding to free parasitic β-tubulin. The spectrum of worms includes nematodes (e.g. the common round worm and the worm causing trichiniasis, Trichinella spiralis) and some cestodes. The drug of choice for the treatment of river blindness (caused by Onchocerca volvulus) is ivermectin. Trematodes (e.g. Schistosoma species causing bilharzia) are sensitive to praziquantel.

Anthracyclins

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Synonyms

Anthraquinones; Antibiotics; Cytotoxics

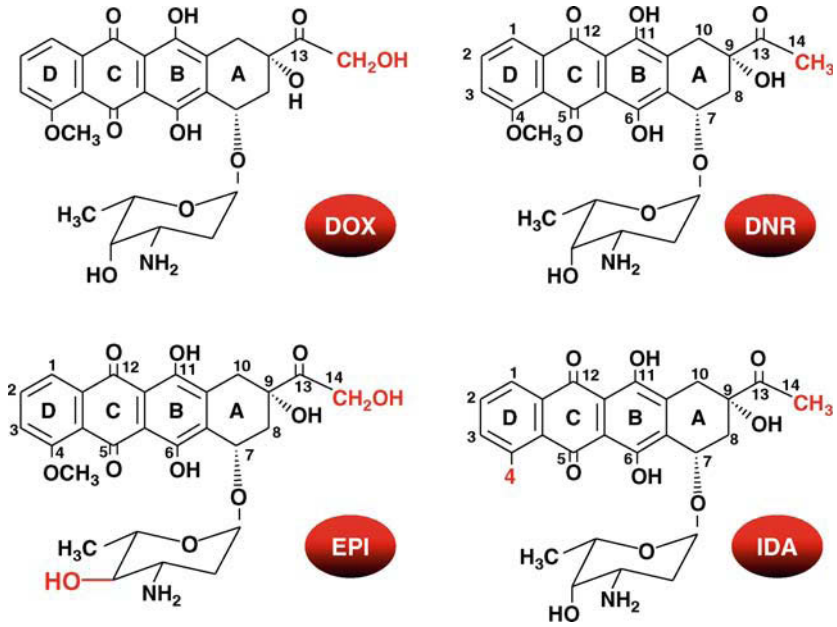
Definition

The ►anthracyclines represent a broad family of antibiotics that exhibit activity in numerous tumors. The first anthracyclines, doxorubicin (DOX) and daunorubicin (DNR), were isolated from *Streptomyces* var *peucetius*; they were shown to be composed of a tetracyclic ring system with adjacent quinone–hydroquinone moieties, a short side chain with a carbonyl group, and an aminosugar bound to the C-7 of the four-ring system. DOX and DNR only differed in the side chain terminus ($-CH_2OH$ in DOX vs. $-CH_3$ in DNR). Second generation anthracyclines, like epirubicin (EPI) and idarubicin (IDA), were obtained after minor chemical modifications of DOX or DNR, respectively (Fig. 1).

Mechanisms of Action

DOX and other approved anthracyclines have long been known to inhibit ►topoisomerase II (topo II). This enzyme promotes the formation of double-strand DNA breaks, which are resealed after changing the twisting status of the double helix of DNA. Topo II is highly important to proliferating cells, as the supercoiling of the DNA double helix is modulated according to the cell cycle phase and transcriptional activity. Anthracyclines stabilize a reaction intermediate in which DNA strands are cut and covalently linked to topo II, eventually impeding DNA resealing. Rings B, C, and D are important determinants of anthracycline intercalation into DNA, while ring A and the aminosugar residue play a major role in the formation and stabilization of the ternary complex. Anthracycline- and topo II-mediated DNA damage is followed by growth arrest in G1 and G2 and apoptosis. This is usually, but not always, relayed by p53 and the consequent induction of the WAF1/CIP1 p21 gene product, a strong inhibitor of cyclin-dependent kinases that favor cell cycle progression through the G1 to S transition.

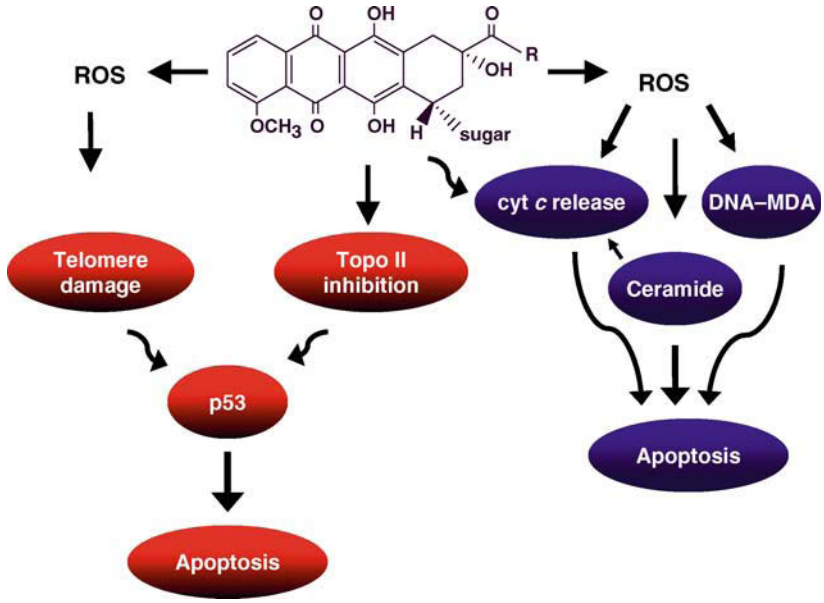
Topo II inhibition remains the most persuasive mechanism to explain the antitumor activity of anthracyclines; accordingly, limited clinical studies showed that tumor



Anthracyclins. Figure 1 Structure of the four main anthracyclines approved for clinical use. The anthracyclines are composed of a tetracyclic ring with adjacent quinone–hydroquinone moieties and a short side chain with a carbonyl group at C-13; an aminosugar is attached by a glycosidic bond to the C-7 of the tetracyclic ring. Doxorubicin (DOX) and daunorubicin (DNR) differ in the side chain terminus (–CH_2OH or –CH_3, respectively). Epirubicin (EPI) is obtained after an axial-to-equatorial epimerization of the hydroxyl group at C-4' in the aminosugar. Idarubicin (IDA) is characterized by the absence of the methoxy group at C-4 in ring D.

response and patient's outcome correlated with apoptosis induced by topo II inhibition. Nonetheless, clinically relevant concentrations of anthracyclines were shown to induce apoptosis also through corollary mechanisms that were not bound to topo II or p53; these mechanisms include, among others: (i) the activation of neutral sphingomyelinases, followed by ceramide formation and converse activation of cell death effectors (c-Jun N-terminal kinase) or downregulation of survival pathways (Akt/protein kinase B), (ii) mitochondrial dysfunction, followed by cytochrome *c* (cyt *c*) release and apoptosome formation, (iii) induction of lipid peroxidation and formation of malondialdehyde-DNA adducts, followed by the reduced activity of cyclin E- and cyclin B- associated kinase activities and growth arrest in both p53-proficient and p53-deficient cells, and (iv) inhibition of the ►proteasome, followed by an accumulation of undegraded ubiquinated proteins that signal apoptosis [1]. The mechanisms (i–iii) are triggered by ►reactive oxygen species (ROS), which are major byproducts of anthracycline metabolism. ROS may also enable anthracyclines to damage and shorten telomeres, long sequences of base repeats that otherwise would delay cell senescence and apoptosis by preventing the degradation and ligation of the end of chromosomes; however, anthracycline-induced telomere damage and dysfunction would be relayed to apoptosis through p53 (Fig. 2).

Anthracycline treatment may be accompanied by the acquisition of a resistance phenotype through a combination of pharmacokinetic and pharmacodynamic mechanisms. On pharmacokinetic grounds, tumor resistance is caused by the reduced accumulation and/or an altered distribution of anthracyclines in tumor cells. A major mechanism of such a kind of resistance rests on the overexpression of drug transporters that belong to the ATP-binding cassette family of proteins and are collectively referred to as ABC proteins (P-glycoprotein/Pgp, ►multidrug resistance protein 1/MRP1, breast cancer resistance protein/BCRP). For many years, it was thought that ABC proteins could confer resistance to anthracyclines by localizing to the plasma membrane of tumor cells and by mediating drug efflux through a canonical ATP-dependent antiporter mechanism. More recently, ►ABC proteins were shown to localize also to the membrane of cytoplasmic acidic organelles like lysosomes, recycling endosomes, and vesicles of the *trans*-Golgi network. Vesicular ABC proteins mediate an [out]→[in] transport of the anthracyclines, which subsequently undergo protonation of their amino residue and remain entrapped in the lumen of the vesicles. The ABC proteins therefore divert anthracyclines away from the nucleus or mitochondria toward pharmacologically unproductive cell sanctuaries like cytoplasmic organelles. Anthracycline-naïve tumor cells do not exhibit such a mechanism of anthracycline



Anthracyclins. Figure 2 Mechanisms of anthracycline-induced apoptosis of tumor cells. ROS, reactive oxygen species; topo II, topoisomerase II; cyt *c*, cytochrome *c*.

sequestration, as they usually lack the vesicular expression of ABC proteins or fail to generate a proton gradient across the envelope of cytoplasmic organelles.

On pharmacodynamic grounds, tumor resistance may be caused by such diverse mechanisms as the mutation or redundancy of topo II, the overexpression and preferred nuclear localization of proteasome α-type subunits (leading to a anomalous degradation of topo II), genetic deletion or loss-of-function mutations of p53, overexpression of ROS-detoxifying enzymes, overexpression of Bcl-2 (leading to a diminished cyt *c* release), etc. However, none of these factors would universally predict the development of anthracycline-resistance in a given tumor or another.

Clinical Use

In spite of their longer than 40 years record of longevity the anthracyclines still rank among the most effective cytotoxics available to the oncologists. DOX and its second generation analogue EPI are essential components of the treatment of breast cancer, childhood solid tumors, soft tissue sarcomas, and aggressive lymphomas. DNR is used to treat acute lymphoblastic or myeloblastic leukemias, while its second generation analogue IDA shows activity also in multiple myeloma, non-Hodgkin's lymphomas, and breast cancer. The broader spectrum of activity of IDA versus DNR is attributed to pharmacokinetic and pharmacodynamic factors such as an increased lipophilicity and cellular uptake, and a stronger stabilization of a ternary anthracycline-topoisomerase II-DNA complex. An additional point of consideration is that DOX, EPI, and DNR must be administered by i.v. boluses, while IDA shows a nonnegligible bioavailability (~10–30%) also when administered orally.

Many other anthracyclines were extracted or synthesized over the last two decades, but only few of them eventually progressed toward a robust program of clinical development. A rather benign conclusion is that the novel anthracyclines or related anthraquinones offered only modest advantages over DOX-EPI or DNR-IDA, with the possible exceptions being nemorubicin (for the locoregional treatment of hepatocellular carcinoma), pixantrone (for the second-line treatment of non-Hodgkin's lymphomas), sabarubicin (for the treatment of non-small cell lung cancer, hormone refractory metastatic prostate cancer, platinum- or taxane-resistant ovarian cancer), valrubicin (for the topical treatment of bladder cancer).

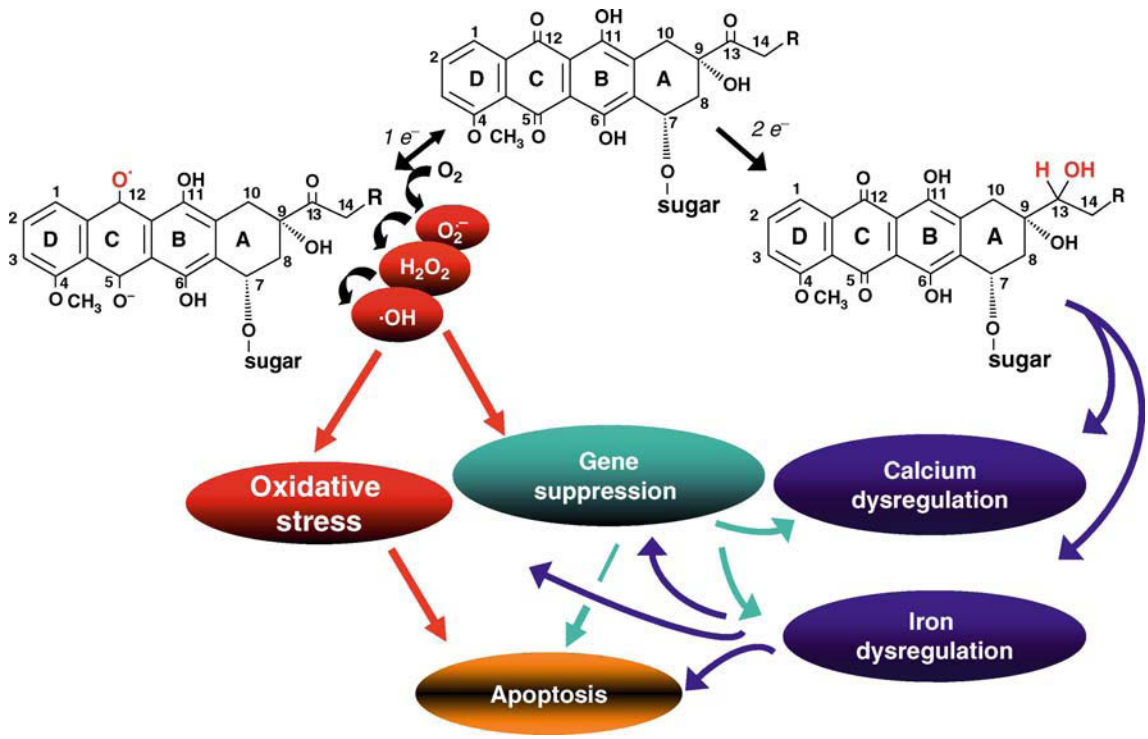
As with any other anticancer agent, the clinical use of anthracyclines is limited by hematologic and nonhematologic toxicities. The main nonhematologic toxicity is represented by a life-threatening dilative cardiomyopathy; in the long-term survivors of childhood cancer, cardiomyopathy may surface in a hypertrophic-restrictive form. The precise mechanisms of anthracycline cardiotoxicity remain a matter of debate. The current thinking is that DOX and other anthracyclines may become cardiotoxic after their conversion to ROS or secondary alcohol metabolites. ROS are formed after a one-electron reduction of the quinone moiety and the consequent formation of a semiquinone that reduces oxygen to superoxide anion $O_2^{\bullet}$, hydrogen peroxide (H_2O_2), hydroxyl radical $^{\bullet}OH$. Due to the lower levels of ROS-detoxifying enzymes in cardiomyocytes as compared with other cell types (and many tumors

as well), anthracyclines may gradually overrule the cardiac defenses and cause oxidative stress, suppression of cardiac-specific genes, apoptosis. Secondary alcohol metabolites are formed after a two-electron reduction of the side chain carbonyl group; they do not only downregulate cardiac-specific genes, but also inactivate calcium- and iron- handling proteins. The two pathways of toxicity, induced by ROS or secondary alcohol metabolites, share multiple links and feedbacks (Fig. 3).

Anthracycline-induced cardiomyopathy exhibits a well-defined dose-dependence, and may progress toward congestive heart failure (CHF) in the face of medication with β-blockers, calcium antagonists, converting enzyme inhibitors, diuretics. In the case of DOX, the threshold to cardiomyopathy and CHF is currently set at ~500 mg/m^2, but there have been concerns about whether cumulative doses <500 mg/m^2 caused nonsymptomatic cardiac dysfunction that could surface at a later time in the form of symptomatic CHF. These concerns are less than speculative if one considers that very many women receive ~300 mg of DOX/m^2 for the neoadjuvant (presurgery) or adjuvant (postsurgery) treatment of early breast cancer. The available evidence suggests that these women do develop nonsymptomatic cardiac dysfunction, usually detected as pathologic or borderline decreases of the ►left ventricular ejection fraction; however, there is no compelling demonstration that such a dysfunction would eventually progress to symptomatic CHF. Clinically, CHF may occur anytime after the completion of a cumulative anthracycline regimen; children tend to develop cardiomyopathy and CHF at longer times, sometime as late as 15 years after the last of several doses of an anthracycline. This latter observation suggests that changes in the lifestyle, environmental factors, and growth-related hemodynamic challenges may trigger the progression of noncardiotoxic injuries toward symptomatic cardiac dysfunction [2].

Cardiotoxicity may develop at lower than expected cumulative doses of anthracyclines in patients with risk factors like hypertension, preexisting arrhythmias or valvular disease, advanced age, prior irradiation of the mediastinum.

A higher than expected incidence of CHF is also observed in patients treated with DOX and other cytotoxics (e.g., the ►taxane paclitaxel) or new generation targeted agents (e.g., the humanized anti-ErbB-2/neu monoclonal antibody trastuzumab) [3]. The cardiotoxic synergism of DOX with taxanes or



Anthracyclins. Figure 3 Mechanisms of anthracycline cardiotoxicity. One-electron (*1 e*$^-$) reduction of the quinone moiety generates a semiquinone that recycles to the parent quinone by redox coupling with oxygen. The consequent cascade of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), induces apoptosis through oxidative stress and gene suppression. Two-electron (*2 e*$^-$) reduction of the side chain carbonyl group generates a secondary alcohol metabolite that also suppresses cardiac-specific genes and inactivates proteins of calcium and iron homeostasis. These mechanisms share multiple links and feedbacks.

Anthracyclins. Table 1 Strategies for reducing anthracycline cardiotoxicity

Strategy	Rationale	Indications
Substituting EPI for DOX	Lower formation of ROS or secondary alcohol metabolite	Same as those of DOX; combination with drugs that stimulate anthracycline conversion to secondary alcohol metabolite or diminish the cardiac defenses against ROS
Liposomal encapsulation of DOX or DNR	Preferred anthracycline delivery to the tumor	Breast cancer, ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma (pegylated liposomal DOX). Breast cancer (uncoated liposomal DOX). AIDS-related Kaposi's sarcoma, acute myeloblastic leukemia, multiple myeloma, non-Hodgkin's lymphomas (uncoated liposomal DNR)
Conjugation of DOX with copolymers or peptides	Recognition of DOX by tumor-specific receptors or proteases	Investigational
Coadministration of dexrazoxane	Chelation of iron in the heart, correction of iron dysregulation or mitigation of free radical formation	Approved for use in patients who continue DOX above 300 mg/m^2 or require another anthracycline after a prior exposure to 300 mg of DOX/m^2
Substituting slow infusions for 5–10 min boluses	Diminished anthracycline C_{max} and cardiac uptake	At the investigator's discretion (doubtful usefulness in pediatric settings)

EPI, epirubicin; DOX, doxorubicin; ROS, reactive oxygen species; DNR, daunorubicin

trastuzumab has been attributed to anomalous increases of the formation of secondary alcohol metabolites or an interruption of salvage pathways against ROS, respectively. While providing a validation of the mechanistic hypotheses of anthracycline cardiotoxicity, these concepts anticipate that a higher than expected incidence of CHF might well occur, if DOX were combined with other biologic agents that blocked proliferation-survival pathways. Recent studies show that bevacizumab, a humanized antibody against the Vascular Endothelial Growth Factor, synergizes with DOX and causes moderate to severe cardiotoxicity at cumulative doses of 300–420 mg of DOX/m^2. Other agents that could cause excess cardiotoxicity upon combination with DOX might include sunitinib (inhibitor of the Vascular Endothelial Growth Factor receptor) and lapatinib (inhibitor of the tyrosine kinase domain of both ErbB-1 and ErbB-2 receptors).

The unabated need for anthracyclines in many clinical settings has formed the basis to develop strategies that optimize their activity and/or tolerability. The reversion of the resistance phenotype has been pursued – with a limited success – through the use of first and second generation PgP inhibitors like verapamil, dexverapamil, cyclosporine A, valspodar, quinidine. Third generation revertants are now available (biricodar, zosuquidar, laniquidar), but the benefits of this strategy remain uncertain [4]. The attenuation of cardiotoxicity is obtained – with a somewhat higher success – through the combination of one or more of the following strategies: (i) substitution of EPI for DOX, as EPI seems to form fewer amounts of ROS and secondary alcohol metabolite, (ii) encapsulation of anthracyclines in uncoated or pegylated ►liposomes that ensure a good drug delivery to the tumor but not to the heart, (iii) conjugation of anthracyclines with chemical moieties that are selectively recognized by the tumor cells, (iv) coadministration of dexrazoxane, an ►iron chelator that diminishes the disturbances of iron metabolism and free radical formation in the heart, and (v) administration of anthracyclines by slow infusion rather than 5–10 min bolus (Table 1). Pharmacological interventions with antioxidants have also been considered, but the available clinical studies do not attest to an efficacy of this strategy.

►Antineoplastic Agents

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Anthraquinones

►Anthracyclins

Antiarrhythmic Drugs

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Synonyms

Antiarrhythmics

Definition

Antiarrhythmic drugs are substances that affect cardiac ionic channels or receptors, thereby altering the cardiac action potential or its generation or propagation. This results in changes of the spread of activation or the pattern of repolarization. Thereby, these drugs suppress cardiac arrhythmia.

These drugs are traditionally classified according to Vaughan-Williams (Table 1): (i) sodium channel blockers; slowing the spread of activation (►antiarrhythmic drugs, class I) with prolongation of the action potential (class IA), with shortening of the action potential (class IB) or without effect on action potential duration (class IC), (ii) β-adrenoceptor antagonists; slowing sinus rhythm and atrioventricular conduction (►antiarrhythmic drugs, class II), (iii) potassium channel blockers; prolonging the action potential (►antiarrhythmic drugs, class III), (iv) calcium channel blockers; mainly slowing atrioventricular conduction (►antiarrhythmic drugs, class IV), and (not included in this classification) (v) digitalis glycosides, (vi) adenosine, (vii) atropine and (viii) symapathomimetic drugs like orciprenaline.

Mechanism of Action

Basic Considerations

Normal rhythmic activity is the result of the activity of the sinus node generating action potentials that are conducted via the atria to the atrioventricular node, which delays further conduction to the His-Tawara-Purkinje system. From the Purkinje fibres, action potentials propagate to the ventricular myocardium. Arrhythmia means a disturbance of the normal rhythm either resulting in a faster rhythm (tachycardia, still rhythmic) or faster arrhythmia (tachyarrhythmia) or slowed rhythm (bradycardia, bradyarrhythmia).

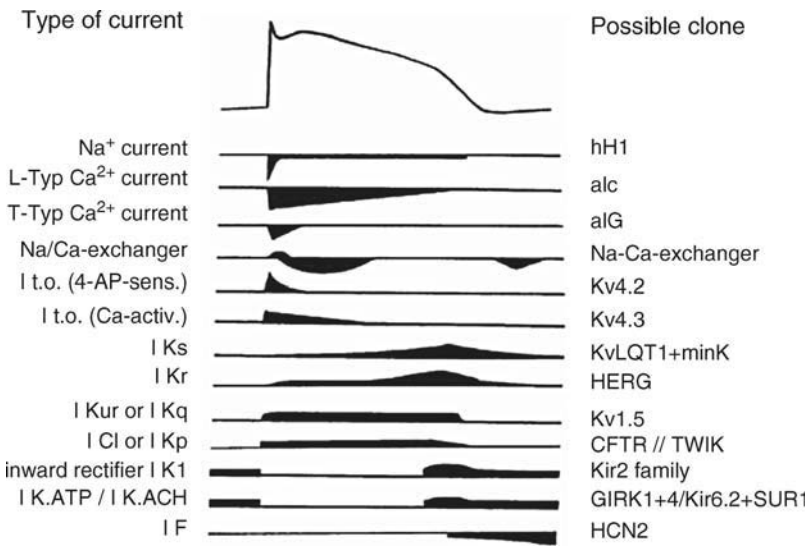
Arrhythmia is either the result of impaired conduction or altered electrical activity. However, in all arrhythmias, conduction and intercellular communication are important since arrhythmia only occurs if the altered electrical activity in one region is transduced to the whole organ.

Antiarrhythmic drugs can either influence electrical activity of the single cell or can interfere with the spread of activation.

In the following, the cardiac action potential is explained (Fig. 1): An action potential is initiated by depolarization of the plasma membrane due to the pacemaker current (I_f) (carried by K^+ and Na^+, which can be modulated by acetylcholine and by adenosine) modulated by effects of sympathetic innervation and β-adrenergic activation of Ca^{2+}-influx as well as by acetylcholine- or adenosine-dependent K^+-channels [in sinus nodal and atrioventricular nodal cells] or to depolarization of the neighbouring cell. Depolarization opens the fast Na^+ channel resulting in a fast depolarization (phase 0 of the action potential). These channels then inactivate and can only be activated if the membrane is hyperpolarized

Antiarrhythmic Drugs. Table 1 Classification of antiarrhythmic drugs according to Vaughan-Williams

Class	Effects	Drugs
I	Block of sodium channel	
I a	With prolongation of action potential	Quinidine, Procainamide, Disopyramide, Ajmaline, Prajmaline
I b	With shortening of action potential	Lidocaine, Mexiletine, Tocainide, Phenytoin, Aprindine
I c	With only little effect on action potential duration	Lorcainide, Flecainide, Propafenone
II	β-adrenoceptor antagonists	Propranolol, Metoprolol and others
III	Block of repolarizing potassium channels, prolongation of action potential	Amiodarone, Dronedarone, Sotalol, Dofetilide, Ibutilide
IV	Block of calcium channels	Verapamil, Diltiazem



Antiarrhythmic Drugs. Figure 1 Transmembrane ionic currents of the cardiac action potential. In the middle of the figure, a typical cardiac action potential is shown as can be obtained from the ventricular myocardium (upper trace). Below, the contribution of the various transmembrane currents is indicated. Currents below the zeroline are inward; currents above the zero line are outward fluxes. In the left column the name of the current is given and in the right column the possible clone; redrawn and modified after [5].

again. This fast upstroke is followed by a short incomplete repolarization (activation of the transient outward rectifier $I_{t.o.}$ carried by K^+, phase 1). Next, the action potential remains quiet constant for about 50–350 ms (plateau phase, phase 2), which is the result of inward Ca^{2+} current (via L-type Ca^{2+} channels) and simultaneous activation of the repolarizing potassium current, the delayed rectifier I_K (which has three components: rapid, $I_{K.r}$, ultrarapid, $I_{K.ur}$, slow component, $I_{K.s}$). This is followed by complete ►repolarization of the membrane to about −80 mV via activation of the delayed rectifier (phase 3), while the Ca^{2+} channels close during this phase. During an action potential it is not possible to elicit a second action potential since the fast Na^+ channel is inactivated. This period is called refractory period [4].

Not all cells in the heart express the fast sodium channel. Thus, sinus nodal and atrioventricular nodal cells lack the fast Na^+ channel and instead generate their action potentials via opening of Ca^{2+} channels. This is the basis for their sensitivity to Ca^{2+} antagonists.

Action potential propagation along the fibre is mainly dependent on the Na^+ channel availability, which is a function of the resting membrane potential. Propagation from cell to cell is realized via intercellular gap junction channels. These channels can be regulated by a number of stimuli. Thus, e.g. low pH, high $[Ca^{2+}]_i$ or low $[ATP]_i$ result in a closure of these channels leading to conduction disturbances and arrhythmias in myocardial infarction. Moreover, the density of these channels can be regulated by e.g. chronic adrenergic stimulation, angiotensin-II or endothelin and has been found to be altered in arrhythmias such as atrial fibrillation or arrhythmogenic conditions like cardiac hypertrophy.

There are several basic mechanisms of arrhythmia:

1. A single cell or group of cells capable of a pacemaker potential may generate extrastimuli (enhanced automaticity).
2. A cell may generate oscillating afterpotentials which reach the threshold for activation of the Na^+ channel (triggered activity).
3. A cell generates late afterdepolarizations (typically induced by catecholamines or digitalis) following a complete repolarization that may elicit an action potential.
4. A cell may produce early afterdepolarizations that are depolarization during incomplete repolarization. This is possible if the action potential is considerably prolonged. This is the typical mechanism for elicitation of Torsade de Pointes arrhythmia, a typical complication of class III antiarrhythmics and many other drugs.
5. Furthermore, under certain conditions (e.g. local unidirectional block) it is possible that the activation wavefront is delayed and encounters areas already repolarized. This may result in a circulating wavefront (= reentrant circuit ►reentrant arrhythmia), from which centrifugal activation waves originate and elicit life-threatening ventricular fibrillation.
6. Block of propagation may occur in the specific conduction system leading to bradyarrhythmia

(sinuatrial block, atrioventricular block, intraventricular bundle block).

Antiarrhythmic treatment is based upon modulation of the ionic currents mentioned above. A principal problem with this therapy is that the electrophysiology of all cells is targeted and not specifically the arrhythmogenic focus. As a consequence, all antiarrhythmics acting at transmembrane ionic channels possess a risk for elicitation of arrhythmia (= proarrhythmic risk).

Molecular Mechanism of Action

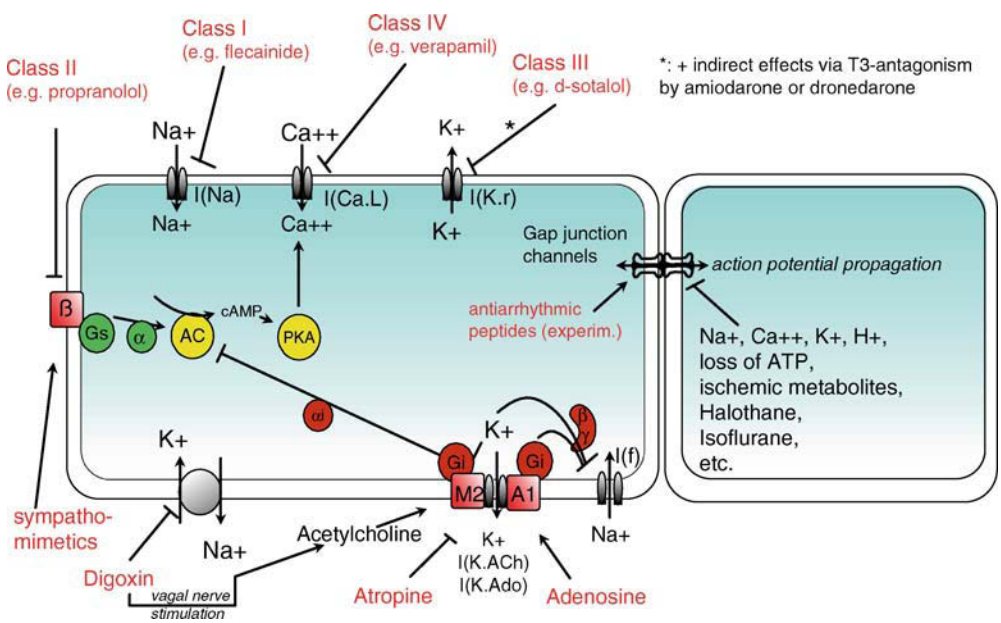
Class I, III and IV antiarrhythmics bind to and block transmembrane ionic channels (Fig. 2). Class I antiarrhythmics block the fast Na^+ channel. This channel switches from a resting state to an open state, and then time- and voltage-dependently inactivates (inactivated state) (Fig. 3). The block of this channel by an antiarrhythmic drug is a ►state-dependent block: a class I compound like lidocaine enters the channel in its open state and binds to the inactivated state, altering the kinetics of recovery from inactivation. If the channel switches to its resting state, the affinity for lidocaine is less and the drug dissociates from the channel. This is the basis for the use-dependence of block: the kinetics of dissociation determines the interval after which a subsequent action potential is not influenced. That means that an action potential early after the foregoing action potential will be suppressed while another after a long interval will not be altered.

Drugs with fast dissociation will only suppress high frequency arrhythmia (high use-dependence). Drugs with a long dissociation time constant will suppress action potentials at normal frequency as well. Class IB drugs exhibit the shortest time constant (0.2–0.4 s; highest use-dependence), while class IC drugs have the longest dissociation time constant (2–250 s; no use-dependence). Class IA antiarrhythmics show an intermediate dissociation time constant (5–50 s).

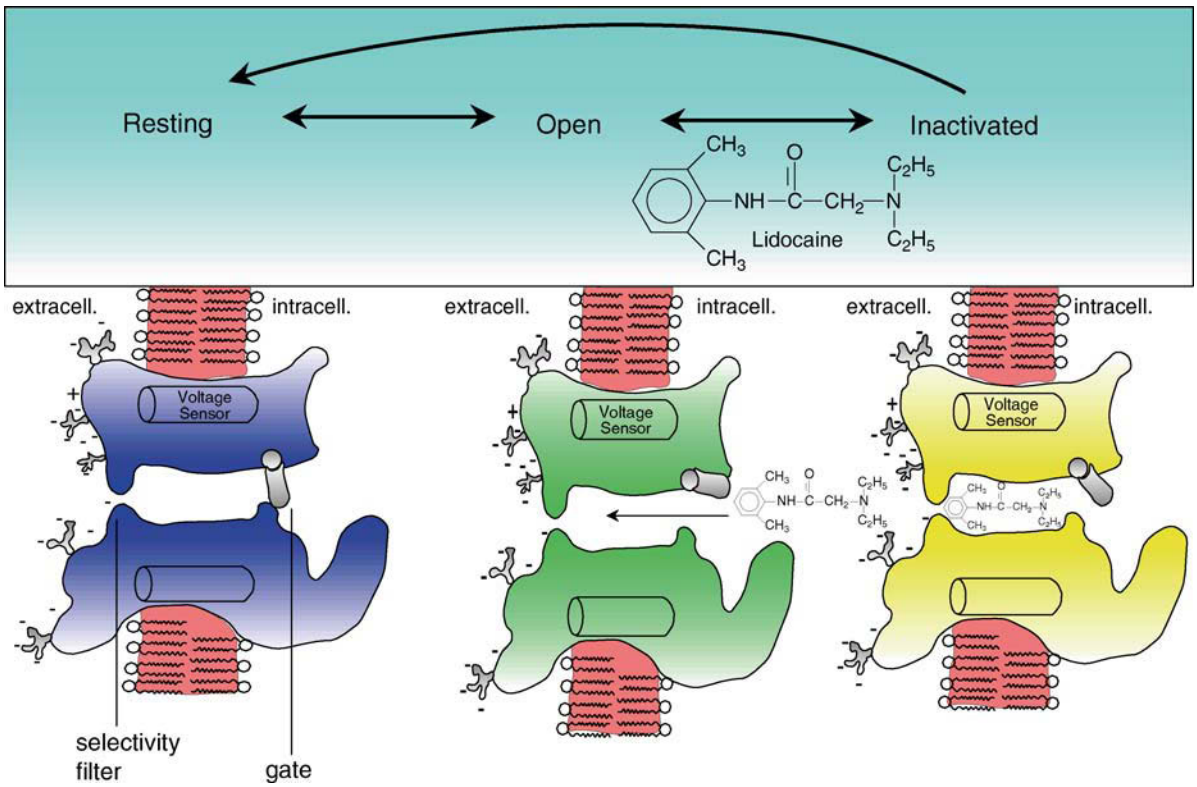
Class I drugs (Na^+ channel blockers) suppress action potential amplitude, reduce fast depolarization (upstroke) velocity and propagation velocity, prolong total refractory period and reduce automaticity. These drugs have no or little influence on slow Ca^{2+} carried action potentials (AV- or sinus nodal cells). All these drugs prolong the ventricular QRS complex, exert negative inotropic effects and a strong proarrhythmic effect especially in patients with structural heart disease and, thus, cannot be used in patients after myocardial infarction.

Besides the class I-typical proarrhythmic risk class IA antiarrhythmics possess a marked proarrhythmic risk for the induction of ►torsade de pointes arrhythmia (life-threatening polymorphic ventricular tachycardia observed with most action potential prolonging drugs).

Quinidine, the classical class IA drug, binds to the open state of the Na^+ channel, and prolongs the action potential by block of the delayed rectifier. In higher concentrations, L-type Ca^{2+} channels are inhibited. Quinidine exerts antimuscarinic effects, thereby accelerating AV-nodal



Antiarrhythmic Drugs. Figure 2 Targets of the various antiarrhythmic drugs. The K^+ channel, regulated by acetylcholine (via M_2 receptors) or adenosine (via A_1 receptors), plays a role only in supraventricular tissues. The action potential which is generated in one cell propagates to the neighbouring cell via the gap junction channel.



Antiarrhythmic Drugs. Figure 3 Binding of class I antiarrhythmic drugs to the cardiac sodium channel. Summary of the modulated receptor hypothesis as an explanation of state-dependent block of Na^+ channels by local anaesthetics such as lidocaine. The Na^+ channel switches between resting and open and inactivated state. On the extracellular side, a selectivity filter controls the ions passing through the channel. On the intracellular side, the inactivation gate can close the channel. A class I antiarrhythmic drug (lidocaine) enters the channel during its open state and binds with its lipophilic moiety to the inactivated state, the hydrophilic part of the molecule extending into the water-filled channel pore blocking it.

conduction and antagonising α-adrenergic effects, which may lead to accelerated AV-conduction and faster ventricular rhythms in situations as atrial fibrillation or atrial flutter. Typical untoward effects include vomiting, diarrhoea, allergies, immunological and haematological effects and hepatitis.

Ajmaline (intravenously only) and its orally applicable propyl-substituted prodrug prajmaline are classified as class IA drugs, but due to their long dissociation time constant can also be considered as class IC compounds.

Further class IA drugs include the open state blockers procainamide and disopyramide with electrophysiological effects similar to those of quinidine; procainamide lacks the antimuscarinic and antiadrenergic effects. Characteristic side effects of procainamide are hypotension and immunological disorders.

Class IB drugs like lidocaine, phenytoin or mexiletine preferentially bind to the inactivated state. Lidocaine, a local anaesthetic, can be used intravenously for antiarrhythmic treatment. It is one of the classical drugs used in emergency medicine for the treatment of ventricular fibrillation. The side effects of lidocaine are typical for local anaesthetics including dizziness, tremor, nystagmus, seizures or nausea.

The antiepileptic drug phenytoin, an orally available class IB antiarrhythmic, is mainly effective in digitalis-induced arrhythmias. This drug exhibits nonlinear pharmacokinetics and a number of side effects including neuropathy, gingival hyperplasia, hepatitis, immunological disorders and suppression of white blood cells.

Class IC antiarrhythmic drugs such as flecainide or propafenone block the Na^+ channel (open state; propafenone: open and inactivated state) with a very long dissociation time constant so that they alter normal action potential propagation. Flecainide increased mortality of patients recovering from myocardial infarction due to its proarrhythmic effects (CAST study). Action potential is shortened in Purkinje fibres but is prolonged in the ventricles.

Propafenone possesses β-adrenoceptor antagonistic effects due to its structural similarity to propranolol.

In high concentrations it blocks calcium channels and, thus, exerts prominent negative inotropic effects. Its adverse effects include proarrhythmic effects, worsening of heart failure and (due to β-adrenoceptor blockade) bradycardia and bronchospasm.

Class II drugs are classical β-adrenoceptor antagonists such as propranolol, atenolol, metoprolol or the short-acting substance esmolol. These drugs reduce sinus rate, exert negative inotropic effects and slow atrioventricular conduction. Automaticity, membrane responsiveness and effective refractory period of Purkinje fibres are also reduced. The typical extracardiac side effects are due to β-adrenoceptor blockade in other organs and include bronchospasm, hypoglycemia, increase in peripheral vascular resistance, depressions, nausea and impotence.

Class III antiarrhythmic drugs block the repolarizing K^+ channel thereby prolonging the action potential duration and lengthen the refractory period. The classical class III antiarrhythmic compounds like sotalol block the rapid component of the delayed rectifier, I_{Kr}. However, at higher heart rates the repolarization is mainly carried by the slow component I_{Ks} and, thus, the action potential prolonging effect of these agents is progressively reduced with increasing heart rate (inverse use-dependence). Class III antiarrhythmics can induce early afterdepolarizations and torsade de pointes, a polymorphic ventricular tachycardia associated with excessive QT prolongation, which can degenerate into ventricular fibrillation.

d,l-Sotalol is a racemate of l-sotalol, a β-adrenoceptor antagonist, and d-sotalol, an inhibitor of both I_{Kr} and I_{Ks}. This substance exhibits a strong inverse use-dependence. Regarding the beneficial antiarrhythmic effects, studies with only l-sotalol showed that the racemate is superior. However, due to β-adrenoceptor blockade d,l-sotalol can induce bronchoconstriction, increase in peripheral vascular resistance, negative inotropy, depressions, hypoglycemia and bronchospasm. A serious side effect is the induction of torsade de pointes arrhythmia (ca. 3%).

Amiodarone blocks several ionic channels; besides predominant blockade of I_{Kr} and I_{Ks}, as well as I_{K1}, $I_{K.ACh}$, I_{sus} I_f, I_{Na}, $I_{Ca.L}$, $I_{Ca.T}$ is inhibited. Moreover, amiodarone acts as an antagonist at both α- and β-adrenoceptors. Inverse use-dependence is less than with sotalol. The action potential prolonging effect is slowly developing (steady state after 2–5 months). From todays understanding the molecular mechanism includes the generation of an inactive triiodothyronine isomere thus acting as a functional T3-antagonist affecting the T3-dependent gene activation, which seems to explain the slowly developing prolongation of the action potential. A small acute prolongation of the action potential is probably dependent on the formation of the metabolite desethylamiodarone. Side effects include defects of vision, corneal depositions, neurological disorders, pigmentation, photosensibilization, torsade de pointes arrhythmia (but less than 1%) and AV-block. Moreover, alterations of thyroid function with both hypo- (due to the inactive T3-isomere) and hyperthyreoidism (due to the iodine content of the drug) and lung fibrosis are observed.

Since alterations of thyroid function by amiodarone are related to the iodine substitution of the drug, the iodine-free derivative dronedarone has been developed with similar electrophysiological effects as amiodarone. It seems to act also as a T3-anatgonist, but does not provoke hyperthyreoidism [1].

Newly developed class III drugs comprise dofetilide, a specific I_{Kr} blocker, and ibutilide, which blocks I_{Kr} and activates the slow I_{Na}. Both drugs lack hemodynamic side effects. These drugs are scheduled for the treatment of atrial fibrillation and atrial flutter. As with class III drugs, they can induce torsade de pointes arrhythmia.

Class IV antiarrhythmic drugs (Ca^{2+} entry blockers) inhibit L-type Ca^{2+} channel. For antiarrhythmic purposes, only those Ca^{2+} channel antagonists are used with higher affinity to the heart (i.e. phenylalkylamines like verapamil, gallopamil and D600, or benzothiazepine derivatives like diltiazem) than to the vasculature (as nifedipine or other 1,4-dihydropyridines, which therefore do not belong to the class IV antiarrhythmics). Class IV antiarrhythmic drugs like verapamil or diltiazem exert the strongest electrophysiological effects on sinus and atrioventricular node, since in sinus and AV-nodal cells I_{Na} is not expressed but action potentials are carried by Ca^{2+}. They reduce sinus rate, slow atrioventricular conduction, prolong refractory period of the AV node and exert a strong negative inotropic and vasodilator effect. Ventricular electrophysiology is only slightly affected, but force of contraction is markedly reduced.

Verapamil is a phenylalkylamine which blocks L-type Ca^{2+} channels in a use-dependent manner. The drug binds to the inactivated state of the channel. Diltiazem is a benzothiazepine derivative with a profile of action most similar to that of verapamil.

Other Antiarrhythmic Compounds

►Cardiac glycosides exert parasympathomimetic effects by activation of vagal nerves leading to slowing of sinus rate and predominantly to prolongation of atrioventricular conduction time. This latter action is used for the control of the ventricular response frequency in the treatment of atrial fibrillation with digitalis (frequency control therapy of atrial fibrillation). In that indication it can be combined with verapamil or ß-adrenoceptor antagonists.

Adenosine activates the atrial A_1-adenosine receptor, which opens the $I_{K.Ado}$ channel leading to

hyperpolarization, slowing of spontaneous depolarization, reduces sinus rate and atrioventricular conduction [3]. The drug has to be administered intravenously. It produces a short cardiac arrest and is used for termination of atrioventricular reentrant tachycardia. Due to its extremely short half-life time (0.6–1.5 s) the effects are only transient. Since adenosine is also a potent vasorelaxant, it may produce pronounced hypotension, and in patients suffering from asthma bronchospasm. The latter effects are transduced via other adenosine receptors. This is the basis for the development of a new A_1-selective agonist, tecadenoson.

The antimuscarinic drug atropine, and its derivative ipratropiumbromide, can also be used for antiarrhythmic treatment. Muscarinic receptors (M_2 subtype) are mainly present in supraventricular tissue and in the AV node. They inhibit adenylylcyclase via G_i proteins and thereby reduce intracellular cAMP. On the other hand, activation of the M_2 receptor leads to opening of hyperpolarizing $I_{K.ACh}$ and inhibits the pacemaker current I_f probably via the βγ-subunit of the G_i protein associated with this receptor. The results are hyperpolarization and slower spontaneous depolarization. Muscarinic receptor antagonists like atropine lead to increased heart rate and accelerated atrioventricular conduction. There are no or only slight effects on the ventricular electrophysiology.

Intravenous administration of magnesium sulfate (1–5 g) is used for the termination of torsade de pointes arrhythmia. The underlying electrophysiological mechanism is not well understood. It includes changes of the current–voltage relationship of I_{K1} and Ca^{2+} channel blockade.

Clinical Use

Clinical uses of antiarrhythmics have been restricted after CAST [2] due to their proarrhythmic risk, and preference is given to electrophysiological methods.

Supraventricular bradycardia is treated by implantation of a pacemaker device or has been treated pharmacologically with atropine. Supraventricular paroxysmal tachycardia is treated with ajmaline or prajmaline. Supraventricular tachyarrhythmias or AV reentrant arrhythmia typically can be terminated using adenosine.

The risk of atrial flutter is a 2:1 transmission to the ventricles generating a high ventricular rate. The therapeutic goal is to reduce transmission to 3:1 or 4:1 by administration of either β-adrenoceptor antagonists, Ca^{2+} channel blockers or amiodarone. Quinidine must not be used in this arrhythmia, since it accelerates AV-conduction due to its vagolytic effect.

The most common arrhythmia in humans is atrial fibrillation. Because of the lack of rhythmic atrial activation, irregular ventricular rhythms and thromboembolism result. There are two possible therapeutic goals: control of heart rate or return to sinus rhythm. For frequency (heart rate) control, β-adrenoceptor antagonists, Ca^{2+} channel blockers and digitalis can be used. For conversion to sinus rhythm, electrophysiological ablation is the therapy of choice. Alternatively, a pharmacological attempt with class IA drugs or class IC drugs can be made. Thereafter, relapse to atrial fibrillation has to be prevented by amiodarone or β-adrenoceptor antagonists. A recent concept is the pill-in-the-pocket concept which means that the patient takes just one dose of oral flecainide (or similarly acting drugs) when experiencing a relapse to atrial fibrillation. It is necessary to include oral anticoagulant therapy with phenprocoumon or warfarin in the treatment strategy of chronic atrial fibrillation to prevent from stroke, which is the main risk of a patient suffering from atrial fibrillation.

Atrioventricular block in general is treated by implantation of an electrical pacemaker. A pharmacological alternative (although no longer used today) was atropine. However, atropine can be used for bridging the time between the onset of symptoms and the definitive implantation of a pacemaker.

Ventricular extrasystoles are treated only if they may degenerate into life-threatening arrhythmia. In milder forms the proarrhythmic risk of the drugs overshadows their benefits. In such cases β-adrenoceptor antagonists may be attempted. For the treatment of ventricular extrasystoles, such as series or runs of extrasystoles, amiodarone or sotalol are used. In the absence of structural heart disease, class I antiarrhythmic drugs can be considered an alternative. However, they may not be administered during the post-infarction period.

Ventricular fibrillation should be terminated by electrical defibrillation. Alternatively, lidocaine can be injected intravenously. In cases with lower frequency, ventricular tachyarrhythmia class I drugs such as ajmaline, flecainide or propafenone are more effective as a result of the use-dependence of lidocaine. For prophylaxis treatment, amiodarone or sotalol may be helpful or the implantation of a cardioverter-defibrillator system. Acute amiodarone (i.v.; in higher doses) can also terminate ventricular tachyarrhythmias. This action, however, seems to be mediated by its I_{Na}-blocking side effects and not (or less) by its class III like effects.

Torsade de pointes arrhythmia can be terminated by intravenous (not oral) administration of large doses of magnesium.

►K^+ Channels
►Voltage-dependent Na^+ Channels
►Voltage-dependent Ca^{2+} Channels
►β-Adrenergic System
►Cardiac Glycosides

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Antiarrhythmic Drugs Class I

Antiarrhythmic drugs are antagonists of the fast Na^+ channel, which slow the propagation of the cardiac action potential. Class I drugs suppress the fast upstroke of the action potential.

►Antiarrhythmic Drugs

Antiarrhythmic Drugs Class II

Class II antiarrhythmic drugs are β-adrenoceptor antagonists such as propranolol, metoprolol or atenolol. β-adrenoceptor antagonists slow sinus rate and atrioventricular conduction and exert negative inotropic effects.

►Antiarrhythmic Drugs

Antiarrhythmic Drugs Class III

Class III antiarrhythmic drugs are drugs which act as K^+ channel antagonists and result in action potential prolongation without effect on the upstroke of the action potential.

►Antiarrhythmic Drugs

Antiarrhythmic Drugs Class IV

Class IV antiarrhythmic drugs are Ca^{2+} channel blockers, which predominantly slow sinus rate and atrioventricular conduction and thus are used in the treatment of supraventricular tachyarrhythmias. These drugs exert a pronounced negative inotropic effect.

►Antiarrhythmic Drugs

Antibiotic Resistance

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Definition

From the mechanistic point of view three basic principles of microbial resistance to drugs are known: inactivation of the drug, alteration of the target, and reduced drug accumulation at the target site. However, several variations on these themes are known.

Basic Mechanisms

The phenomenon of bacterial resistance to antibiotics was already known by the pioneers of the era of antibiotics, like Paul Ehrlich, who coined the term "selective toxicity" as the basic principle of antimicrobial therapeutics, as well as Gerhard Domagk, the inventor of the sulfonamide drugs, and Sir Alexander Fleming, the discoverer of the penicillins. When penicillin G was introduced into clinical practice in 1944, as many as 5% of the isolates of *Staphylococcus aureus* were resistant to penicillin, while 5 years later the percentage was 50%.

That bacterial resistance predates the era of clinical use of antibiotics by several hundred millions of years is the recent result of genomic sequence data mining from antibiotic-producing microorganisms. These are supposed to be the "inventors" of antibiotic resistance genes which they had "developed" to protect themselves from the lethal action of their own antibiotics [4].

Sharing these resistance genes with other bacteria, including human pathogens, by ►gene transfer mechanisms has resulted in a wide spread and in the adaption of these foreign gene sequences to the genetic codon usage of the respective pathogen. This is the preferred

strategy to provide bacteria with resistance determinants to naturally occurring antibiotics [1].

In addition, due to their the short generation times resulting in high cell densities within a short time, bacteria can easily acquire mutations in genes encoding antibiotic targets. The usually haploid bacterial genome assures that resistant variants of these targets are phenotypically expressed and, thus, mediate resistance. With this strategy bacteria are able to respond to novel chemical structures of synthetic antibacterials.

Over 4 decades, between 1960 and 2000, the development of new antibiotics used well characterized basic structures for partial synthetic modifications, primarily to overcome resistance by increasing the pharmacodynamic properties and, secondarily, to improve the pharmacokinetic profile of older compounds. However, bacteria rapidly responded by acquiring additional genetic alterations either as mutations or by accumulating resistance genes as part of mobile genetic elements (►integrons) on transferable ►resistance plasmids.

Inactivation of the Drug

The inactivation of a drug is an enzymatic process which results either in the cleavage of a chemical bond, e.g. by proteases or esterases, or the formation of a new chemical bond, e.g. by transferring phosphate (phosphotransferase), acetate (acetyltransferase), or nucleotidylate (nucleotidyltransferase) moieties to a functional group of the antibiotic. The cleavage causes the loss of function of the antibiotic. Chemical modification can eliminate a specific molecular interaction between drug and target or can alter the physico-chemical properties of a drug, e.g. electrical charge, that has an impact on the ability of a drug to penetrate the bacterial membrane(s).

Proteases

An example for proteases are the β-lactamases that hydrolyse a peptide bond in the essential β-lactam ring of penicillins, cephalosporins, carbapenems and monobactams and, thereby, irreversibly inactivate the drug. β-lactamases share this mechanism with the ►penicillin binding proteins (PBPs), which are essential enzymes catalyzing the biosynthesis of the bacterial cell wall. In contrast to the PBPs which irreversibly bind β-lactams to the active site serine, the analogous complex of the drug with β-lactamases is rapidly hydrolyzed regenerating the enzyme for inactivation of additional β-lactam molecules.

According to their genetic relationship and their biochemical mechanism of action β-lactamases are divided into enzymes of the serine-protease type containing an active-site serine (molecular class A, C, and D enzymes) and those of the metallo-protease type (molecular class B enzymes), which contain a complex bound zinc ion.

The further classification of the over 400 different enzymes described so far into different subclasses is based upon different parameters, like substrate profile, molecular mass, isoelectric point, stability against β-lactamase inhibitors, localization of the encoding gene on a plasmid or on the chromosome (Fig. 1). According to this classification the group 1 enzymes are cephalosporinases of Gram-negative pathogens (most enterobacteria and ►*Pseudomonas aeruginosa*) which usually are chromosomally encoded. In many bacteria, like several enterobacteria (Citrobacter, Enterobacter, Serratia, Providencia, Morganella) their expression can transiently be induced in the presence of β-lactams, however, this induction is not of clinical relevance. Instead, the constitutive overexpression of the genes due to a mutation inactivating a genetic repressor of the β-lactamase will result in a high level of resistance to cephalosporins and penicillins, but not carbapenems. Enzyme inhibitors, like clavulanic acid are not active against these enzymes.

The group 2 contains the greatest number of enzymes showing the broadest spectrum of substrates. The majority of these enzymes belongs to the plasmid-encoded families TEM, SHV, CTX-M and OXA. Each family consists of one or a few progenitor enzymes with narrow substrate profile, e.g. TEM-1, TEM-2, and TEM-13, and several descendents which have acquired mutations resulting in the extension of the substrate profile (►extended spectrum β-lactamases, ESBL). This is accomplished either by widening the binding pocket or by introducing amino acids with side chains allowing for the stabilization of the drug–enzyme interaction. These group 2 enzymes are usually inhibited by clavulanic acid, however, some variants exist, which are resistant to the action of β-lactamase inhibitors (IR-β-lactamase), but have lost their broad substrate spectrum.

For the CTX-M family which has been rapidly growing during the last three years genomic data have identified the genome of *Kluyvera* species as the genetic source of progenitor enzymes (e.g. encoding CTX-M1, CTX-M2 or CTX-M9).

►Metallo-enzymes belonging to group 3 naturally show a very broad substrate spectrum including all β-lactams except monobactams and are not inhibited by clavulanic acid, but by complexing agents, like EDTA. This can only be exploited for diagnostic purposes.

Esterases

Hydrolysis of macrolides by products of the *ere* genes detected in enterobacteria is only of scientific interest, while esterases VGB-A and VGB-B encoded by the *vgb* type genes mediate clinically relevant resistance in staphylococci to the B compound (quinupristin) of the streptogramin combination quinupristin–dalfopristin.

Classification of β-lactamases - by molecular and functional features

Molecular class	Ambler group	Enzymatic aktivity	Localisation and way of distribution of gene	Examples
1	C	Cephalosporinase	Chromosome, Gram-negatives	AmpC
2a	A,D	Penicillinase	*S.aureus, P. aeruginosa*	BlaZ
2b	A	"broad spectrum"	Plasmid, Gram-negatives	TEM-1
		TEM-1,-2; SHV-1		SHV-1
2be	A	"extended	Plasmid, Gram-negatives	TEM-3 to 161
		spectrum"	Chromosome, *K. oxytoca*	SHV-2 to 104
				CTX-M1 to n
2br	A	"inhibitor resistant"	Plasmid, Gram-negatives	TEM-30 to 41
				SHV-10
2c	A	Carbenicillinase	Plasmid, Gram-negatives	CARB-1
		PSE; CARB		
2d	D	Cloxacillinase	Plasmid, Gram-negatives	OXA type
2e		Cephalosporinase	Chromosome, *P. vulgaris*	CepA
2f	A	Carbapenemase, ceph-, pen-ase	Chromosome, *E. cloacae, S.marcescens*	SME-1, MNC-A, KPC-1
3	B	Metalloenzym	Chromosome, *B. cereus*	L1, CfiA, IMP-1, VIM-1
4	?	Penicillinase	Chromosome, *P. cepacia*	

Antibiotic Resistance. Figure 1 According to Bush, Jacoby and Medeiros [2] four molecular classes of β-lactamases can be discriminated based upon biochemical and molecular features. Classes 1, 2, and 4 included serine-proteases, while metallo enzymes are included in class 3. The substrate spectrum varies between different subclasses and the corresponding genes can be part of an R-plasmid leading to a wider distribution or are encoded chromosomally in cells of specific species.

Acetyltransferases

The preferred substrates of acetyltransferases are amino-groups of antibiotics, like chloramphenicol, streptogramin derivatives, and the various aminoglycosides. The modification is believed to block a functional group involved in the drug-target-interaction. All acetyltransferases use acetyl-coenzyme A as cofactor.

The major mechanism of resistance to chloramphenicol is mediated by the chloramphenicol acetyltransferases (CAT enzymes) which transfer one or two acetyl groups to one molecule of chloramphenicol. While the CAT enzymes share a common mechanism, different molecular classes can be discriminated. The corresponding genes are frequently located on integron-like structures and are widely distributed among Gram-negative and – positive bacteria.

Resistance to the A compound of streptogramins is mediated by products of the *vat* genes (*v*irginiamycin *a*cetyl*t*ransferase), of which at least five molecular classes are known (*vatA-E*). These genes are located on plasmids of *Enterococcus faecium* and frequently in combination with a gene encoding a streptogramin hydrolase (*vga/vgb*) in staphylococci.

Enzymes transferring an acetyl moiety to one specific of several amino-groups of the aminocyclitol–aminoglycoside antibiotics (e.g. gentamicin, amikacin, kanamycin) are called aminoglycoside acetyltransferases (AAC). These enzymes are also widely distributed among Gram-positive and Gram-negative pathogens and are classified according to the position of the amino group modified (e.g. AAC3, AAC6′). They are further subdivided into different molecular classes based upon substrate profile (e.g. AAC6′-I, AAC6′-II) and amino acid sequence homology (e.g. AAC6′-IIa, AAC6′-IIb). A summary of relevant aminoglycoside modifying enzymes (AMEs) mediating resistance to clinically used aminoglycosides is given by Shaw et al [3].

Nucleotidyltransferases, Phosphotransferases

Beside AAC enzymes two different enzyme classes, nucleotidyltransferases (ANT enzymes), and phosphotransferases (APH enzymes) modify the hydroxyl groups of aminocyclitol–aminoglycoside antibiotics.

Alteration of the Target

Target alterations usually result in the reduction of the affinity for a drug. This strategy enables the bacteria to respond to alterations in the selective pressure due to, e.g. novel antibiotics within a short period of time. Genetic basis for a target alteration are either point mutations in the respective target gene or acquisition of a DNA fragment containing information for a an altered less susceptible target. Alternatively, targets can be modified enzymatically.

Target Alteration by Point Mutations

The major mechanism of resistance to fluoroquinolones is the acquisition of point mutations in the genes *gyrA* and *parC* encoding the respective A subunits of both targets, ►topoisomerases II and IV. Only a few codons are affected which are located in the so-called quinolone resistance-determining region (QRDR) between ala-67 and gln-106 (*E. coli* numbering of *gyrA*). To achieve a high level of resistance, at least two *gyrA* and one *parC* mutations are required.

Other examples include rifampin resistance due to mutations in the *rpoB* gene encoding the β-subunit of RNA polymerase, or oxazolidinone resistance due to a G2576T mutation in the gene for the 23S rRNA as central part of the 50S large ribosomal subunit. Macrolide resistance is based upon the alteration of nucleotide A2058 by a point mutation.

Target Alteration by Enzymatic Modification

An alternative strategy to modify nucleotide A2058 of the 23S rRNA is used by the majority of pathogens: the production of a specific methyltransferase which couples one or two methyl groups to the N6 amino group thereby reducing the affinity of macrolides to their target. Since this residue is also involved in the binding of other antibiotics, like streptogramin B and lincosamine, this mechanism is termed MLS_B type [4].

Another more sophisticated mechanism of enzymatic alteration of the target is the transfer of the complete regulon encoding a D-alanyl-D-lactate-ligase, like VanA, as basis for transferable resistance to glycopeptide resistance. In cooperation with some accessory factors the structure of the basic units of the bacterial cell wall, the disaccharide-pentapeptide with a C-terminal D-alanine, is transformed into a variant carrying a C-terminal D-lactate which prevents the binding of glycopeptide antibiotics at physiological concentrations and, thus, causes glycopeptide resistance. Such regulons reside on transposons allowing for the rapid exchange of the genetic information between different enterococcal species and even to staphylococci.

Target Modification by Acquisition of Genes Encoding Resistant Target

In contrast to macrolides, the targets of β-lactams, the penicillin binding proteins (PBPs) require several mutations in order to become resistant while simultaneously maintaining their viable function as cell wall transpeptidases/transglycosidases. Thus, in order to achieve clinically relevant resistance *Streptococcus pneumoniae* uses a unique strategy to rapidly accumulate several point mutations. Due to its natural competence for transformation during respiratory tract infections *S. pneumoniae* cells can acquire and insert into their chromosomes genetic material from closely related species, like viridans group streptococci. Since these cells carry genes for PBPs with reduced sensitivity to β-lactams due to the presence of several genetic variations, transformation/recombination generate so-called ►mosaic genes encoding a β-lactam-resistant variant protein, PBPX.

Staphylococcus aureus cells can acquire large DNA fragments containing the *mecA* gene which encodes a complete new penicillin binding protein 2A (PBP 2A), as part of a transposon. PBP2A can substitute the natural set of penicillin-sensitive PBPs thereby mediating a complete cross resistance to all β-lactam antibiotics.

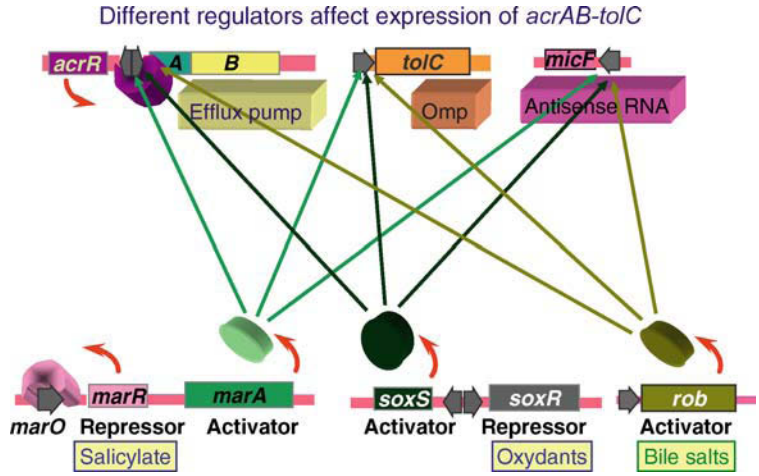
Reduced Drug Accumulation at Target Site

Uptake of nutrients from the environment, release of cell signalling molecules and virulence factors as well as disposal of toxic compounds are essential for metabolic active microorganisms. In contrast to Gram-positive bacteria having a cytoplasmic membrane only, Gram-negative bacteria are surrounded by an additional outer membrane, which forms a strict barrier between the intracellular space and the environment. While lipophilic compounds can directly penetrate the membranes' lipid bilayers, hydrophilic molecules require transmembrane proteins forming water-filled pores for passive diffusion into the cell or energy-driven transmembrane pumps for active efflux.

Two mechanisms are operating alone or in concert to minimize the antibiotic concentration at the intracellular target site: Downregulation of the expression of the pore proteins, also called porins, and upregulation of one or a set of several unspecific efflux pumps. However, the impact of these mechanisms on the resistance is low, since due to the essential function of porins for uptake of nutrients their reduction is limited and to avoid disturbances of membrane integrity due to extensive overproduction of mdr efflux pumps these are subjected a strict regulation.

Resistance Due to Multiple-Drug Resistance (mdr) Efflux

Currently, five different molecular classes of mdr efflux pumps are known [5]. While pumps of the the ATP-binding cassette (ABC) transporter superfamily are driven by ATP hydrolysis, the other four superfamilies called resistance-nodulation-division (RND), major facilitator superfamily (MFS), multidrug and toxic compound extrusion (MATE), and small multidrug resistance transporter (SMR) are driven by the proton-motive force across the cytoplasmic membrane. Usually a single pump protein is located within the cytoplasmic membrane. However, the RND-type pumps which are restricted to Gram-negative bacteria consist of two additional components, a periplasmic membrane fusion protein (MFP) which connects the efflux pump to an outer



Antibiotic Resistance. Figure 2 The major mdr efflux pump of *Escherichia coli* belongs to the RND superfamily and consists of the pump AcrB, the membrane fusion protein AcrA, and the porin TolC. The expression of the genes *acrAB* is under control of the local repressor AcrR, while *tolC* and *acrAB* are additionally regulated by several global transcriptional activators, like MarA induced by salicylate, SoxS derepressed by oxidants, and by Rob directly activated by bile salts.

membrane porin. This architecture allows the disposition of the antibiotics outside the cell (Fig. 2). Efflux is due to an enzymatic activity and therefore saturable.

The combined intrinsic activities of different efflux pumps play a major role for the intrinsic resistance of Gram-negative bacteria to macrolides and oxazolidinones as well as to the intrinsic resistance of *Pseudomonas aeruginosa* against a broad range of disinfectants and antibiotics.

Acquired resistance has been observed by constitutive upregulation of mdr efflux pump expression due to a mutation inactivating a respective repressor or inducibly, caused by molecules transiently inactivating repressor molecules upon binding. Depending upon the substrate spectra of the respective subset of efflux pumps upregulated, a multiple drug resistance (mdr) phenotype is expressed, which in combination with a specific resistance mechanism can contribute to a clinically relevant level of resistance.

Resistance to Tetracyclines Due to Specific Efflux Pumps

For some naturally occurring antibiotics, like chloramphenicol and tetracyclines, specific drug efflux pumps have been detected in antibiotic-producing bacteria. The tetracycline specific efflux pumps have been detected in many bacterial pathogens and are the major mechanism of resistance to these drugs. Several of the corresponding structural genes are inducibly expressed: In the presence of tetracyclines in the growth medium a few drug molecules enter the cell by diffusion, bind to the pump repressor and, thus, induce tet efflux pump expression.

►β-Lactam Antibiotics
►Ribosomal Protein Synthesis Inhibitors
►Quinolones
►Microbial Resistance to Drugs

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Antibiotics

Originally, the term antibiotics referred to substances produced by microorganisms that suppressed the growth of other organisms. Today, the term antibiotics often includes synthetic antimicrobial agents.

►Microbial Resistance to Drugs
►β-Lactam Antibiotics

►Ribosomal Protein Synthesis Inhibitors
►Anthracyclins

Antibodies

Antibodies are involved in the humoral immune response. They recognize foreign substances (antigens) and trigger immune responses by the host. For the former, they possess interaction sites for a specific antigen. These interaction sites (Fab portions) are highly variable between antibodies produced by different clones of B cells. For the latter, they possess a constant region (Fc portion). Engineered antibodies are increasingly used for the treatment of human diseases.

►Immune Defense
►Humanized Monoclonal Antibodies

Antibodies to Cyclic-citrullinated Peptides (Anti-CCPs)

It has long been known that antiperinuclear factor and antikeratin antibodies have high specificity for rheumatoid arthritis. Both these antibodies recognize epidermal fillagrin, a protein involved in the cornification of the epidermis. The amino acid target of these antibodies is citrulline, which is derived from the amino acid arginine after peptide translation under the influence of the enzyme peptidyl arginine deiminase. Antibodies directed at cyclic citrullinated peptides have a similar sensitivity to rheumatoid factor, but higher specificity.

►Rheumatoid Arthritis

Antibody-dependent Cellular Cytotoxicity (ADCC)

A mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies. The typical ADCC involves activation of natural killer (NK) cells and is dependent on the recognition of antibody-coated cells by Fc receptors on the surface of the NK cell. The Fc receptors recognize the Fc (constant) portion of antibodies such as IgG, which bind to the surface of a target cells. Following binding NK cells release cytokines such as IFN-γ and cytotoxic granules containing perforin and granzymes that enter the target cell and promote cell death by triggering apoptosis.

►Immune Defence
►Interferons
►Tyrosine Kinase Inhibitors

Anticancer Drugs

►Antineoplastic Agents

Anticoagulants

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Synonyms

Oral anticoagulants, usually coumarin derivatives (e.g., warfarin, phenprocoumon); heparin, either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH); danaparoid (heparinoid); fondaparinux (indirect factor Xa-inhibiting pentasaccharide); drotrecogin α (recombinant human activated protein C [APC]); direct thrombin inhibitors (DTIs), including hirudin derivatives (e.g., lepirudin, desirudin) or analogues (e.g., bivalirudin) and small molecule active site inhibitors (e.g., argatroban, ximelagatran)

Definition

Anticoagulants inhibit ►coagulation by preventing ►thrombin generation and, ultimately, fibrin formation [1]. They represent one of the two major classes of antithrombotic drugs, the other being antiplatelet agents. Anticoagulants are widely used to treat and prevent thrombosis involving arteries, veins, and intracardiac chambers.

In general, arterial thrombi are platelet-rich ("white clots") and form at ruptured atherosclerotic plaques, leading to intraluminal occlusion of arteries that can result in end-organ injury (e.g., myocardial infarction, stroke). In contrast, venous thrombi consist mainly of fibrin and red blood cells ("red clots"), and usually form in low-flow veins of the limbs, producing deep vein thrombosis (DVT); the major threat to life results when lower extremity (and, occasionally, upper extremity) venous thrombi embolize via the right heart chambers into the pulmonary arteries, i.e., pulmonary embolism (PE).

Mechanism of Action

Overview of Coagulation

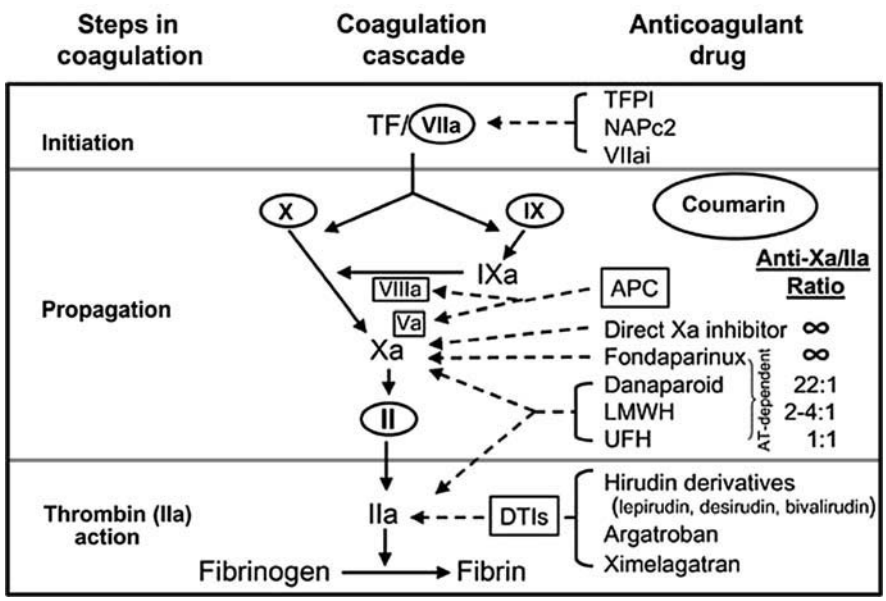
Figure 1 shows a simplified scheme of the coagulation cascade. Coagulation is usually triggered physiologically when ►tissue factor (TF), usually found in extravascular sites, binds to circulating factor VII(a) following vessel injury. TF/VII(a) complexes activate factor X, generating factor Xa. Factor Xa, together with a cofactor (factor Va), forms "prothrombinase" on phospholipid surfaces on activated platelets. Prothrombinase generates the key procoagulant enzyme, thrombin (factor IIa), from prothrombin (factor II). Various positive feedback loops help to convert a small procoagulant stimulus into a thrombin burst. For example, TF/VII(a) complexes also activate factor IX to IXa, which acts with a cofactor (VIIIa) to form the "tenase" complex that activates factor X to Xa. Other positive feedback loops initiated by thrombin include activation of factors V to Va, VIII to VIIIa and XI to XIa (not shown in Fig. 1).

Coagulation is regulated by three major inhibitory systems. (i) Antithrombin (AT, formerly, antithrombin III) inhibits circulating thrombin, Xa, IXa, XIa and TF/VII(a). However, AT does not inhibit thrombin bound to fibrin ("clot-bound thrombin") or surface-bound Xa. (ii) The protein C natural anticoagulant pathway is triggered when thrombin binds to a receptor (thrombomodulin, TM) on endothelial cell surfaces: TM-bound thrombin activates protein C to APC, which together with a cofactor (protein S) degrades factors Va and VIIIa, thus downregulating thrombin generation in the TM-rich microcirculation. (iii) Tissue factor pathway inhibitor (TFPI) binds to and inhibits factor Xa; subsequently, TFPI/Xa complexes inhibit VII(a) within VII(a)/TF.

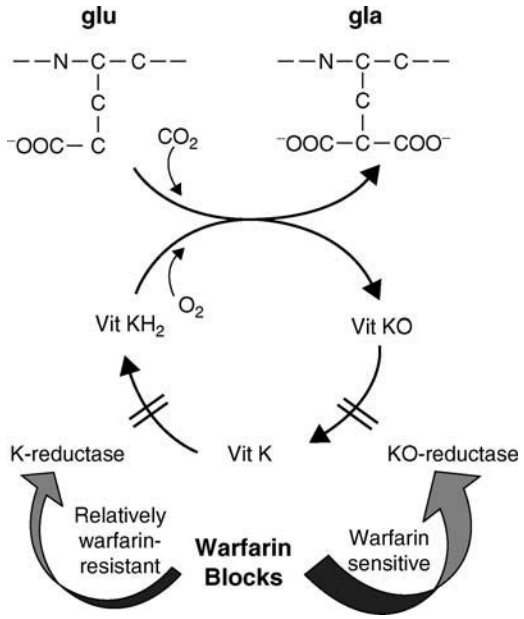
The most commonly used anticoagulants – coumarins and heparins – interfere with various steps, involving "propagation" of the coagulation cascade. Several newer agents inhibit thrombin directly. Drugs that inhibit initiation of coagulation are under investigation.

Oral Anticoagulants (Coumarins)

Most oral anticoagulants are coumarin derivatives that act via ►vitamin K antagonism ([2]; Fig. 2). Vitamin K



Anticoagulants. Figure 1 Effects of anticoagulants on the coagulation cascade. Coumarin agents alter the synthesis of four procoagulant zymogens (VII, X, IX, II), shown within circles. The other anticoagulants affect various coagulation factors (dotted arrows). Abbreviations: APC, activated protein C; AT, antithrombin; DTIs, direct thrombin inhibitors; LMWH, low-molecular-weight heparin; NAPc2, nematode anticoagulant protein; TF, tissue factor, TFPI, tissue factor pathway inhibitor; UFH, unfractionated heparin; VIIai, active site-blocked VIIa. (Modified from [1], with permission from Chest.)



Anticoagulants. Figure 2 Coumarin (Warfarin) and the vitamin K cycle. Abbreviations: glu, glutamate; gla, γ-carboxyglutamate. (Modified from [2], with permission from Chest.)

is required for posttranslational modification of certain glutamate (glu) residues in four procoagulant factors (II, VII, IX, X). Addition of a carboxyl group (COO-) to each glu residue (to form γ-carboxyglutamate, or gla, residues) causes these vitamin K-dependent factors to become functional ►zymogens (proenzymes), as they now can bind to phospholipid surfaces via Ca^{2+}-recognizing gla regions. Protein C and protein S are vitamin K-dependent anticoagulant factors.

The two most widely used coumarins are warfarin (US, Canada, and UK) and phenprocoumon (continental Europe). The long half-life (60 h) of prothrombin means that coumarin cannot achieve therapeutic anticoagulation for at least 5 days following initiation. Thus, for patients with acute thrombosis, oral anticoagulants are usually started only when the patient is receiving a rapidly active agent, usually UFH or LMWH.

Disadvantages of oral anticoagulants include a narrow therapeutic index (bleeding risk), their highly variable dose–response relation (ongoing need for monitoring), embryopathy (if administered during the first trimester of pregnancy), and potential to induce microvascular thrombosis (coumarin necrosis syndromes).

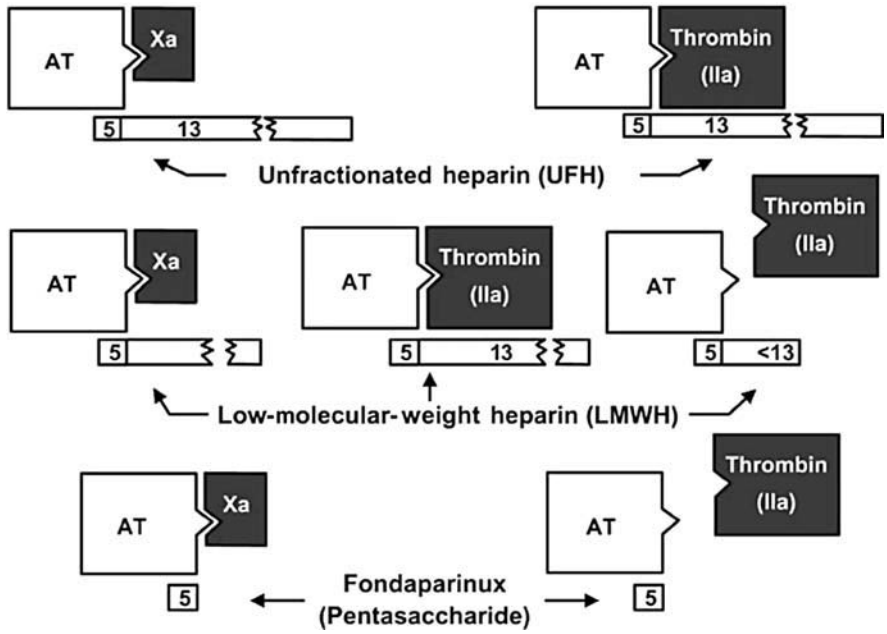
Maintenance doses widely vary among patients (e.g., from 1 to 20 mg/day for warfarin), and are influenced by diet (variable vitamin K intake) and medications that affect coumarin metabolism (decreased drug clearance: e.g., cotrimoxazole, amiodarone, erythromycin; increased clearance: e.g., barbiturates, carbamazepine, rifampin). Thus, regular monitoring is needed even during long-term maintenance therapy. This is performed using the ►prothrombin time (PT), which is usually expressed as the ►international normalized ratio (INR).

AT-dependent Anticoagulants: Heparins, Danaparoid, and Fondaparinux

Heparin is a highly sulfated ►glycosaminoglycan [3]. Usually obtained from pig intestine or beef lung, UFH contains polymer varying from 3,000 to 30,000 Da (mean, 15,000 Da; range, 10–90 monosaccharide units). Chemical or enzymatic methods can be used to make LMWH preparations that vary from 1,000 to 10,000 Da (mean, 4,500 Da; range, 3–30 monosaccharide units). A specific five saccharide sequence ("AT-binding pentasaccharide") present within up to one third of UFH chains binds to AT, greatly increasing the efficiency of AT to inactivate thrombin, Xa, IXa, XIa, and TF/VII(a). AT is most efficient at inactivating thrombin and Xa, as shown by higher second-order rate constants (8,900 and 2,500 $M^{-1}s^{-1}$, respectively compared with values of 300–450 for VII(a)/TF, IXa and XIa, respectively). Catalysis by UFH increases AT-mediated inhibition 1,000-fold.

Besides containing the specific AT-binding pentasaccharide sequence, heparin molecules must be at least 18 monosaccharide units long to bind to both AT and thrombin; in contrast, AT bound to any pentasaccharide-containing heparin – even with a chain length <18 monosaccharide units – will inhibit factor Xa. Thus, whereas UFH catalyzes inhibition of thrombin and Xa equally well, LMWH preferentially inhibits factor Xa (usual anti-Xa/anti-IIa ratio, 2–4:1) (Fig. 3). LMWH preparations (e.g., ardeparin [Normiflo], dalteparin [Fragmin], enoxaparin [Lovenox], reviparin [Clivarin], tinzaparin [Innohep]) differ in both jurisdictional availability and composition, and cannot be assumed to be interchangeable.

The ►activated partial thromboplastin time (aPTT) is usually used to monitor the anticoagulant effect of UFH, with the target aPTT level corresponding to an anti-factor Xa level of 0.35–0.70 U/mL (i.e., a ratio of patient/control aPTT of 1.5–2.5 for many aPTT reagents). However, prolongation of the aPTT is not sufficiently great to permit monitoring of LMWH therapy by this test. Nevertheless, since the shorter LMWH polymers have less nonspecific binding to plasma proteins, LMWH anticoagulation is quite predictable. Thus, weight-adjusted LMWH dosing without monitoring is standard practice. Particularly during inflammation (high levels of UFH-binding proteins), high doses of UFH may be needed to prolong the aPTT and anti-factor Xa levels into the therapeutic range (heparin "resistance"). Anticoagulant monitoring of LMWH using anti-factor Xa levels may be needed in renal failure as LMWH accumulates.



Anticoagulants. Figure 3 Relative effects of UFH, LMWH, and fondaparinux on AT-mediated inhibition of factor Xa and thrombin (IIa). Whereas UFH catalyzes inhibition of Xa and thrombin equally well, only LMWH chains of 18 saccharide units or longer catalyze thrombin inhibition; thus, the anti-Xa/anti-IIa ratio of LMWH preparations ranges from 2:1 to 4:1. In contrast, fondaparinux exclusively inhibits Xa. (Modified from [3], with permission from Chest.)

Danaparoid (Orgaran; mean MW, 6,000 Da) is a mixture of nonheparin glycosaminoglycans derived from pig gut (dermatan sulfate, heparan sulfate, chondroitin sulfate). The anti-Xa/anti-IIa ratio (22:1) is even greater than seen with LMWH. The anti-IIa effect may be mediated in part by dermatan sulfate, which catalyzes thrombin inhibition by heparin cofactor II.

Fondaparinux, the factor Xa-binding pentasaccharide (Arixtra, MW 1,728 Da), is prepared synthetically, unlike UFH, LMWH and danaparoid, which are obtained from animal sources. Despite only inactivating free factor Xa, clinical trials indicate that fondaparinux is an effective antithrombotic agent, both for venous thromboembolism prophylaxis and treatment, as well as for acute coronary syndrome and ST elevation myocardial infarction [4].

In addition to the AT-dependent agents discussed above, various direct Xa inhibitors (e.g., tick anticoagulant peptide, antistatin, DX-9065a) are undergoing clinical testing. Unlike fondaparinux, these drugs also inhibit surface-bound Xa within prothrombinase.

Activated Protein C

Protein C is a vitamin K-dependent natural anticoagulant activated by thrombin to form APC in the presence of the endothelial receptor, TM. APC proteolyzes factors Va and VIIIa, thus downregulating thrombin generation. APC may also have anti-inflammatory properties, as recombinant human APC (drotrecogin α, Xigris) reduces mortality in ►septicemia. Nonactivated protein C concentrates, prepared from pooled plasma, are also available for use in patients with congenital or acquired protein C deficiency.

Direct Thrombin Inhibitors

There are two major classes of DTIs: hirudin derivatives and small molecule active site inhibitors. Hirudin is a 65-amino acid polypeptide produced by the medicinal leech, which binds irreversibly and with high affinity to both the active site and exosite I (fibrinogen binding site) regions of thrombin, resulting in stable noncovalent hirudin–thrombin complexes (dissociation constant, $\sim 10^{-14}$ M). Hirudin binds to both circulating and clot-bound thrombin. Lepirudin (Refludan, MW ~7,000 Da) and desirudin (Revasc) closely resemble hirudin. In contrast, bivalirudin (Angiomax, MW ~2,180 Da) is a 20-amino acid oligopeptide consisting of the active site and exosite I regions of hirudin connected by a short "spacer." All three agents are obtained by recombinant technology.

Two small molecule DTIs are argatroban (Novastan, MW 527 Da) and the oral thrombin inhibitor, ximelagatran (Exanta, MW 474 Da) Ximelagatran is an inactive pro-drug: after absorption, it is metabolized to the active DTI, melagatran [MW 430 Da]. Concerns regarding hepatotoxicity have prevented (xi)melagatran

from successful regulatory and marketplace adoption. In general, levels of DTIs are monitored indirectly using the ►aPTT (usual target therapeutic range, about 1.5–2.5-times baseline aPTT). The DTIs prolong the INR in the order: argatroban > bivalirudin > lepirudin.

Factor VII(a)/Tissue Factor Pathway Inhibitors

Recombinant TFPI (tifacogin) directly inhibits VII(a)/TF complexes. Unlike recombinant APC, TFPI did not reduce mortality in clinical trials of septicemia. Recombinant nematode anticoagulant protein (NAPc2) is a small hookworm protein that binds to a noncatalytic site on both X and Xa, thus inhibiting VII(a)/TF. The half-life of NAPc2 is long (48 h), resembling that of factor X. Active site-blocked VIIa (factor VIIai) achieves an anticoagulant effect by competing with VII(a) for binding to TF.

Clinical Use (Including Side Effects)

Both UFH and LMWH are used when rapid anticoagulation is needed, such as acute venous thromboembolism (DVT and/or PE), acute coronary insufficiency (acute myocardial infarction or unstable angina), or for ►percutaneous coronary intervention (PCI). UFH is also used for intraoperative anticoagulation during cardiac surgery employing cardiopulmonary bypass (CPB) as well as during vascular surgery. ►Protamine sulfate is used to reverse UFH anticoagulation after heart surgery.

Treatment of DVT or PE consists of therapeutic-dose heparin, given as intravenous UFH or subcutaneous LMWH or fondaparinux with overlapping oral anticoagulation. Until the early 1990s, UFH was usually given alone for 5 days, followed by at least 5 days of UFH/coumarin overlap, then several months of coumarin anticoagulation. Now, coumarin is often started within 24 h of initiating UFH or LMWH. Duration of coumarin typically ranges from as low as 6 to 8 weeks (small calf-vein DVT in a transient prothrombotic situation, such as postsurgery) to indefinite (multiple prior DVTs complicating a chronic hypercoagulability state). Often, treatment of DVT or PE employing LMWH followed by oral anticoagulants occurs exclusively in an outpatient setting.

Prevention of DVT and PE (antithrombotic prophylaxis) is another common indication for UFH, LMWH or coumarin, especially following surgery or immobilizing trauma. Fondaparinux is approved for prevention of DVT and PE after hip and knee surgery, and following abdominal surgery.

Coumarin is also widely used for long-term anticoagulation in chronic atrial fibrillation (particularly to avoid cardioembolic strokes), to prevent DVT or PE in patients with chronic hypercoagulability (e.g., congenital AT or protein C deficiency), or to prevent atherothrombosis in patients with atherosclerosis. Coumarin is often unsuccessful in patients with hypercoagulability states, such as immune heparin-induced thrombocytopenia or cancer-associated disseminated intravascular coagulation. In contrast, LMWH therapy is often appropriate for patients with cancer-associated hypercoagulability or to prevent or treat thrombosis during pregnancy.

Danaparoid, lepirudin, and argatroban are important options for rapid anticoagulation when UFH or LMWH are contraindicated (e.g., heparin-induced thrombocytopenia). Desirudin is approved in some jurisdictions for antithrombotic prophylaxis after hip replacement surgery. Bivalirudin is an alternative to heparin for anticoagulation during ►PCI; both bivalirudin and argatroban are approved for anticoagulation during PCI in patients in whom heparin is contraindicated because of acute or previous heparin-induced thrombocytopenia.

Side Effects

Bleeding is the most common adverse effect of anticoagulants [1–3] and is often associated with overdosing. When bleeding occurs during anticoagulation within the target therapeutic range, factors such as recent surgery or gastrointestinal lesions often coexist. For bleeding caused by coumarin overdosing, vitamin K will reverse anticoagulation beginning at least 4 h after administration. More urgent reversal can be achieved by coagulation factor replacement, using plasma or prothrombin complex concentrates. Rapid reversal of UFH is achieved by ►protamine sulfate (1 mg protamine for 100 U heparin). However, only about 60% of the anticoagulant effect of LMWH is neutralized by protamine. Specific antidotes are not available for danaparoid, fondaparinux, DTIs, or inhibitors of the VII(a)/TF pathway. Thus, careful patient selection and anticoagulant monitoring are usually needed to reduce bleeding risk with these newer agents.

Unusual adverse effects sometimes occur with coumarin [2] or heparin [5]. For example, coumarin-induced skin necrosis is a rare complication of oral anticoagulants characterized by (sub)dermal microvascular thrombosis that usually begins 3–6 days after commencing coumarin. Typically, central tissue sites such as the breast, abdomen, and thigh are affected. Congenital abnormalities of the protein C natural anticoagulant pathway are implicated in some patients. A related syndrome of microvascular thrombosis can lead to limb gangrene in some patients treated with oral anticoagulants during heparin-induced ►thrombocytopenia and hypercoagulability. This syndrome of coumarin-induced venous limb gangrene has been linked to severe protein C depletion during use of warfarin to treat DVT, complicated by metastatic cancer or heparin-induced thrombocytopenia.

As many as 3–5% of postoperative patients who receive UFH for 2 weeks develop heparin-induced thrombocytopenia, also known as HIT. This hypercoagulable state is caused by IgG antibodies that recognize complexes between heparin and platelet factor 4 (a platelet α-granule protein). Paradoxically, patients with HIT remain at high risk for thrombosis, even when heparin is discontinued or heparin is replaced with coumarin. To avoid coumarin-induced venous gangrene, alternative anticoagulants such as danaparoid, lepirudin, or argatroban should be given, and coumarin delayed until thrombocytopenia has resolved. Vitamin K should be given to reverse coumarin if HIT is diagnosed after warfarin or another vitamin K antagonist has been given. Long-term UFH treatment can cause ►osteoporosis, likely because heparin both decreases bone formation by osteoblasts and increases bone resorption by osteoclasts. Both HIT and osteoporosis are less likely to occur with LMWH.

Coumarins are generally contraindicated for use during pregnancy, particularly the first trimester. This is because γ-carboxyglutamate (gla), containing proteins are found in bone. Thus, pharmacologic vitamin K antagonism can cause embryopathy (chondrodysplasia punctata). LMWH is an attractive option for many pregnant women who require anticoagulation.

►Antiplatelet Drugs
►Coagulation/Thrombosis
►Fibrinolytics

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Anticonvulsants

►Antiepileptic Drugs

Antidepressant Drugs

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Synonyms

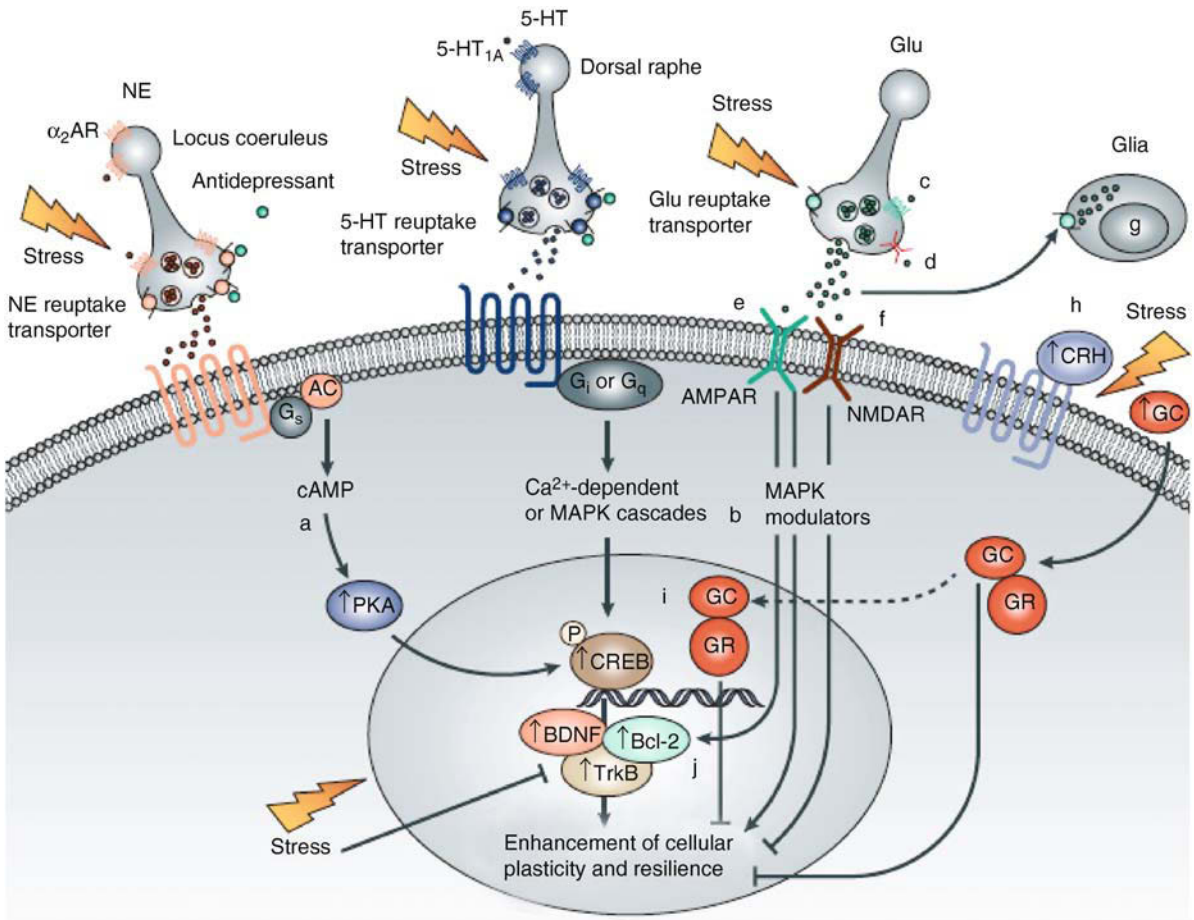
Antidepressants; Mood elevators; Psycho energizers

Definition

Antidepressants are small heterocyclic molecules entering the circulation after oral administration and passing the blood–brain barrier to bind at numerous specific sites in the brain. They are used for treatment of depression, panic disorders, generalized anxiety disorder, social phobia, obsessive compulsive disorder, and other psychiatric disorders and nonpsychiatric states.

Mechanism of Action

Most available antidepressants enhance neurotransmission of ►biogenic amines, mainly ►norepinephrine and ►serotonin, to a lesser extent dopamine. Once released from specialized vesicles at the presynaptic nerve terminal ►neurotransmitters enter the synaptic cleft and bind to respective receptors at the postsynaptic cell membrane, thus modulating the associated signaling cascades (Fig. 1). Additionally, some of them bind to presynaptically localized receptors that regulate the amount of transmitter released. The cell membrane of presynaptic nerve terminals also contains ►reuptake transporters that clear the synaptic cleft from biogenic amines. Once reshuffled into the presynaptic compartment the neurotransmitter is degraded by ►monoamine oxidase (MAO). These two molecular processes, reuptake through specific transporters and enzymatic degradation by MAO, are targeted by most of the antidepressant drugs. For example, the ►selective serotonin reuptake inhibitors (SSRI; citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline) and the so called "dual acting drugs" (the selective serotonin/noradrenaline reuptake inhibitors venlafaxine and duloxetine and the noradrenergic/selective serotonergic drug mirtazapine), which became the mainstay for the treatment of the majority of depressed patients either prevent clearance of serotonin and/or noradrenaline from the synaptic cleft by blocking the presynaptic transporter and thus amplify receptor-mediated events postsynaptically or antagonize specific pre- and postsynaptic receptors. Analogous effects are those by norepinephrine reuptake inhibitors, while MAO-inhibitors act by reducing norepinephrine or serotonin degradation and thus increase the releasable amount of



Antidepressant Drugs. Figure 1 Effects of stress as a model for depression and the reversal by the use of antidepressants. Multiple intracellular targets might be involved in the regulation of plasticity and resilience by antidepressants, which block extracellular transporters. Adapted from [1].

neurotransmitter from the respective vesicles. These drugs do not exert prompt antidepressant effects as it takes weeks or months until clinical amelioration occurs. The exact mode of action by which antidepressants work is still not resolved but there is consensus that their primary action, i.e., binding to cell membrane transporters, triggers a manifold of events, which we are only beginning to decipher (Fig. 1; [1]). Ingredients of St. John's wort have serotonin-/noradrenaline-/GABA- and glutamate reuptake inhibiting properties. The older tricyclic antidepressants like amitriptyline are also noradrenaline and/or serotonin reuptake inhibitors but also show antagonistic effects on different cerebral receptors like α1-adrenergic, cholinergic, histaminergic receptors.

One such hypotheses submits that most antidepressants enhance the expression of cyclo-AMP response element binding protein (CREB), which is a transcription factor that after phosphorylation binds to cyclo-AMP response elements localized in the promoter region of many genes including that coding for ▶brain derived neurotrophic factor (BDNF) [2]. The latter neurotrophin was found to be decreased in the hippocampus of chronically stressed rats, serving as animal model of depression. When treated with antidepressants, BDNF expression increases, possibly through enhanced phospho-CREB driven transactivation of the *BDNF* gene. This hypothesis is in keeping with the frequently observed reduction of depressed patients hippocampus volume (estimated by magnetic resonance imaging), a limbic brain structure pertinent for cognitive function, and expressing BDNF at high levels. Some preliminary studies support that antidepressants increase adult neurogenesis in this brain area, a phenomenon also associated with increased levels of phospho-CREB. The hypothesis that phospho-CREB is involved in adult neurogenesis is also strengthened by experiments with transgenic mice overexpressing a dominant negative isoform of CREB where the Ser^{133} is mutated preventing phosphorylation-induced transactivation of CREB. Overexpression of mutant CREB prevented decreased neurogenesis in adult

hippocampus. While many pieces of this hypothesis are in line with an antidepressant-induced enhancement of neurogenesis, evidence is lacking that this effect is the same through which antidepressants regulate emotional states. Morphological studies on brains of depressives failed to detect evidence for neuronal deterioration in the hippocampus. Moreover the increase of *BDNF* gene transcription as induced by antidepressants is possibly an unspecific response to a xenobiotic molecule. Whether increased transcription of BDNF conveys antidepressant effects is yet not proven, as mouse mutants where BDNF production is lowered by heterozygous gene deletion failed to show behavioral abnormalities. Also, data on drug-induced changes in BDNF peptide concentrations are not giving a clear picture.

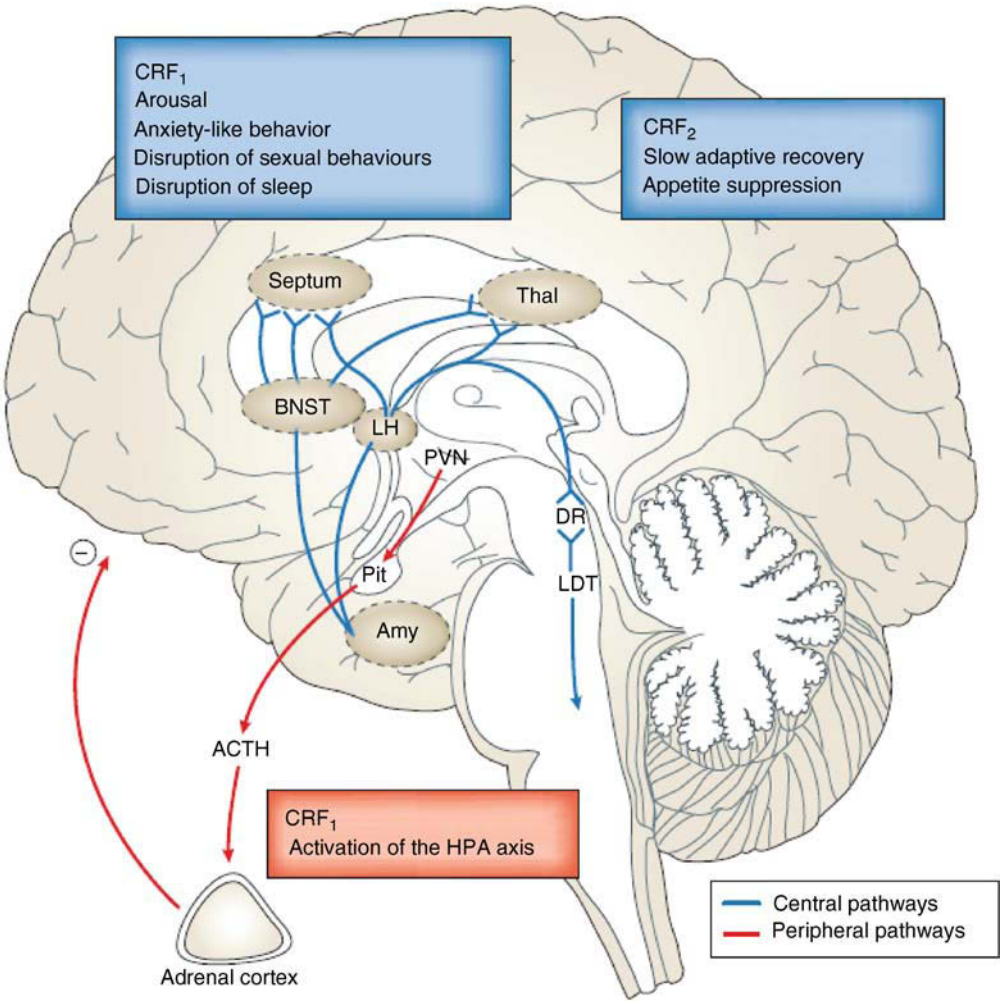
Another hypothesis derives from the clinical observation that impaired stress hormone regulation is a cardinal symptom among patients with an acute major depressive episode. If stress hormones (primarily cortisol secreted by adrenocortical glands and corticotropin released from the pituitary) are monitored longitudinally in these patients, those who respond to drug treatment show a trend towards neuroendocrine normalization while those where stress hormone regulation continues to be altered have a much worse outcome, i.e., they fail to respond or they relapse. Studies using transgenic mice with glucocorticoid receptor impairment show some behavioral and functional features reminiscent of depression. Some of these abnormalities disappear under antidepressants, which is in line with a drug-induced improvement of corticosteroid receptor function. When ligand-activated, these gluco- and mineralocorticosteroid receptors form homo- and heterodimers that interact with ►corticotropin releasing hormone (CRH) in many ways (Fig. 2). This is of relevance in this context because clinical and basic studies have shown that overexpression of CRH in many brain areas is causally related to development and course of depression. The effect of antidepressants has therefore consequences upon CRH secretion and it is believed that these antidepressants may work through this corticosteroid receptor driven signaling pathway, suppressing the depressogenic and anxiogenic effects of CRH acting through CRH type 1 receptors (CRHR1). Thus, the antidepressant-induced behavioral and neuroendocrine changes in patients together with their observed molecular actions upon stress hormone signaling pathways have triggered the search for new pharmacological approaches to understand how antidepressants might work and ultimately to discover better drugs.

In the absence of a robust pathogenetic model for depression, hypotheses-driven research has limitations that hopefully can be overcome by unbiased approaches. The availability of cDNA microarrays allowing one to study a huge amount of genes, which are simultaneously regulated in the brains of mice under long-term treatment with antidepressants will shift the emphasis from the "usual suspects" (such as serotonin and its receptors) to yet unheard candidate genes [3].

Clinical Use and Side Effects

Antidepressants were serendipitously discovered in the 1950s and the first generation of these drugs was constituted by tricyclic molecules. The refinement among the second and third generation of these drugs resulted in molecules that have less side effects, are better tolerated and consequently enjoy much better acceptance. In fact, the percentage of Americans treated for depression tripled nationwide. Simultaneously patient visits to doctors for depression fell by a third through the last 5 years. Such figures do not yet apply for Europe, where alternative treatments, especially herbals (St. Johns Wort is the best selling antidepressant in Germany), continue to play a major role. Given the personal and socioeconomic burden of depression the under-treatment of this disabling clinical condition seems neither ethical nor prudent.

While antidepressants have proven to be effective drugs, several drawbacks and caveats need to be resolved. This can be most likely achieved by enforced ►pharmacogenetic approaches (►pharmacogenetics) in combination with refined clinical research: Matching patients to the antidepressant that is most likely to be effective and less likely to harm through adversive reactions is the main goal of all modern therapies. Patient characteristics including sex, age, anxiety level, premedication, and family history (genetic load) do not predict better or worse response to a particular antidepressant drug or drug class. However, the fact that all drugs are equally effective between comparison groups does not mean that they are equally effective for individual patients. It is now hoped that combination of clinical data, including functional assessments, e.g., neuroendocrine, neuroimaging, neuropsychology together with information from genotyping, i.e., identification of a collection of single nucleotide polymorphisms (SNPs) will ultimately lead to choosing a first-line antidepressant based upon individual data. The most frequently examined candidate gene codes for the serotonin transporter. A 44-bp insertion or deletion results in a long and a short variant of this gene; the s-variant is associated with a twofold decreased expression and transport activity in vitro. Individuals in an epidemiological sample with one or two copies of the short allele of the serotonin transporter promoter polymorphism showed more depressive symptoms, diagnosable depression, and tendency to commit suicide in relation to stressful life events than individual homozygous for the long allele.



Antidepressant Drugs. Figure 2 The corticotropin-releasing factor system in depression. Corticotropin releasing factor (CRF) from the paraventricular nucleus (PVN) of the hypothalamus is released into the hypophyseal portal system and triggers the release of corticotrophin (ACTH) from the anterior pituitary via stimulation of CRF1 receptors. ACTH, in turn, stimulates the secretion of glucocorticoid hormones (cortisol in humans or corticosterone in rodents) from the adrenal cortex. Increased glucocorticoid levels suppress hypothalamic CRF expression via negative feedback through hippocampal and hypothalamic glucocorticoid receptors. The neurotransmitter action of CRF on CRF1 receptors throughout the limbic system mediates anxiogenic effects of stress. By contrast, its neurotransmitter action on CRF2 receptors in more discrete regions of the brain might reduce anxiety-like behavior in a delayed fashion (adapted from [2]).

Unfortunately, twin studies were not concordant in lending support to this result [4].

In practice, a genotype-guided medication selection is yet not in reach, but several minor innovations emerging from hypothesis-driven research are. The current antidepressive pipeline contains three promising candidates: ►Substance P, a peptide from the tachykinin family, which binds preferentially at the NK_1-receptor, was suspected to play a role in causality of depression. Several clinical studies testing NK_1-receptor antagonists showed promising results and a number of pharmaceutical companies are developing drugs antagonizing NK- receptors. NK_2-receptor antagonists might also be effective in the treatment of depression. Another neuropeptide is CRH that seems to be causally related to symptoms of depression through activation of CRHR1, which led to the development of CRHR1 antagonists as potential antidepressants. A first clinical study supported such a possibility. Finally, glutamate antagonists like ketamine might have some use in treatment-resistant depression.

Another new development of immediate clinical usefulness is the analysis of genetic variability in the cytochrome P450 enzyme system in patients, which

may elucidate clinically relevant changes in drug metabolization and adverse reactions. For example, if a patient receives an SSRI such as Prozac, which blocks the P4502D6 enzyme, and an antiarrhytmic, which is metabolized by the same enzyme, a fatal increase of the cardiotropic drug may occur. Other possible candidates involved in pharmacokinetics are P-glycoproteins, which are important regulators of a drug's blood–brain barrier passage. It was recently shown that antidepressants are substrates of P-glycoprotein, which, if overexpressed, can extrude the antidepressant out of the brain cells into the circulation thus preventing central effects that may lead to therapy resistance.

Side effects of antidepressants usually occur during the first days of treatment and tend to diminish over time. The side-effect profile can be easily derived from the transporter-binding profile. Serotonergic drugs might cause headaches, appetite loss, nervousness, sweating and sexual dysfunction and noradrenergic drugs palpitations, sweating, anxiety, and drowsiness. Anticholinergic antidepressants show side effects like constipation, blurred vision, memory dysfunction, dry mouth, while antihistaminergic drugs exhibit side effects like sedation, hypotension, and weight gain. Antiadrenergic properties are associated with postural hypotension and reflex tachycardia.

►Neurotransmitter Transporters
►Noradrenaline Transporter
►Serotoninergic System
►Monoamine Oxidases

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Antidiabetic Drugs

Antidiabetic drugs is the general term for drugs that lower blood glucose concentrations and are used in the treatment of diabetes mellitus. Antidiabetic drugs are typically categorized as either oral (sulphonylureas, prandial insulin releasers, metformin, thiazolidinediones, alpha-glucosidase inhibitors) which are used to treat most type 2 (non-insulin-dependent) diabetic patients, or insulin (given parenterally) which is used to treat all type 1 and some type 2 diabetic patients.

►Diabetes Mellitus
►Insulin
►Antidiabetic Drugs other than Insulin

Antidiabetic Drugs other than Insulin

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Synonyms

Oral hypoglycaemic agents; Oral blood glucose-lowering drugs; Insulin secretagogues; Antihyperglycaemics

Definition

Antidiabetic drugs are used to treat hyperglycaemia in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus. They are used in conjunction with non-pharmacological interventions involving diet, exercise and health education. Insulin therapy is essential for all type 1 patients and is often used to treat more advanced stages of type 2 diabetes. Other antidiabetic drugs are mostly used to treat type 2 diabetes, which accounts for more than 85% of all cases of diabetes. The classes of antidiabetic drugs other than insulin are sulphonylureas, prandial insulin releasers (also termed meglitinides), the biguanide metformin, thiazolidinediones (TZDs), α-glucosidase inhibitors, incretin mimetics, gliptins (also termed dipeptidyl peptidase-4 inhibitors or incretin enhancers) and the amylin analogue pramlintide (Table 1) [1].

Mechanism of Action

Type 2 diabetes is a heterogeneous and progressive endocrine disorder associated with insulin resistance (impaired insulin action) and defective function of the insulin-secreting β-cells in the pancreatic islets of Langerhans. These endocrine disorders give rise to widespread metabolic disturbances epitomised by hyperglycaemia. The present classes of antidiabetic agents other than insulin act to either increase insulin secretion, improve insulin action, slow the rate of intestinal

Antidiabetic Drugs other than Insulin. Table 1 Classes of antidiabetic drugs other than insulin and their main mechanisms of action

Class	Examples[a]	Main mechanism of action	Route
Sulphonylureas	Chlorpropamide, glibenclamide[b], gliclazide, glimepiride, glipizide, gliquidone, tolazamide, tolbutamide	Stimulate insulin secretion (typically 6–24 h)	Oral
Prandial insulin releasers (meglitinides)	Repaglinide, nateglinide	Stimulate insulin secretion (rapid and short-acting < 6 h)	Oral
Biguanide	Metformin	Improve insulin action	Oral
Thiazolidinediones	Pioglitazone, rosiglitazone	Improve insulin action (PPARγ agonists)	Oral
α-Glucosidase inhibitors	Acarbose, miglitol, voglibose	Slow rate of carbohydrate digestion	Oral
Incretin mimetic	Exenatide	Mimic GLP-1[c]: enhance prandial insulin secretion	SC injection[d]
Gliptins (DPP-4 inhibitors)	Sitagliptin	Inhibit DPP-4[e]: enhance prandial insulin secretion	Oral
Amylin analogue	Pramlintide	Suppress glucagon secretion and slow gastric emptying	SC injection[c]

[a]Availability of agents and prescribing instructions vary between countries.
[b]Glibenclamide is called glyburide in some countries.
[c]Glucagon-like peptide-1.
[d]Subcutaneous injection.
[e]Dipeptidyl peptidase-4.

carbohydrate digestion, enhance the incretin effect, suppress glucagon secretion or slow gastric emptying.

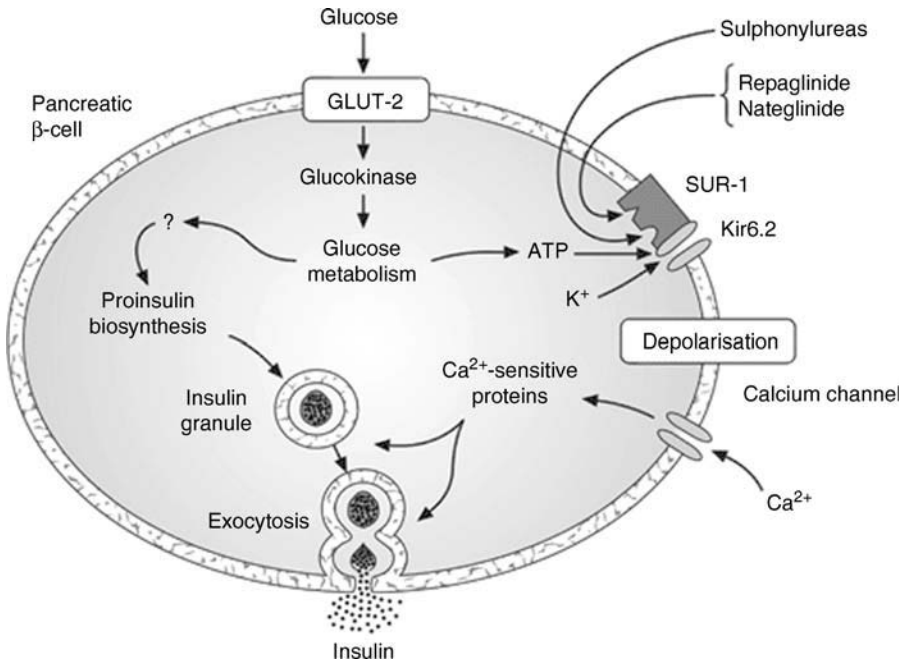
Sulphonylureas

The first sulphonylureas were introduced in the 1950s. They stimulate insulin secretion by a direct effect on pancreatic β-cells. Sulphonylureas enter the β-cell and bind to a site at the cytosolic face of the sulphonylurea receptor (SUR). The SUR-1 isoform is expressed by the β-cell. It forms part of a transmembranal complex that includes ATP-sensitive Kir6.2 potassium efflux channels (K-ATP channels). The binding of a sulphonylurea to SUR-1 produces a conformational change that closes K-ATP channels, favouring local depolarisation of the plasma membrane. This opens voltage-dependent L-type calcium channels, increasing calcium influx and raising the cytosolic free calcium concentration. In turn, this activates calcium-dependent signalling proteins controlling the contractile activities of microtubules and microfilaments that mediate exocytosis of insulin granules. Preformed insulin granules adjacent to the plasma membrane are released first (first-phase insulin release). Newly formed granules contribute to the secretory pool within 1 h of continued stimulation. Increased insulin release is sustained as long as drug stimulation is maintained, provided the β-cells are functionally competent (Fig. 1).

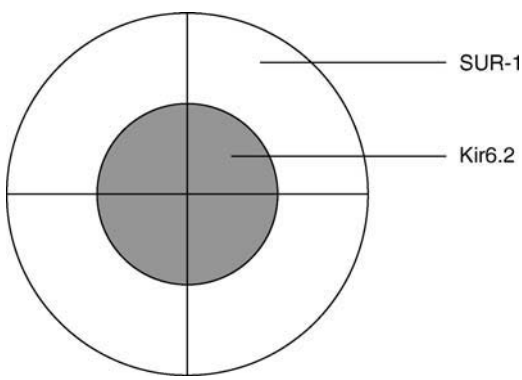
The SUR–Kir6.2 complex is a non-covalently bonded octamer (4 × SUR/4 × Kir6.2), with the pore-forming Kir6.2 channels located at the centre (Fig. 2). SUR molecules are members of the ATP binding cassette proteins (ABC proteins). Each SUR-1 molecule comprises 17 transmembrane domains, 2 cytosolic nucleotide binding domains and cytosolic binding domains for sulphonylurea, benzamido and other ligands. The Kir6.2 channel also has cytosolic binding regions, including one for ADP/ATP. Sulphonylureas bind to the sulphonylurea site with high affinity (e.g. *Ki* for glibenclamide in low nanomolar range), being dependent on a 'U' shape to the ligand with 5.5 Å between the hydrophobic rings.

By closing K-ATP channels, sulphonylureas induce insulin release by activating a step along the normal pathway of glucose-induced insulin secretion. Activation of insulin secretion is therefore independent of glucose, provided there is sufficient glucose metabolism to stimulate proinsulin biosynthesis and service the energy requirements for the cellular processing and exocytosis of insulin. Hence sulphonylureas can stimulate insulin secretion at low glucose concentrations, creating the risk of hypoglycaemia. Sulphonylureas will also increase the amount of insulin secreted at any level of stimulation by glucose, subject to adequate β-cell function. Additionally, sulphonylureas may potentiate insulin release that is stimulated by glucose and other nutrients. This may involve SUR molecules located within the membranes of insulin granules and activation of certain isoforms of protein kinase C.

Although the main therapeutic effect of sulphonylureas is increased insulin secretion, there is evidence that



Antidiabetic Drugs other than Insulin. Figure 1 Sulphonylureas stimulate insulin release by pancreatic β-cells. They bind to the sulphonylurea receptor (SUR-1), which closes Kir6.2 (ATP-sensitive) potassium channels. This promotes depolarisation, voltage-dependent calcium influx, and activation of calcium-sensitive proteins that control exocytotic release of insulin.



Antidiabetic Drugs other than Insulin. Figure 2 Octameric structure (4 × SUR/4 × Kir6.2) of the SUR–Kir6.2 complex.

these drugs exert weak extra-pancreatic effects. The latter effects include suppression of hepatic gluconeogenesis, possibly by suppression of a kinase which leads to increased formation of fructose-2, 6-bisphosphate. This stimulates phosphofructokinase and suppresses fructose-1, 6-bisphosphatase, thereby increasing glycolytic flux and suppressing gluconeogenic flux. Sulphonylureas might also enhance insulin-stimulated glucose transport by increasing translocation of GLUT-4 glucose transporters to the plasma membrane in adipocytes and muscle. However, these effects appear to require supra-therapeutic concentrations of sulphonylureas and are probably not therapeutically relevant. Sulphonylureas have been reported to reduce the hepatic extraction of insulin and to act on pancreatic α-cells to transiently stimulate and then suppress glucagon secretion.

The increase in insulin concentrations produced by sulphonylureas lowers blood glucose concentrations through decreased hepatic glucose output and increased glucose utilisation, mostly by muscle (►insulin, ►insulin receptor).

Prandial Insulin Releasers (Meglitinides)

This class comprises the meglitinide analogue repaglinide (introduced in 1998) and the structurally related D-phenylalanine analogue nateglinide (introduced in 2001). These agents have a benzamido group that binds to a site on SUR-1 that is distinct from the sulphonylurea site, but probably in close proximity and capable of binding interference. Some sulphonylureas also have a benzamido moiety (e.g. glibenclamide, glimepiride, glipizide) but the binding affinity for the sulphonylurea site has a higher affinity. Binding of repaglinide or nateglinide to the benzamido site closes the K-ATP channels and induces insulin secretion via the same pathway described for sulphonylureas.

Repaglinide and nateglinide are rapidly absorbed; their binding durations to SUR-1 are much shorter than sulphonylurea binding, and their hepatic metabolism

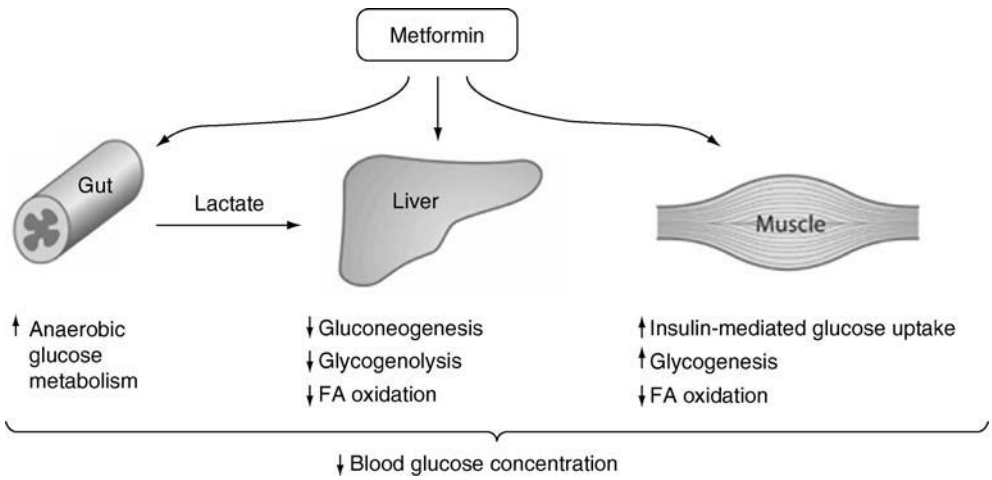
and subsequent elimination are faster. Consequently, repaglinide and nateglinide are faster-acting and shorter-acting insulin releasers than sulphonylureas. They can be taken immediately before a meal, and quickly stimulate insulin secretion to coincide approximately with the period of meal digestion, hence the categorisation of 'prandial insulin releasers'.

Biguanide

Metformin is the main compound in this class, introduced in the late 1950s. Other biguanides, namely phenformin and buformin have been widely discontinued. The antihyperglycaemic effect of metformin results partly from a direct improvement of insulin action and partly from actions that are not directly insulin dependent. A presence of insulin is required for the therapeutic efficacy of metformin, but the drug does not stimulate insulin release and is often associated with a small decrease in basal insulin concentrations in hyperinsulinaemic patients. Metformin has a variety of metabolic effects: The main antihyperglycaemic actions involve a reduction of excess hepatic glucose production, increased insulin-mediated glucose utilisation predominantly by muscle, decreased fatty acid oxidation and increased splanchnic glucose turnover.

Metformin restrains hepatic glucose production principally by suppression of gluconeogenesis. The mechanisms involve potentiation of insulin action and decreased hepatic extraction of certain gluconeogenic substrates such as lactate. In addition, metformin reduces the rate of hepatic glycogenolysis and decreases the activity of hepatic glucose-6-phosphatase. Insulin-stimulated glucose uptake and glycogenesis by skeletal muscle is increased by metformin mainly by increased movement of insulin-sensitive glucose transporters (GLUT-4) into the plasma membrane. Metformin also appears to increase the transport function of glucose transporters and increases the activity of glycogen synthase. Further actions of metformin include insulin-independent suppression of fatty acid oxidation in liver and muscle, and insulin-independent increase in anaerobic glucose metabolism by the intestine. Lactate produced in this way is recycled to glucose by the liver. Thus metformin acts to a modest extent via several different effects to lower blood glucose concentrations (Fig. 3).

Metformin enters some cell types (e.g. liver) at least in part via the organic cation transporter 1. The drug improves insulin sensitivity by increasing insulin-stimulated tyrosine kinase activity of the β-subunit of the insulin receptor, possibly by reducing phosphatase-mediated receptor dephosphorylation. Metformin also increases insulin signalling at more distal steps in the postreceptor cascades. Although metformin can increase insulin receptor binding when insulin receptor numbers are depleted, this does not appear to have a significant impact on insulin action. The mediating steps that enable metformin to interface with insulin-signalling pathways are not resolved. Metformin has been shown to alter membrane fluidity in hyperglycaemic states and to alter the activities of several metabolic enzymes (listed above), apparently independently of insulin. Emerging evidence suggests that metformin can activate adenosine monophosphate-activated protein kinase (AMPK) via an LKB1-dependent mechanism. Very high concentrations of metformin that occur in the intestine could increase anaerobic glucose metabolism by suppression of the respiratory chain at complex I.



Antidiabetic Drugs other than Insulin. Figure 3 The antihyperglycaemic effect of metformin involves enhanced insulin-mediated suppression of hepatic glucose production and muscle glucose uptake. Metformin also exerts non-insulin-dependent effects on these tissues, including reduced fatty acid oxidation and increased anaerobic glucose metabolism by the intestine. FA, fatty acid; ↑, increase; ↓ decrease.

Thiazolidinediones

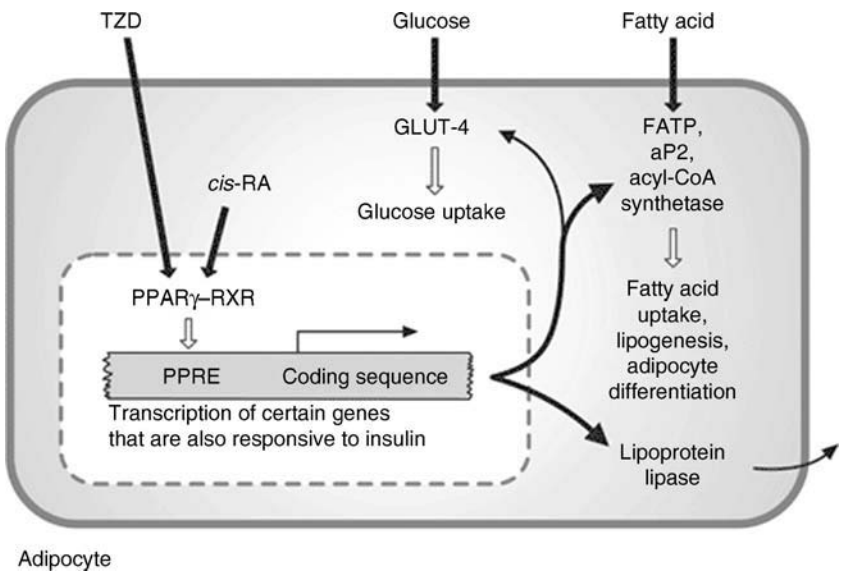
Two TZDs introduced in 1999 are presently available, pioglitazone and rosiglitazone. Another TZD, troglitazone has been withdrawn. TZDs improve insulin sensitivity and their principal mechanism of action is stimulation of the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ). PPARγ (►PPARs) is a member of the nuclear receptor superfamily for retinoid, steroid and thyroid hormones. PPARγ exists as a heterodimer with the retinoid X receptor (RXR). Binding of a TZD to PPARγ together with binding of *cis*-retinoic acid to the RXR moiety produces a conformational change that prompts dissociation of co-repressors. The activated heterodimer then binds to the peroxisome proliferator response element (PPRE), which is a sequence (AGGTCAXAGGTCA) located in the promoter region of the responsive genes. Recruitment of co-activators including PGC-1 and assembly of the RNA polymerase complex follows, initiating transcription (Fig. 4). Many of the responsive genes are also activated by insulin, hence the ability of TZDs to improve insulin sensitivity [2].

PPARγ is strongly expressed in adipocytes, and stimulation by TZDs promotes adipogenesis, predominantly in preadipocytes from subcutaneous depots. Increased transcription of transporters and enzymes involved in fatty acid uptake and lipogenesis increases the deposition of lipid in these adipocytes (Table 2). This appears to facilitate a reduction in hyperglycaemia by reducing circulating concentrations of non-esterified (free) fatty acids and triglycerides. The consequent effect on the glucose-fatty acid (Randle) cycle is to reduce the availability of fatty acids as an energy source, thereby favouring the utilisation of glucose. Additionally, TZDs increase transcription of GLUT-4 glucose transporters that directly facilitates glucose uptake. Reducing free fatty acid concentrations also reduces the production of lipid metabolites, which suppress early postreceptor steps in the insulin-signalling pathway. TZDs may further improve insulin signalling by increasing production of the adipocyte hormone adiponectin, decreasing production of the adipocyte cytokine tumour necrosis factor-α (TNFα), and decreasing production of the adipocyte hormone resistin (and possibly leptin), which have been implicated in the pathogenesis of insulin resistance.

There is weak expression of PPARγ in muscle, liver and other tissues, enabling TZDs to support the effects of insulin in these tissues, notably increased glucose uptake in muscle and reduced glucose production in liver. TZDs may also affect nutrient metabolism by skeletal muscle through a direct mitochondrial action that is independent of PPARγ.

α-Glucosidase Inhibitors

The first member of this class, acarbose, was introduced in the early 1990s. α-Glucosidase inhibitors slow the intestinal process of carbohydrate digestion by competitive inhibition of the activity of α-glucosidase enzymes located in the brush border of the enterocytes



Antidiabetic Drugs other than Insulin. Figure 4 Thiazolidinediones stimulate the PPARγ moiety of the PPARγRXR nuclear receptor complex, which then binds to a response element, leading to transcription of certain genes that are also responsive to insulin. These facilitate increased uptake of fatty acids, lipogenesis and adipogenesis. PPARγ, peroxisome proliferator-activated receptor-γ; RXR, retinoid X receptor; PPRE, peroxisome proliferator response element; TZD, thiazolidinedione; *cis*-RA, *cis*-retinoic acid; GLUT-4, glucose transporter isoform-4; FATP, fatty acid transporter protein; aP2, adipocyte fatty acid binding protein.

(Fig. 5). Acarbose also causes a modest inhibition of pancreatic α-amylase activity. The principal α-glucosidase enzymes are glucoamylase, sucrase, maltase and dextrinase. The inhibitors bind to these enzymes with much higher affinity than their natural disaccharide and oligosaccharide substrates. Hence, when bound to the inhibitor, the enzyme fails to cleave the disaccharides and oligosaccharides into their absorbable monosaccharides. The available α-glucosidase inhibitors, acarbose, miglitol and voglibose, show different binding affinities for the enzymes, giving them different activity profiles. For example, the affinity profile of acarbose is glycoamylase > sucrase > maltase > dextrinase. Miglitol is a more potent inhibitor of sucrase, and voglibose of other α-glucosidases [3].

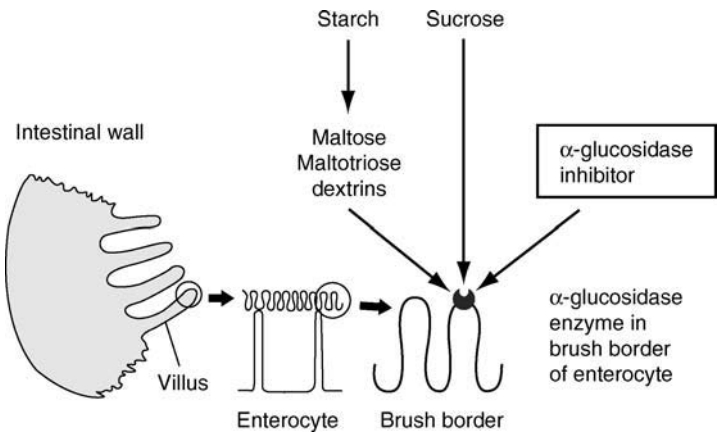
When α-glucosidase activity is inhibited, carbohydrate digestion is prolonged and takes place further along the intestinal tract. This in turn delays and spreads the period of glucose absorption, which reduces the extent of the postprandial rise in blood glucose concentrations. The effectiveness of α-glucosidase inhibitors is dependent on the consumption of a meal rich in complex carbohydrate.

Incretin Mimetics

The first incretin mimetic 'exenatide' was introduced in 2005. It is an analogue of the gut hormone glucagon-like peptide-1 (GLP-1). This therapy is based on the so-called 'incretin' effect, which is the enhanced insulin response to nutrients that occurs after a meal (compared with the insulin response to similar plasma nutrient levels created by intravenous administration). The incretin effect is due to hormonal and neural stimuli produced by the gut during meal digestion which increase glucose-induced insulin secretion, and thereby reduce prandial glucose excursions. The main incretin hormones are GIP (glucose-dependent insulinotropic polypeptide), produced by K-cells in the mucosa of the duodenum and jejunum, and GLP-1 from L-cells located mostly in the mucosa of the ileum. Both GIP and GLP-1 increase glucose-stimulated insulin secretion. Additionally, GLP-1 reduces glucagon secretion from pancreatic α-cells in a glucose-dependent manner, slows gastric emptying and exerts a satiety effect (Table 3). Animal and in vitro studies have suggested that GIP and GLP-1 might increase neogenesis and proliferation of β-cells and reduce β-cell apoptosis, but

Antidiabetic Drugs other than Insulin. Table 2 Tissue expression, ligands, genes activated, and biological actions of the peroxisome proliferator-activated receptor-γ (PPARγ)

Tissue expression	Mainly white and brown adipose tissue; weak expression in liver, muscle, gut, macrophages, pancreatic β-cells and haemopoietic tissues
Natural ligands	Certain unsaturated fatty acids and prostaglandin metabolites
Synthetic ligands	Thiazolidinediones and some non-steroidal antiinflammatory drugs
Gene activated	Lipoprotein lipase; fatty acid transporter protein; adipocyte fatty acid binding protein; acyl-CoA synthetase; malic enzyme; GLUT-4 glucose transporter; phosphoenolpyruvate carboxykinase
Biological actions	Adipocyte differentiation; fatty acid uptake; lipogenesis; glucose uptake; other effects on nutrient metabolism which lower hepatic glucose production



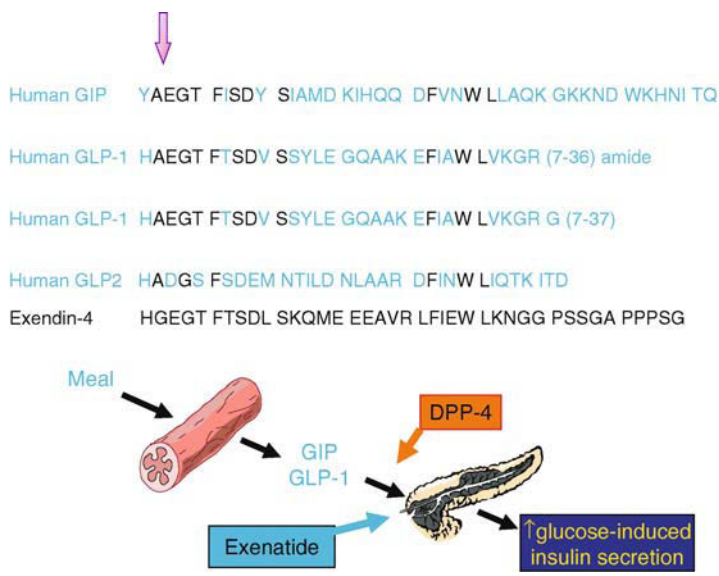
Antidiabetic Drugs other than Insulin. Figure 5 α-Glucosidase inhibitors slow the rate of intestinal carbohydrate digestion by competitive inhibition of α-glucosidase enzymes in the brush border of enterocytes. The α-glucosidase inhibitors have a higher affinity for the α-glucosidase enzymes than the natural disaccharide and oligosaccharide substrates.

it is uncertain whether these hormones can preserve β-cell mass in human type 2 diabetes [4].

The incretin effect is reduced in type 2 diabetes, and this is attributed, at least in part, to reduced secretion of GLP-1. The biological actions of GLP-1 remain essentially intact in type 2 diabetes, but administration of extra GLP-1 is not a practical therapeutic option because the peptide is degraded rapidly ($t½ < 2$ min) by the enzyme dipeptidyl peptidase IV (DPP-4). DPP-4 cleaves the N-terminal dipeptide from many of the peptides that have either an alanine or a proline residue penultimate to the N-terminus (Fig. 6). Exenatide (exendin-4) is a GLP-1 analogue with a 52% sequence homology in which the penultimate N-terminal alanine residue of GLP-1 is replaced by glycine. This confers resistance to degradation by DPP-4, giving exenatide protracted biological activity of about 5–7h after subcutaneous injection. Since exenatide interacts with the same receptor as GLP-1 and retains the same profile of biological effects as GLP-1, exenatide enables the incretin effect to be enhanced. It is noteworthy that DPP-4 exists free in the circulation and tethered to the external surface of endothelia and other epithelial cells in most tissues: it is also the CD26

Antidiabetic Drugs other than Insulin. Table 3 Actions of the incretin hormones GIP (glucose-dependent insulinotropic polypeptide, gastric inhibitory peptide) and GLP-1 (glucagon-like peptide-1)

	GIP	GLP-1
Pancreatic		
↑ *Glucose-induced insulin secretion*	Yes	Yes
↑ Proinsulin biosynthesis	Yes	Yes
↑ β-Cell survival (rodents)	Yes	Yes
↓ Glucagon secretion	–	Yes
Other actions		
↓ Gastric emptying	No *(slight)*	Yes
↓ Appetite/feeding	No	Yes
↓ Weight gain	No	Yes
↑ Myocardial glucose metabolism	–	Yes?
Type 2 Diabetes	Incretin effect reduced	
Postprandial response	About normal	Reduced (*late phase*)
Insulin-releasing effectiveness	Reduced	Retained (*mostly*)



Antidiabetic Drugs other than Insulin. Figure 6 The incretin mimetic, exenatide is a long-acting analogue of the gut hormone GLP-1. Arrow indicates the site of action of dipeptidyl peptidase-IV (DPP-4). Exenatide is resistant to degradation by DPP-4, allowing protracted GLP-1 mimetic activity that enhances the 'incretin' effect.

T-cell activating antigen although the aminopeptidase and immunological functions do not appear to interfere with each other.

Exenatide was discovered in the saliva of a lizard from Arizona – the Gila monster (*Heloderma suspectum*). For therapeutic purposes exenatide is usually administered by twice daily subcutaneous injection before the main meals. Because the insulin-releasing and glucagon-suppressing effects of exenatide (like GLP-1) are glucose dependent, there is low risk of severe hypoglycaemia. The satiety effect is often associated with some weight loss, and the slowing of gastric emptying can cause nausea, at least during initial therapy. About one third of patients develop antibodies to exenatide, but biological activity of the molecule is rarely affected.

Gliptins

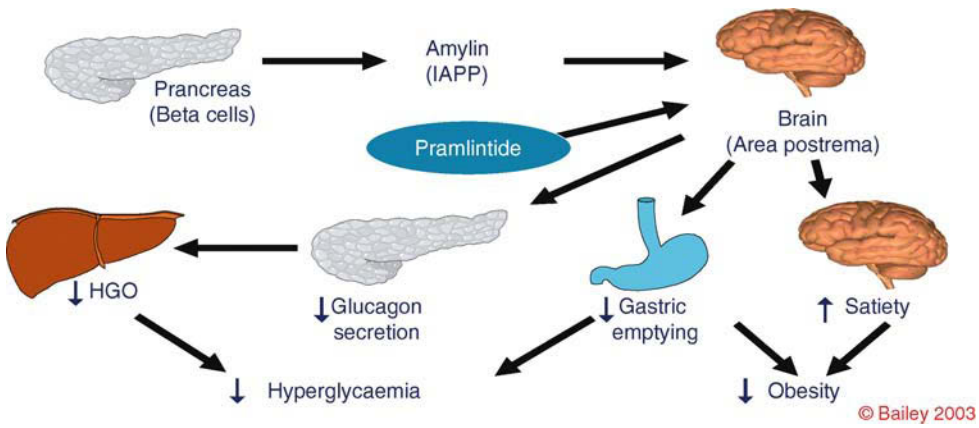
Gliptins (also termed dipeptidyl peptidase-IV inhibitors, DPP-4 inhibitors or incretin enhancers) are selective inhibitors of DPP-4 (described above). They enhance endogenous incretin activity by preventing the rapid degradation of GLP-1 and GIP (Fig. 6). The first gliptin 'sitagliptin' became available in the USA and UK in 2007. Since there are many other natural substrates for DPP-4 including neuropeptide Y (NPY), peptide YY (PYY), gastrin releasing polypeptide (GRP), substance P, insulin-like growth factor-1 (IGF-1), vasostatin-1 and several chemokines, gliptins have the potential to influence the hunger–satiety system, gastrointestinal motility, growth, vascular reactivity and immune mechanisms. However neither CD26 knockout mice nor the DPP-4-specific inhibitors used in animals or humans have yet shown any substantive untoward effects.

In clinical studies, selective DPP-4 inhibition increased active circulating concentrations of GLP-1 and GIP by two- to threefold. This was associated with increased glucose-induced insulin secretion and suppression of glucagon secretion, although changes in satiety and gastric emptying have not been reported.

Pramlintide

Pramlintide was introduced in the USA in 2005 as an adjunct to insulin therapy. It is a soluble analogue of the islet hormone amylin (islet amyloid polypeptide, IAPP) that is normally co-secreted from the pancreatic β-cells with insulin and C-peptide in response to nutrient stimuli. Paradoxically, amylin has been a suspect in the demise of β-cells in type 2 diabetes due to its accumulation and polymerisation to form insoluble fibrils in the islets. However normal amylin secretion appears to contribute to glucose homeostasis. Amylin acts centrally, probably via receptors in the area postrema (where there is no blood–brain barrier), dorsal raphe and nucleus accumbens. The central effects induce satiety and initiate a vagally-mediated suppression of prandial glucagon secretion and a slowing gastric emptying (Fig. 7). In type 1 diabetes and advanced stages of type 2 diabetes there is a lack or substantial reduction of amylin. Thus replacement therapy with a non-aggregating analogue of amylin can be used to complement insulin therapy in type 1 and advanced type 2 diabetic patients.

The structure of pramlintide (Fig. 8) differs from human amylin by the substitution of three residues with proline residues, retaining biological potency but preventing self aggregation. Pramlintide is not used alone: it is administered by subcutaneous injection as an adjunct to insulin therapy. Since pramlintide requires a more acidic pH than insulin it has to be given as a separate injection to insulin, usually just before the main meals. The suppression of glucagon secretion and to a lesser extent the slowing of gastric emptying are the main immediate actions of pramlintide that reduce blood glucose. The satiety effect is typically associated with a long-term reduction of food intake and body weight: reduced adiposity in obese type 2 diabetes generally improves metabolic control. It is advised to reduce the mealtime insulin dose during initiation of pramlintide therapy to reduce the risk of interprandial hypoglycaemia. Antibodies to pramlintide have been



Antidiabetic Drugs other than Insulin. Figure 7 Mechanisms of action of the amylin analogue pramlintide.

identified in some patients although these do not appear to affect biological activity.

Clinical Use

Type 2 (non-insulin-dependent) diabetes typically emerges in middle or later life. Unlike type 1 diabetes in which there is total loss of pancreatic β-cells and a critical need for exogenous insulin administration, type 2 diabetes is associated with a continued presence of β-cells and continued insulin production. However insulin resistance usually develops as a prelude to type 2 diabetes and creates a demand for a compensatory increase in insulin secretion. Eventually, the β-cells are unable to produce sufficient extra insulin to overcome the insulin resistance. This results in impaired insulin-mediated glucose uptake by muscle, failure of insulin to suppress hepatic glucose production and consequently hyperglycaemia. Pancreatic β-cells of type 2 diabetic patients become increasingly sluggish in their responsiveness to raised glucose concentrations, and eventually β-cell function becomes severely impaired, leading to a state of hypoinsulinaemia and greater hyperglycaemia. The toxic effects of hyperglycaemia on the permeability of small blood vessels and nerve function result in the long-term microvascular and neuropathic complications of diabetes (retinopathy, nephropathy and neuropathy). The additional effects of other metabolic disturbances associated with insulin resistance (the so-called 'metabolic syndrome') are largely responsible for the long-term cardiovascular complications of type 2 diabetes [5].

Achieving and maintaining blood glucose concentrations as close to normal as possible reduces the morbidity and premature mortality of the long-term complications of type 2 diabetes. All treatments begin with non-pharmacological measures (diet, exercise and healthy living), but compliance is limited, and lasting glycaemic control occurs in only a small minority of patients. Patients are usually started on one oral antidiabetic drug. Recent studies suggest that metformin offers additional advantages beyond glycaemic control to reduce long-term cardiovascular complications. Thus, this is often the first oral agent to be used. The mechanisms of action of metformin also prevent weight gain and avoid overswings into hypoglycaemia. Alternatively, a sulphonylurea or a prandial insulin releaser may be favoured as the first oral antidiabetic agent if substantial β-cell failure is suspected. The

Amylin	KCNTA TCATQ RLANF LVHSS NNFGA ILSST NVGSNT
Pramlintide	KCNTA TCATQ RLANF LVHSS NNFGP ILPPT NVGSNTY

Antidiabetic Drugs other than Insulin. Figure 8 Structure of human amylin and its soluble analogue pramlintide.

Antidiabetic Drugs other than Insulin. Table 4 Main exclusions, adverse events and precautionary monitoring required for clinical use of oral anti-diabetic drugs

Class[a]	Main exclusions	Main adverse events	Monitoring[b]
Sulphonylureas	Severe liver or renal disease[c]	Hypoglycaemia	–[b]
Prandial insulin releasers (meglitinides)	Severe liver or renal disease[c]	Hypoglycaemia[d]	–[b]
Metformin	Renal or liver disease; any predisposition to hypoxia	Gastro intestinal upsets; risk of lactic acidosis if wrongly prescribed	Creatinine, Hb or Vit B12[b]
Thiazolidinediones	Cardiac failure; liver disease	Oedema, anaemia, heart failure, fractures in women	LFT[b]
α-Glucosidase inhibitors	Chronic intestinal disease	Gastrointestinal upsets	LFT[b,e]
Incretin mimetic	Severe renal or gastrointestinal disease	Nausea, hypoglycaemia if used with another antidiabetic agent	–[b]
Gliptins	Severe renal disease	Abdominal pain, hypoglycaemia when used with another antidiabetic agent	Creatinine[b]
Amylin analogue	Gastroparesis	Hypoglycaemia, nausea	–[b]

Hb, haemoglobin; Vit B12, vitamin B12; LFT, liver function test.

[a]The dosage of each antidiabetic drug should be increased until either the target level of glycaemia is achieved or the last dosage increment produces no additional effect.

[b]Appropriate monitoring of glycaemic control using fasting or random blood glucose, glycated haemoglobin (HbA1c) or fructosamine (glycated albumin) should be undertaken for all patients receiving antidiabetic drugs.

[c]Depending upon pathways of metabolism and elimination of individual members of the class.

[d]Prandial insulin releasers are less likely to produce severe or prolonged episodes of hypoglycaemia than sulphonylureas.

[e]Liver function should be checked in patients on high dose acarbose.

prandial insulin releaser would be preferred for individuals with either mainly postprandial hyperglycaemia or irregular meal patterns which predispose to interprandial hypoglycaemia when taking a sulphonylurea. An α-glucosidase inhibitor can be used if the hyperglycaemia is modest and predominantly restricted to postprandial periods. The TZDs are slower to take effect than other agents, presumably due to their largely genomic mode of action. In Europe TZDs are used mainly as an alternative to metformin if metformin is not tolerated, or as add-on (combination) therapy with metformin. Nateglinide, or a gliptin (DPP-4 inhibitor) or a GLP-1 analogue (incretin mimetic) are presently recommended as second-line agents to be used in combination with another differently acting agent, when that agent alone does not achieve glycaemic control. The amylin analogue pramlintide is not available in Europe: it is used as add-on therapy to insulin to improve glycaemic control without increasing the insulin dose and without weight gain.

Type 2 diabetes is a progressive disease with continued insulin resistance and gradually declining β-cell function. Thus, hyperglycaemia increases with disease duration and glycaemic control becomes ever more difficult to maintain. If two or possibly three *differently acting* antidiabetic agents listed above do not achieve glycaemic control then it is apposite to switch to insulin therapy (►insulin, insulin receptor).

The main limitations and precautions for the use of oral antidiabetic drugs are listed in Table 4.

►Diabetes Mellitus
►Insulin Receptor
►Glucose Transporters
►ATP-dependent K^+Channel
►PPARs

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Antidiarrhoeal Agents

Antidiarrhoeal drugs are used for the symptomatic treatment of diarrhoea (the frequent passage of liquid faeces). Commonly used antidiarrhoeal drugs are opioids including codeine, diphenoxylate and loperamide. They reduce the motility of the intestine. Other antidiarrhoeal agents (chalk, charcoal, methyl cellulose) probably act by adsorbing toxins or microorganisms causing diarrhoea. Bismuth subsalicylate is used for the treatment of traveller's diarrhoea. It mainly reduces fluid secretion in the bowel.

Antidiuretic Hormone

►Vasopressin/Oxytocin

Antidysrhythmic Drugs

►Antiarrhythmic Drugs

Anti-emetic Drugs

►Emesis

Antiepileptic Drugs

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Definition

►Epilepsy is a chronic neurological disorder that affects about 0.6–0.8% of the general population worldwide. The clinical hallmark of epilepsy is

recurrent seizures (►seizure), which disrupt normal brain function. A large number of different types of epilepsies and epileptic syndromes have been distinguished. Many specific syndromes start in infancy and are accompanied by further developmental, neuropsychological and metabolic alterations, mostly of unknown origin. Generally, epilepsies with a focal origin (focal epilepsies) are discriminated from epilepsies with a generalized beginning (primary generalized epilepsies).

At the cellular level, focal and general convulsions correspond to synchronized high-frequency ►discharges (►discharge of neurons) of large groups of neurons, which disrupt normal information processing. Depending on the areas of the CNS recruited into the abnormal discharge, clinical symptoms observed during focal seizures may vary considerably. Thus, discharges within limited areas of the motor cortex may lead only to mild motor seizures, while seizure activity in the ►temporal lobe may cause complex semiologies that include behavioral automatisms and loss of consciousness. Focal seizures without loss of consciousness are termed simple partial seizures, whereas focal seizures with loss of consciousness are named complex partial seizures. In some epilepsies, initially focal seizures spread to involve most of the cerebral cortex (secondary generalized seizures).

Primary generalized seizures are also heterogeneous with respect to their clinical features. Such seizures can impose as ►absence epilepsy, which is characterized by a brief interruption of consciousness due to highly synchronized neuronal activity involving thalamocortical networks without increases in neuronal firing rate. On the other hand, ►tonic-clonic convulsions with loss of consciousness are often also primarily generalized.

Basic Mechanisms

Both focal and generalized epilepsies are heterogeneous with respect to their etiology and the principles of therapy.

Basic Mechanisms Underlying Focal Epilepsies

A large group of focal epilepsies arises as a consequence of developmental lesions, CNS tumors, trauma or inflammatory processes, which may be located in neocortical areas as well as the mesial temporal lobe. In a second group of patients, no such causal factor can be identified. Very frequently, such epilepsies arise from a focus within the ►hippocampus, which shows characteristic neuropathological and molecular changes. Only few focal epilepsies seem to be due to a mutation in ion channel ►(ion channels) genes. In contrast, a large number of generalized epilepsies is thought to have a genetic basis, and the chromosomal localization or the gene mutation has been identified in some of these disorders.

Many patients with focal epilepsies respond well to antiepileptic drugs, but a sizeable portion continues to have seizures even in the presence of optimal therapeutic drug concentrations. For unknown reasons, patients with an epileptic focus residing in the temporal lobe (Temporal Lobe Epilepsy, TLE) often develop pharmaco-resistant epilepsy. Therefore, considerable attention has been focused on unraveling the cellular changes underlying hyperexcitability in this form of epilepsy. Identifying such changes is of obvious importance in determining promising novel therapeutic strategies.

In focal epilepsies a number of functional and morphological changes are observed which may act in concert to support enhanced excitability. Such changes have been intensively investigated in order to develop targets for drug design.

- Altered density of voltage-dependent ion currents in neurons: Such changes may considerably affect the firing properties of neurons. They may also affect how neurons integrate a given synaptic input.
- Altered synaptic properties: Numerous changes in the properties of inhibitory (GABAergic) and excitatory (glutamatergic) synapses have been reported. While the simple adage of an imbalance between inhibitory and excitatory neurotransmission in epilepsy is not generally applicable, some forms of inhibition are lost or impaired in epilepsy. Likewise, an increased function of glutamate receptors has been demonstrated in some brain areas.
- Formation of novel aberrant synapses, axonal sprouting: In addition to altered properties of inhibitory and excitatory synapses, numerous synapses are newly formed in chronically epileptic tissue. In some regions, as in the dentate gyrus, the subiculum and area CA1 of the hippocampus, excitatory neurons form recurrent synapses terminating within the same region. This and other forms of recurrent sprouting are thought to constitute a positive feedback pathway facilitating seizure generation in this area. Very little is known about the elementary properties of newly formed synapses.
- Altered properties of glial cells: Glial cells are centrally involved in regulating the size of the extracellular space and the composition of the extracellular milieu, amongst other important tasks. In particular, glial cells normally take up K^+ released by neurons during repetitive neuronal activity. Preventing excessive increases in the extracellular K^+ concentration is important because they may enhance excitability of surrounding neurons. In chronic epilepsy, one of the numerous changes occurring in glial cells is the loss of the capacity to take up K^+.

Clearly, the largest difficulty in chronic focal epilepsy is to identify amongst the numerous changes that might plausibly affect excitability those that are most important in mediating hyperexcitability. Because of the lack of molecular targets with a proven causal role in mediating seizures, design of anticonvulsant drugs has been driven mainly by considering which drugs potently limit excitability in normal brain tissue or normal animals. It must be also stressed that, in focal epilepsies, our knowledge extends mainly to the cellular changes that underlie hyperexcitability in the chronic stage of the disease. The factors governing the development of the epileptic condition in humans are much less clear, and the design of substances aimed at inhibiting the progression of epilepsy is in its first stages.

Primary Generalized Epilepsies

Primary generalized epilepsies are a heterogeneous group of diseases. Some of the generalized epilepsies are hereditary, and several genetic mutations of ion channels or membrane receptors linked to this disorder have been identified. In others, the pathogenesis is less clear. Absence epilepsies present with a characteristic 3/s discharge in the electroencephalogram, and the mechanism for similar aberrant discharges have been well studied in animal models. It is thought that thalamic projection neurons that have the capacity to generate burst discharges mediated by low-threshold Ca^{2+} channels provide a phasic excitation of interneurons. These interneurons in turn inhibit thalamic projection neurons via GABAreceptors, resulting in a pronounced hyperpolarization. This hyperpolarization removes inactivation of low-threshold Ca^{2+} channels, subsequently enabling these neurons to generate a new, Ca^{2+} channel-dependent burst discharge. Thus, rhythmogenesis seems to rely on the interplay between low-threshold Ca^{2+} channel-dependent bursting and GABA-mediated inhibition. Accordingly, absence epilepsies respond well to substances blocking low-threshold Ca^{2+} channels (ethosuximide, trimethadione), as well as to some GABA-antagonists (which are still in an experimental stage for this indication).

Substances Acting on Voltage-dependent Ion Channels

With few exceptions, information on the anticonvulsant pharmacology of specific ion channel subunits analyzed in expression systems is scarce. Hitherto, a first understanding of the mechanism of action of most antiepileptic drugs has evolved from analyses of somatic ion channel pharmacology either in isolated neurons from human or rodent neurons, or cell culture models.

Voltage-dependent Na^+ Channels

A large number of anticonvulsant drugs commonly in use for focal epilepsies act on fast voltage-dependent Na^+ channels at clinically relevant concentrations (carbamazepine, phenytoin, lamotrigine). Most of these anticonvulsant drugs display three distinct effects on Na^+ channels:

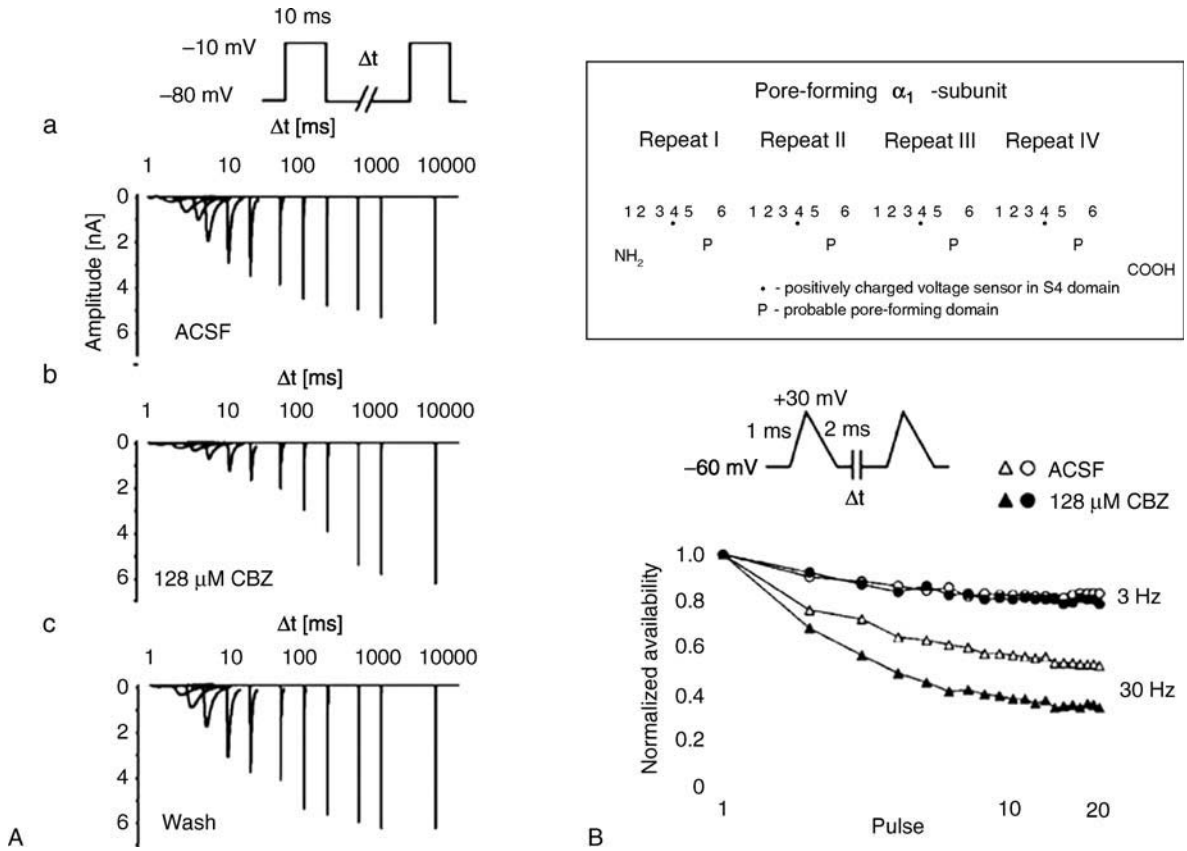
- A shift of the voltage-dependence of inactivation to a hyperpolarizing direction, resulting in a lower fraction of channels available for activation at action potential threshold.
- A reduction of the peak Na^+ channel conductance.
- A pronounced slowing of Na^+ channel recovery from the inactivated state.

The latter effect results in a prolongation of the time required after an action potential for inactivated Na^+ channels to become available again. This prolongation would be expected to inhibit repetitive firing only if the time between action potentials is not long enough to permit recovery of Na^+ channels, i.e. at high discharge frequencies. Indeed, phenytoin, carbamazepine and lamotrigine have been shown to preferentially inhibit high frequency but not low frequency firing (see Fig. 1). It has to be noted that this mechanism is most probably invoked not only at somatodendritic Na^+ channels, but also at presynaptic Na^+ channels. In the latter case, application of one of the antiepileptic drugs mentioned above would be expected to preferentially inhibit transmitter release induced by high frequency presynaptic action potentials.

In addition to inhibiting fast voltage-dependent Na^+ currents, many anticonvulsants also suppress persistent Na^+ currents, in some cases even more efficiently. This mechanism may also be important in the anticonvulsant action of these substances because persistent Na^+ currents are thought to give rise to high frequency burst discharges in some neurons.

Voltage-dependent Ca^{2+} Channels

A number of anticonvulsant drugs also display effects on Ca^{2+} channels. In most cases, effects on Ca^{2+} channels with a depolarized threshold of activation are small at clinically relevant concentrations. In the case of gabapentin, binding to a Ca^{2+} channel accessory subunit has been demonstrated, but whether this binding affects channel function is unknown. In contrast, Ca^{2+} channels with a hyperpolarized threshold of activation (low-threshold channels) are sensitive to a number of drugs (i.e. ethosuximide, trimethadione through its metabolite dimethadione, phenytoin, lamotrigine). As stated above, the activity of ethosuximide and trimethadione against absence epilepsy is thought to be due to their inhibition of low-threshold Ca^{2+} channels. The differing anticonvulsant profile of lamotrigine and phenytoin may be due to the fact that the three pore-forming subunits underlying low-threshold Ca^{2+} channels are differentially sensitive to anticonvulsant drugs.



Antiepileptic Drugs. Figure 1 Effects of carbamazepine on voltage-dependent Na^+ channels. (a) Fast recovery from inactivation can be analyzed in double pulse experiments using the whole-cell patch clamp technique. Recordings shown are from rat hippocampal dentate granule neurons. Inactivation is induced with a conditioning pulse (10 ms, –10 mV), after which recovery of Na^+ channels from inactivation is monitored by a test pulse applied at various intervals following the conditioning pulse (inset, a). Representative traces after various recovery intervals are displayed on an exponential time scale. Application of 128 μM CBZ causes a marked slowing of the time course of recovery. (b) Use-dependent block of Na^+ channels by CBZ. Trains of mock action potentials were applied as voltage commands at different frequencies. Application of CBZ reduces Na^+ channel availability preferentially during high-frequency stimulation. Inset: General molecular structure of pore-forming α subunits of voltage-dependent Na^+ channels.

Voltage-dependent K^+ Channels

Up-modulation of voltage-dependent K^+ channels may be a plausible mechanism to reduce cellular excitability and action potential-dependent neurotransmitter release. However, the number of novel antiepileptic drugs developed that target potassium channels is small. Interestingly, it has recently been discovered that a mutation resulting in a moderate loss of function of KCNQ2/3 K^+ channels causes a focal form of epilepsy. The novel anticonvulsant retigabine, which enhances the activity of this very channel type, displays a high clinical efficacy in these patients.

Substances Acting on Neurotransmitter Receptors

A large fraction of anticonvulsants are based on the attempt to boost inhibitory synaptic transmission in order to restore the balance between inhibition and excitation in epileptic tissue. The first drug using this mechanism of action was phenobarbitone, which was introduced into clinical practice in 1912. Today, there are at least three different targets of anticonvulsant drugs at the synaptic level, all centered on the main inhibitory transmitter GABA (γ-aminobutyric acid).

GABAergic Synapses

Based on the key elements in synaptic inhibitory transmission, three classes of drugs can be distinguished:

- GABA receptor modulators. These substances yield a potentatiation of synaptic responses to GABA by changing the affinity of the GABA receptor

(benzodiazepines) or enhancing the open probability of this ligand-gated ion channel (barbiturates). Benzodiazepines are especially useful against status epilepticus but are also used as an adjunctive therapy in partial and generalized seizures. Clinically used substances are clobazepam, clonazepam, clorazepate, diazepam, lorazepam, midazolam and nitrazepam. Barbiturates (esp. phenobarbitone) are used in tonic-clonic and partial seizures, in status epilepticus and in neonatal seizures. Chronic treatment with benzodiazepines and barbiturates is complicated due to the sedative side effects and, most importantly, development of tolerance.

- GABA-uptake blockers. Block of GABA-uptake prolongs the presence of the transmitter in the synaptic cleft and thereby strengthens the postsynaptic effects of synaptically released GABA. Tiagabine, a derivative of nipecotic acid, is in clinical use as an add-on therapy against simple and complex partial seizures. Like all substances that generally increase GABAergic transmission, tiagabine has sedative side effects.
- Blockers of GABA catabolism. Blocking the GABA-degrading enzyme GABA-transaminase increases the concentration of GABA in synaptic terminals and enhances or stabilizes the inhibitory transmission. A "new" anticonvulsant designed for this purpose is γ-vinyl-GABA (Vigabatrin), but it should be noted that valproate has the same effect. Vigabatrin is used as an adjunctive therapy in partial and secondary generalized seizures. It is very efficient against infantile spasms and is being used in Lennox-Gastout (together with sodium valproate and benzodiazepines). Side effects of vigabatrin include neuropsychiatric (especially mood) disturbances as well as retinopathic changes. Novel experimental approaches are aimed at increasing GABA synthesis, rather than blocking its degradation, by potentiating the action of the GABA-synthesizing enzyme glutamate decarboxylase.

Antiepileptic Drugs. Table 1 Summary of the known spectrum of actions of a selection of antiepileptic drugs

	Voltage-dependent Ion channels			Neurotransmitter receptors			NT release	Other mechanisms
	Na^+	Ca^{2+}	K^+	GABA	NMDA	AMPA		
Phenytoin	+++	++ (T)	+ (DR)	?	?	?	++	Calmodulin and cyclic nucleotide-dependent second messenger systems
Carbamazepine	+++	–	?	–	+	?	?	Adenosine receptors
Lamotrigine	+++	++	–	–	–	–	?	
Valproic acid	+++	+	+	?	?	?	++	Increase in brain GABA, decrease in brain aspartate
Ethosuximide	+ ($I_{Na,p}$)	+++ (T)	+	?	?	?	–	Inhibition of Na^+/K^+ ATPase
Trimethadione	?	+++ (T,L)	?	?	?	?	?	
Phenobarbital	–	+	?	+++	?	?	++	
Diazepam	+	–	–	+++	–	–	–	
Felbamate	++	?	?	?	+++	?	?	
Gabapentin	–	+ (HVA)	+	–	–	–	+	Increased GABA synthesis, GDH, GAD, GABA-aminotransferase
Vigabatrin	?	?	?	?	?	?	+++	GABA-transaminase inhibition
Tiagabine	?	?	?	?	?	?	+++	
Losigamone	?	++	?	++	?	?	?	
Ramecemide	?	?	?	?	?	?	?	
Topiramate	++	?	?	++	–	?	?	
Levetiracetam	–	–(T), + (HVA)	?	?	?	?	?	GABA-T, GAD
Retigabine	?	?	+++ (KCNQ)	?	?	?	?	

This synopsis refers only to actions demonstrated within or close to therapeutic concentrations of drugs. Abbreviations: (+) to (+++) weak to strong efficacy, (–) no efficacy, (?) not investigated. HVA: high threshold Ca^{2+} channels, T: T-type Ca^{2+} channels, L: L-type Ca^{2+} channels, I_{NaP}: persistent sodium current, DR: delayed rectifier K^+ channels, KCNQ: KCNQ subtypes of K^+ channels.

One of the oldest antiepileptic drugs, bromide, has been reported to boost inhibition by an unknown mechanism. Bromide is still in use in certain cases of tonic-clonic seizures and in pediatric patients with recurrent febrile convulsions and others. The mechanism of action may include a potentiation of GABAergic synaptic transmission, although the precise target is not known.

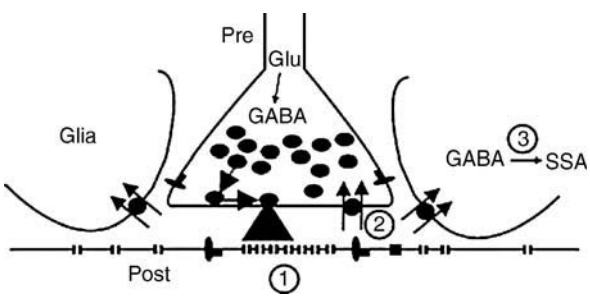
Excitatory Amino Acid Antagonists

The complementary approach to boosting inhibition, i.e. antagonizing the effects of the excitatory neurotransmitter ►glutamate (►GABAergic System), has been less fruitful so far. Antagonists of the NMDA-subtype of glutamate receptors show anticonvulsant activity in animal experiments but have not been introduced into clinical use due to severe neuropsychological side effects. An exception may be felbamate, which seems to exert at least part of its effect by a block on NMDA receptors. Antagonists of two other glutamate receptor subclasses (AMPA- and Kainate-receptors) are under development. Topiramate, a new anticonvulsant drug, partially blocks kainate-receptors and thus may provide the first example of an AED with effects against excitatory neurotransmission.

Substances with Unknown or Mixed Mechanism of Action

It should be pointed out that most anticonvulsants have more than one effect on neuronal excitability or synaptic transmission. A prominent example is valproic acid, which affects GABAergic transmission (probably by enhancing cellular GABA-content), glutamatergic synaptic transmission by reducing synthesis of excitatory amino acids as well as voltage-dependent ion channels (see Table 1).

Drugs with unknown mechanism of action are gabapentin, bromides (but see above effects on GABAergic transmission) and adrenocorticotropic hormone (ACTH), which is used in infantile spasms.



Antiepileptic Drugs. Figure 2 The GABAergic synapse as a target of anticonvulsent drugs. GABA is synthesized from glutamate in the presynaptic terminal and is packed into small synaptic vesicles. After release, GABA activates postsynaptic ion channels ($GABA_AR$, marked "1") which mediate the chloride influx and thereby the inhibition of the postsynaptic cell. From the synaptic cleft, GABA is removed into the presynaptic terminal and into a adjacent glia cells by GABA-uptake ("2"). A fraction of the transmitter is degraded into succinyl-semialdehyde by GABA-transaminase ("3"), which is present in glia cells as well as in neurons. Pre-and postsynaptic metabotrophic $GABA_BR$ are indicated by elipsoid bodies in the cell membrane but are no major target for anticonvulsants presently in use. GABAergic drugs against epilepsy act as positive modulators of the $GABA_AR$ ("1"), blockers of GABA-uptake ("2") or inhibitors of GABA degredation ("3").

►GABAergic System
►Ionotropic Glutamate Receptors
►Voltage-dependent Ca^{2+} Channels
►Voltage-gated K^+ Channels

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Antiestrogen

Antiestrogens are estrogen/estrogen receptor antagonists.

►Selective Sex Steroid Receptor Modulators
►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor

Antifibrinolytic Drugs

►Fibrinolytics

Antifungal Drugs

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Synonyms

Antimycotic drugs; Antimycotics; Fungicides

Definition

►Fungi cause diseases in plants, animals and humans. Antifungal drugs (fungicides) are therefore used in agriculture, animal and human medicine. In this article, only antifungal drugs used for human chemotherapy are described.

Antifungal drugs inhibit the growth of fungi in tissue (►fungistatic activity) by a number of different mechanisms; some of the agents even kill the fungal cell (►fungicidal effect). Antifungal drugs are used for the treatment of established fungal diseases; however, in immunosuppressed patients at high risk they are also used as prevention or empiric therapy.

Antifungal drugs are classified according to their mode of action and/or their chemical class. Four chemical classes have mainly contributed to the actual armentory of antifungal drugs: the broadest class is the one of ►azoles (imidazoles and triazoles), followed by ►polyenes, allylamines and ►morpholines. Some individual compounds are used in dermatology.

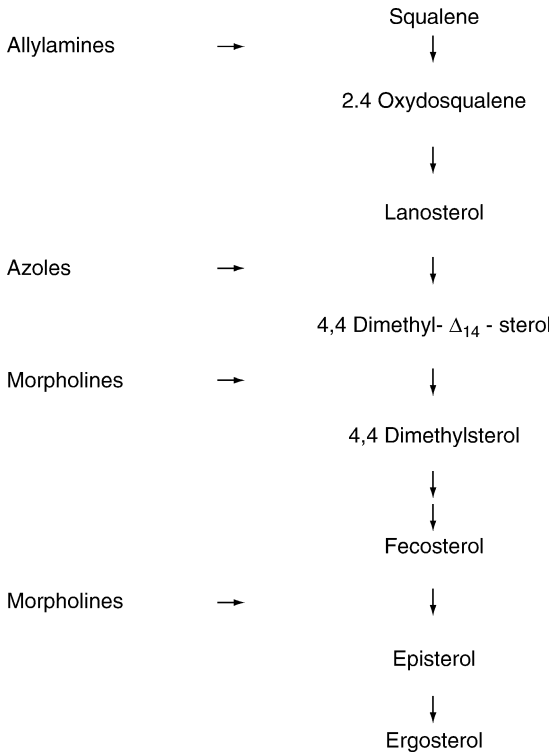
Mechanism of Action

The difficulty of killing the eukaryotic fungal cell without damaging the host is perhaps more akin to the problems of cancer chemotherapy than those of antibacterial treatment. Biochemical studies have identified a number of potential targets for antifungal chemotherapy, including cell wall synthesis, membrane sterol biosynthesis, nucleic acid synthesis, metabolic inhibition and macronuclear biosynthesis. The cell wall synthesis is the only fungal-specific target, since the fungal cell wall has a unique molecular structure; all other pathways (enzymatic steps) in the fungal cell are closely related to the ones used in human cells. (Fig. 1)

Sterol Biosynthesis Inhibitors

Most antifungals on the market (►azoles, allylamines, morpholines, Tolnaftate, Tolciclate) interfere with the various enzymatic steps involved in the cascade of ergosterol synthesis from squalene to ergosterol; this pathway being the Achille's heel of the fungal cell. Ergosterol is the essential component of the fungal membrane and exerts two functions: it is the bulk membrane component and it regulates cell growth and proliferation. All sterol biosynthesis inhibitors induce depletion of the essential ergosterol and accumulation of a wrong sterol moiety, consequently disturbing the function of the cell membrane. The morphological changes seen in all cells treated with a sterol biosynthesis inhibitor are similar, all including thickening of the cell wall by chitin deposits.

The imidazoles and triazole (►azoles) (for example, ketoconazole, itraconazole (ITRA), fluconazole (FLU), voriconazole) interfere with cytochrome P_{450}-dependent lanosterol C_{14} demethylase, leading to depletion of ergosterol and accumulation of lanosterol in the membrane. At the molecular level, one of the nitrogen atoms of the azole ring binds to the haem moiety of cytochrome P_{450}. Only compounds with higher specific binding to the fungal cytochrome than to the human one can be used as systemic antifungal drugs. Compared to the imidazoles, the triazoles have a much higher affinity for fungal cytochrome than for human cytochrome P_{450} enzyme steps. In addition to the main interactions with the P_{450} cytochrome, azoles may inhibit cytochrome C oxidase and peroxidative enzymes; they may also interfere with phospholipids. The fact that miconazole and ITRA are fungicidal is thought to be the result of a direct membrane interaction, leading to the loss of cytoplasmic constituents.



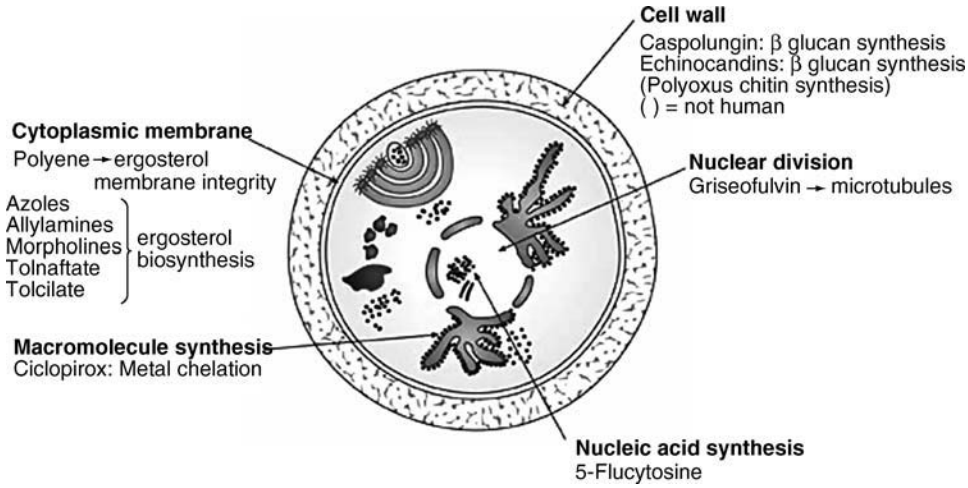
Antiepileptic Drugs. Figure 1 Antifungal drugs.

Allylamines

Allylamines (terbinafine, naftifine) interfere with the ergosterol pathway at the level of squalene epoxidase leading to the depletion of ergosterol and the accumulation of squalene. Again, only compounds with a higher specificity for the fungal enzyme than for the human enzyme can be used for systemic use. A clear correlation exists between growth inhibition and degrees of sterol biosynthesis inhibition; the fungicidal effect is more correlated to the intracellular accumulation of squalene. (Fig. 2).

Morpholines

Morpholines (amorolfine) interfere at two levels of the ergosterol pathway, the Δ_{14}-reductase and the Δ_7-,



Antiepileptic Drugs. Figure 2 Antifungal drugs – targets of antifungal activity.

Δ_8-isomerase leading to depletion of ergosterol and accumulation of an unplanar sterol ignosterol. With the inhibition of two steps in the same pathway, a natural synergistic effect is built into the molecule so that the risk of appearance of resistant mutants is low and efficacy high.

Drugs Binding Directly to Ergosterol (amphotericin B, nystatin, candicidin).

The current model for the mechanism of the ►polyene amphotericin B (Amph B) is based on the formation of a 1:1 Amph B/Ergosterol aggregate, which associates into a transmembrane channel with a large –OH lined aqueous pore down the middle. The result of the interaction between Amph B and the sterols is the disturbance of the ergosterol function leading to increased permeability, disruption of the proton gradient and leakage of potassium. The fungicidal effect, however, has been linked to irreversible inhibition of the membrane ATPase.

5-Fluorocytosine

5-Fluorocytosine (5FC), a mock pyrimidine, is the only antifungal drug that acts as true antimetabolite. 5FC is taken up into the fungal cell, deaminated to 5-fluorouracil (5FU) which is the active principle responsible for the killing of the fungal cell. Fungi lacking the cytosine deaminase are resistant to 5FC. Intracellularly, 5FU acts along two different pathways: it is incorporated as 5-flurouridine monophosphate into the RNA and it inhibits after conversion to 5-fluorodeoxyuridine monophosphate, the thymidylate synthetase, leading to inhibition of DNA synthesis. 5FU itself cannot be used for antifungal therapy due to its toxicity for mammalian cells.

Glucan Synthase Inhibitors

Echinocandins (i.e. caspofungin), semisynthetic lipopeptides, inhibits the synthesis of β-(1,3)-D-glucan, an integral component of the fungal cell wall not present in mammalian cells.

Griseofulvin

Griseofulvin is the first antimycotic drug detected that is only active against ►dermatophytes. Its activity manifests as nuclear and mitotic abnormalities followed by distortions in the hyphal morphology.

Hydroxypyridones (Ciclopirox, Rilopirox)

The primary mode of action of this class of antimycotics is interference with uptake and accumulation of products required for cell membrane synthesis. In higher concentrations it causes a disturbance of the cellular permeability. Some investigations show an interaction with Fe(III)- ions; the compounds acting as chelators. Very high concentrations interfere with the function of fungal mitochondria.

Clinical Use (including Side Effects)

Fungal diseases divide themselves into three classes: superficial (topical, local) mycoses (►dermatomycoses and gynaecological infections), subcutaneous and organ mycoses. This division is important not especially for microbiological reasons, but in the view of the different problems arising during treatment. Superficial mycoses are not life threatening, but they are irritating. Subcutaneous mycoses are also not life threatening but are associated with a high morbidity, and deep mycoses, especially in immunosuppressed patients, are life threatening showing a high mortality rate in patients, if untreated. The treatment schedules (dose, duration of treatment, galenical formulation) are strongly dependent on the localisation of the fungal disease, on the pathogenicity of the fungi, and on the conditions of the host. Additionally, the diagnosis of the disease is not always guaranteed; therefore, a clear-cut, simple description of clinical usage for antifungal drugs is not possible.

Due to the divergence of fungal diseases, there is neither single best treatment nor a superior drug for all diseases. However, a superior drug does exist for dermatomycoses caused by dermatophytes, namely the allylamine terbinafine (TER). For the treatment of deep mycoses in immunosuppressed patients the most efficacious drug is the polyene Amph B.

The therapy of ►dermatomycoses and acute vaginal infections is unproblematic. A large choice of various drugs (all chemical classes and compounds discussed above) in different galenical formulations (crèmes, tinctures, sprays, ovula, powder, shampoo, nail lacquer and tablets) exists. All drugs topically applied – used in various treatment schedules – show high efficacy and a low incidence of adverse reactions. For the treatment of onychomycoses without matrix involvement two nail lacquers (a morpholine and hydroxypyron) are on the market showing high efficacy with low (<1%) adverse reactions after topical therapy for several months. A combination therapy with a topical and a systemically applied antifungal drug is the most efficacious and the most economic therapy for onychomycosis with matrix involvement. Systemic therapy is indicated if the dermatomycosis is widespread. The highest cure rate is achieved with TER. TER is well tolerated in adults and children. In ca. 5% of the patients, mild and reversible side effects have been observed: gastrointestinal, skin, central nervous system, respiratory events and loss of taste. For acute vaginal candidosis the treatment of choice with the highest compliance and best efficacy is one daily dose of FLU.

Three fungal infections – Madura feet (mycetoma), chromomycosis and ►sporotrichosis – fall into the category of subcutaneous mycoses, their distribution is mainly in tropical and subtropical areas. The ideal treatment for madura feet caused by fungi is not yet established; the azoles are of some benefit, however, neither the optimal drug, dose, nor the treatment schedules are known. Chromomycosis responds well to ITRA monotherapy or the combination of 5FC plus ITRA. ITRA has been set up as standard therapy for cutaneous and lymphatic sporotrichosis.

Systemic Mycoses

Systemic mycoses are caused either by true pathogenic fungi (endemic in distinct areas of USA/South America) or by opportunistic fungi that induce severe infections in immunosuppressed patients. The arsenal for the treatment of deep organ mycoses is relatively small: Amph B, 5FC, azoles (FLU, ITRA, voriconazole (NDA filing)) and CAS.

The polyene Amph B (intravenous formulation) has the broadest spectrum, is fungicidal and shows its superiority in immunosuppressed patients. Its only drawback is its infusion-related toxicity and its negative influence on renal function. Acute reactions to Amph B – usually fever chills, rigor and nausea – can be ameliorated by concomitant administration of meperidine, acetomiophen or hydrocortisone. Additionally, there is the possibility of tailoring time and duration of infusion. Prevention of the chronic tubular injury is feasible by salt loading. Encapsulation of Amph B into liposomes or complexing of the drug with other lipid carriers brings a major reduction of nephrotoxicity. Three lipid-associated forms are now available (Ambisome, Amph B lipid complex and Amph B colloidal dispension). Due to its toxic side effects, Amph B is not widely used for prevention; it is, however, often used as empiric therapy with high success rates.

Due to the rapid appearance of resistance, 5FC is only used as a combination partner for the intensive therapy of established severe fungal infections caused by *Candida spp.*, *Cryptococcus neoformans* and *Aspergillus* sp. Anorexia, nausea, vomiting, diarrhoea and or abdominal pain occur in 6% of the patients. Of greater concern is the potential for bone marrow depression (seen in 5% of the patients, all with elevated 5FC levels).

Azoles

Generally the azoles are well tolerated in children and adults; mild side effects like nausea and vomiting are seen in <5% of the patients treated with FLU.

Attention has to be given to the problem of interaction between azoles and other drugs; these are based on two mechanisms:

- inhibition of absorption of the azoles leading to lower bioavailability or
- interference with the activity of hepatic microsomal enzymes, which alters the metabolism and plasma levels of azole, the interacting drug or both. This latter induces often increased toxicity of the concomitant drug.

With FLU, only few drug interactions are seen, namely with rifampicin (reduction of FLU), phenytoin, cyclosporin, tolbutamide and warfarin (increasing levels of concomitant drug). The interactions with ITRA are more significant than with FLU: H2 antagonists and all drugs increasing intragastric pH decrease the absorption of ITRA. Interactions due to hepatic enzymes are seen with rifampicin (reducing the levels of ITRA to undedectable levels), phenytoin, isoniazid, carbamazepine, phenobarbital, midazolam, triazolam, digoxin, lovastatin terfenadine, warfarin and cyclosporin. The list of interacting drugs is still increasing.

Oral FLU is well established as first line therapy for oropharyngeal candidosis and *Candida* oesophagitis and for maintenance therapy in AIDS patients with meningeal cryptococcosis. FLU (oral or intravenous) is also efficacious in candidemia without neutropenia. It shows efficacy in prevention (attention: *Aspergillus* sp. are not in the spectrum) and empiric therapy. ITRA, being fungicidal against *Aspergillus* sp., shows

promising results in aspergillosis, especially under intravenous therapy, and is used as maintenance therapy in AIDS patients with histoplasmosis. ITRA is the first line therapy for histoplasmosis and blastomycosis in HIV-negative patients. A combination of ITRA plus 5FC may be the optimal therapy of phaeohyphomycoses.

The glucan synthase inhibitor caspofungin (intravenous formulation) is new on the market for the treatment of invasive aspergillosis in patients whose disease is refractory to, or who are intolerant of, other therapies. During the clinical trials fever, infused vein complications, nausea, vomiting and in combination with cyclosporin mild transient hepatic side effects were observed. Interaction with tacrolismius and with potential inducer or mixed inducer/inhibitors of drug clearance was also seen.

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Antigen

An antigen is a molecule recognised by specific receptors on cells of the immune system such as B lymphocytes.

►Immune Defense
►Humanized Monoclonal Antibodies

Antigen Presentation

Antigen presentation is the key mechanism allowing T-lymphocytes to survey whether intracellular pathogens exist in the cells of the body. As T-lymphocytes can only recognize antigen in the form of peptides presented on specific molecules termed major histocompatibility complex (MHC), antigen-presenting cells instruct T-cell reaction and thus the development of adaptive immunity. Professional antigen-presenting cells (MHC class II positive) are dendritic cells, monocytes/macrophages, and B-lymphocytes.

►Immune Defense

Antigen-presenting Cells

Antigen-presenting cells (APCs) are cells of the immune system that are able to process and present foreign antigens to effector cells. The antigen is presented in the context of an MHC-I or MHC-II molecule on APCs in the presence of so-called co-stimulatory molecules to activate the effector cells.

►Immune Defense
►DNA Vaccination and Genetic Vaccination

Antigen Receptors

Each T- and B-lymphocyte carries one type of receptor, which recognizes one specific antigen. T-cells carry the heterodimeric T-cell antigen receptor consisting of an alpha and beta chain. This receptor recognizes a peptide presented by a MHC (major histocompatibility complex) molecule. B-cells express an immunoglobulin on their surface, which can recognize epitopes on antigens of different sizes and qualities without the need for presentation. Both forms of antigen receptors are created by random genetic rearrangement during the ontogeny of each individual lymphocyte. Both types of antigen receptors require additional transmembrane molecules for signal transduction.

►Immune Defense

Anti-gout Drugs

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Synonyms

Drugs for the treatment/management of ►gout and/or ►hyperuricemia

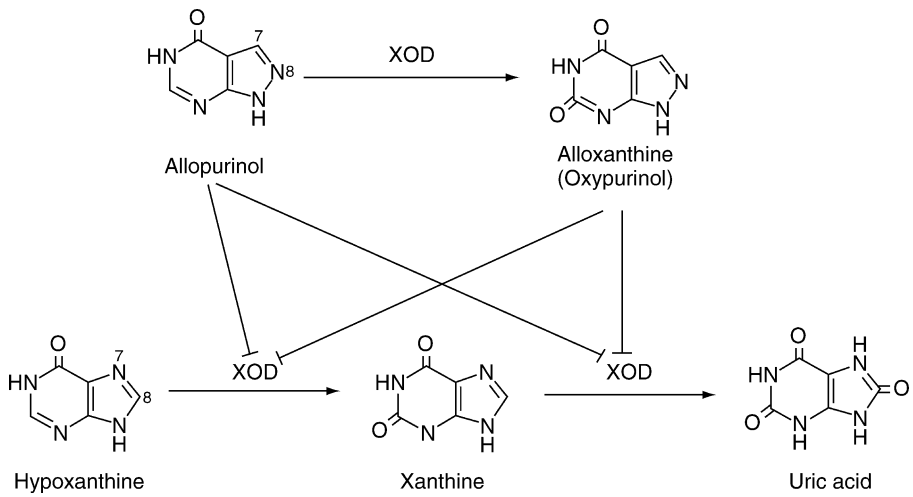
Definition

Pathophysiology and Clinical Manifestations of Gout

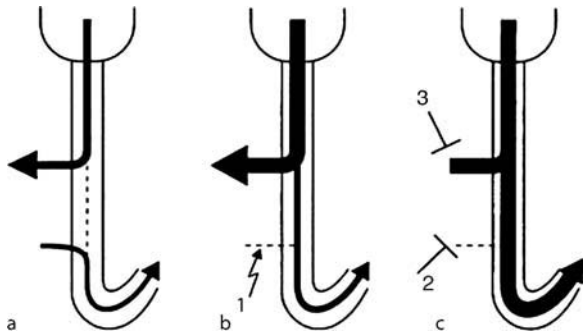
►Uric acid is the end product of purine catabolism in man. Purines originate from food and the degradation of nucleic acids and nucleotides. ►Xanthine oxidase (XOD) is the key enzyme in purine degradation. XOD converts hypoxanthine to xanthine, and xanthine to uric acid, respectively (Fig. 1). Uric acid is filtered in the glomerulus of the kidney, is almost completely absorbed in the proximal tubules and secreted more distally (Fig. 2). At physiological pH (<7.4), uric acid exists predominantly in its ionic form (urate). At lower pH, the fraction of uric acid molecules (protonized form) increases. This is important because uric acid possesses a lower solubility than urate. Thus, a decrease in pH, as it occurs in inflammed tissue and in the tubules, facilitates the formation of uric acid crystals, which are the initial cause of gout. In most mammals, the enzyme uricase converts uric acid to the more soluble allantoin, but humans do not express uricase. Of importance for therapeutic intervention is the fact that xanthine and hypoxanthine are more soluble than uric acid. Specifically, by preventing uric acid formation through XOD inhibition, the excretion of xanthine and hypoxanthine increases, and the risk of uric acid crystal formation decreases. An increase of the serum uric acid concentration above 416 μmol/L is referred to as hyperuricemia and is associated with an increased risk of uric acid crystal formation and acute attacks of gouty arthritis. With a serum uric acid level of 535 μmol/L, the annual incidence of gouty arthritis is 4.9–5.7%.

Hyperuricemia can have genetic causes or acquired causes. A defect of hypoxanthine-guanine phosphoribosyl transferase is the cause of Lesch–Nyhan syndrome, resulting in increased uric acid production. Among the genetically caused defects, impaired renal uric acid secretion is a very common cause of gout. Myeloproliferative diseases, a purine-rich diet (e.g. meat, beer, beans, peas, oatmeal, or spinach), obesity, and alcoholism are common causes of acquired hyperuricemia and result from increased uric acid production. Renal diseases and the application of certain drugs such as the tuberculostatic drug pyrazinamide, thiazide diuretics, loop diuretics, or acetylsalicylic acid at doses of up to 1–2 g/day, and the immunosuppressant cyclosporin A are acquired causes of impaired uric acid secretion.

Gout is the consequence of hyperuricemia and is caused by uric acid deposits in joints, tendons, bursae, kidney and urinary tract. In the USA, the prevalence of gout is ~1% for all ages and both sexes. The prevalence of gout is higher in men than in women and exceeds 5% in men ≥65 years. These epidemiologic data are important for drug therapy since older patients are more sensitive to side effects of anti-gout drugs than younger patients. In the initial stage, gout is characterized by asymptomatic hyperuricemia. In the second stage, the disease manifests itself by acute gouty arthritis. The third (intercritical) stage is asymptomatic, and the fourth stage is characterized by progressive uric acid deposits in joints, tendons, bursae, kidney, and urinary tract (tophus formation). Uric acid deposits result in the deformation and loss of function of joints



Anti-gout Drugs. Figure 1 Xanthine oxidase-catalyzed reactions. Xanthine oxidase converts hypoxanthine to xanthine and xanthine to uric acid, respectively. Hypoxanthine and xanthine are more soluble than uric acid. Xanthine oxidase also converts the uricostatic drug allopurinol to alloxanthine. Allopurinol and hypoxanthine are isomers that differ from each other in the substitution of positions 7 and 8 of the purine ring system. Although allopurinol is converted to alloxanthine by xanthine oxidase, allopurinol is also a xanthine oxidase inhibitor. Specifically, at low concentrations, allopurinol acts as a competitive inhibitor, and at high concentrations it acts as a noncompetitive inhibitor. Alloxanthine is a noncompetitive xanthine oxidase inhibitor. XOD: xanthine oxidase.



Anti-gout Drugs. Figure 2 Reabsorption and secretion of uric acid in the proximal renal tubulus. (a) Normal situation. Uric acid is completely reabsorbed in the proximal segment of the renal tubulus and secreted more distally. (b) Situation in untreated hyperuricemia. In most genetically caused cases of gout, uric acid secretion is defective (1). (c) Situation in hyperuricemia under treatment with uricosouric drugs (2); Inhibition of uric acid secretion by uricosuric drugs at low doses (3). Inhibition of uric acid secretion and reabsorption by uricosuric drugs in therapeutic doses. The inhibition of uric acid secretion with low doses of uricosuric drugs can further increase blood levels of uric acid and induce attacks of acute gouty arthritis.

and recurrent episodes of urate lithiasis. Uric acid deposits in the kidney and urate lithiasis can ultimately result in renal failure.

Figure 3 illustrates important pathophysiologic events leading to acute gouty arthritis. Once the concentration of uric acid exceeds its solubility, uric acid crystals form in the synovial fluid of joints. Subsequently, the uric acid crystals are phagocytosed by synoviocytes that form the inner cell layer of joints. Next, synoviocytes release numerous mediators of inflammation including leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2), platelet-activating factor (PAF), histamine, interleukins (ILs) 1, 6, and 8, and tumor necrosis factor-α that in conjunction with products of the complement cascade (C5a and C3a) and kinins (bradykinin) induce an inflammatory response. Moreover, LTB_4, PAF, C5a, and IL-8 attract polymorphonuclear leukocytes (neutrohpils). Neutrophils migrate into affected joints along a concentration gradient of these inflammatory mediators (chemotaxis). Accordingly, LTB_4, PAF, C5a, and IL-8 are also referred to as chemoattractants. Once present in joints, neutrophils phagocytose uric acid crystals. Uric acid crystals and chemoattractants trigger the release of cytotoxic lysosomal enzymes, NADPH oxidase-catalyzed formation of reactive oxygen species, LTB_4 formation and the release of other proinflammatory molecules from neutrophils. The latter molecules attract additional neutrophils and mononuclear phagocytes. Moreover, neutrophils generate lactate that decreases the pH within the joint and further accelerates uric acid crystal formation. Oxygen radicals and lysosomal enzymes cause damage to tissues. Thus, the presence of uric acid crystals in joints triggers a vicious cycle, resulting in an extremely painful inflammation. A typical localization of acute gouty arthritis is the first metatarsal joint of the foot (podagra). The diagnosis of acute gouty arthritis is confirmed by the detection of urate crystals in the joint or tophus.

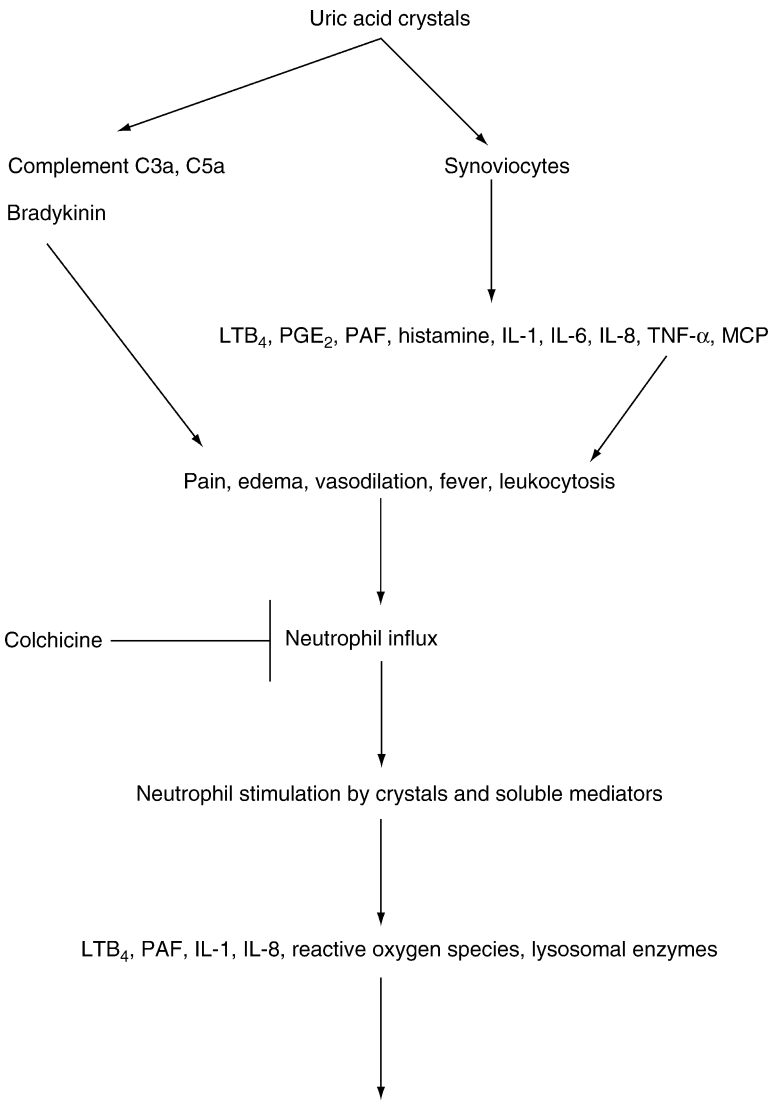
Anti-gout Drugs

Figure 4 shows the structures of commonly employed anti-gout drugs. The treatment of acute gouty arthritis aims at rapidly reducing the pain and inflammatory reaction. This aim can be achieved by treatment with colchicine. In addition, ►nonsteroidal anti-inflammatory drugs (NSAIDs), ►glucocorticoids, and adrenocorticotropic hormone (ACTH) can be used to treat acute gouty arthritis. However, since NSAIDs and glucocorticoids are used in numerous other commonly occurring inflammatory conditions, they are not *per se* considered specific anti-gout drugs. Glucocorticoids can be given systemically (orally, intramuscularly, or intravenously) or locally into afflicted joints. The long-term goals of gout treatment are the prevention of acute gouty arthritis, the prevention of urate lithiasis and renal failure and the resorption of existing uric acid deposits in the joints and urinary tract. The long-term therapy aims at reducing the serum concentration of uric acid below 357 μmol/L. Therapy with the ►uricostatic drug allopurinol and the ►uricosuric drugs benzbromarone, sulfinpyrazone, or probenecid can accomplish the long-term goals. These drugs are well tolerated in most patients. Uricostatic and uricosuric drugs can be combined. Additionally, low doses of colchicine can be used to prevent the occurrence of acute gouty arthritis. However, as is unfortunately often the case with classic diseases, there are only few well conducted clinical studies assessing the clinical efficacy and safety of anti-gout drugs.

Mechanism of Action

Colchicine

Colchicine is an alkaloid from the autumn crocus *Colchicum autumnale*. Colchicine binds to the cytoskeletal protein ►tubulin and, thereby, prevents microtubule formation. As a result, colchicine inhibits neutrophil chemotaxis and the influx of these cells into areas containing uric acid crystals (Fig. 3). Colchicine also inhibits neutrophil phagocytosis. As a result, colchicine interrupts the vicious cycle of inflammation in gouty arthritis. However, because of its mechanism of action, colchicine is most effective only when given in the early stages of gouty arthritis, i.e. within 24 h. Otherwise, the inflammatory reaction may be too advanced. Specifically, colchicine is effective in >90% of patients when given within the first few hours after the start of the attack, but after 24 h, the responsiveness decreases to 75%. Given the very significant side effects of colchicine, it is absolutely crucial to initiate colchicine therapy as early as possible.



Anti-gout Drugs. Figure 3 Important pathophysiologic events in acute gouty arthritis. Uric acid crystals activate the complement cascade, the formation of kinins and the release of various mediators of inflammation from synoviocytes that phagocytose uric acid crystals. The combined action of the released mediators induces a strong inflammatory reaction that is further enhanced by neutrophils. Neutrophils migrate along a concentration gradient to loci in which C5a, LTB_4, PAF and IL-8 are produced (chemotaxis). Accordingly, C5a, LTB_4, PAF and IL-8 are also referred to as chemoattractants. Neutrophils phagocytose uric acid crystals. Upon exposure to uric acid crystals and chemoattractants, neutrophils release various mediators of inflammation, reactive oxygen species and lysosomal enzymes. The concerted effects of all theses compounds amplify the inflammatory reaction even further. Colchicine interrupts the vicious cycle of inflammation predominantly by inhibiting neutrophil chemotaxis. IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; LTB_4, leukotriene B_4; MCP, monocyte chemoattractant protein; PAF, platelet-activating factor; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α.

Allopurinol

Allopurinol is an analog of hypoxanthine and is converted to alloxanthine by XOD. Both allopurinol and hypoxanthine inhibit XOD (Fig. 1). Alloxanthine is a noncompetitive inhibitor of XOD as is allopurinol at high concentrations. At low concentrations, allopurinol is a competitive inhibitor of XOD. As a result of XOD inhibition, the formation of the poorly soluble uric acid is reduced, whereas the formation of the more soluble metabolites hypoxanthine and xanthine is increased. Because of the good solubility of hypoxanthine and xanthine, formation of hypoxanthine/xanthine crystals is a rare complication of allopurinol treatment. Another consequence of XOD inhibition is the accumulation of the precursor of xanthine, inosine. Inosine inhibits the key enzyme of *de novo* purine synthesis,

phosphoribosyl-pyrophosphate amidotransferase. The allopurinol metabolite allopurinol ribonucleotide also inhibits phosphoribosyl-pyrophosphate amidotransferase. Inhibition of purine biosynthesis contributes to the anti-hyperuricemic effects of allopurinol.

Uricosuric Drugs

Depending on the dose applied, uricosuric drugs inhibit tubular reabsorption and tubular secretion of uric acid in the kidney differentially (Fig. 2). At low (subtherapeutic) doses, uricosuric drugs inhibit uric acid secretion without inhibiting reabsorption. Therefore, low doses of uricosuric drugs can actually increase serum levels of uric acid and trigger acute attacks of gouty arthritis. At higher, i.e. therapeutic doses, uricosuric drugs inhibit both tubular secretion and tubular reabsorption. Since inhibition of tubular reabsorption is quantitatively more important than inhibition of tubular secretion, the net effect is an increased renal elimination of uric acid. In order to avoid formation of uric acid crystals in the kidney and urinary tract, it is important that the pH of the urine is kept >6.0. This goal can be achieved by the oral administration of potassium sodium hydrogen citrate, sodium bicarbonate or acetazolamide. In addition, it is mandatory that the patient drinks at least 3L per day to avoid formation of uric acid crystals.

New Drugs

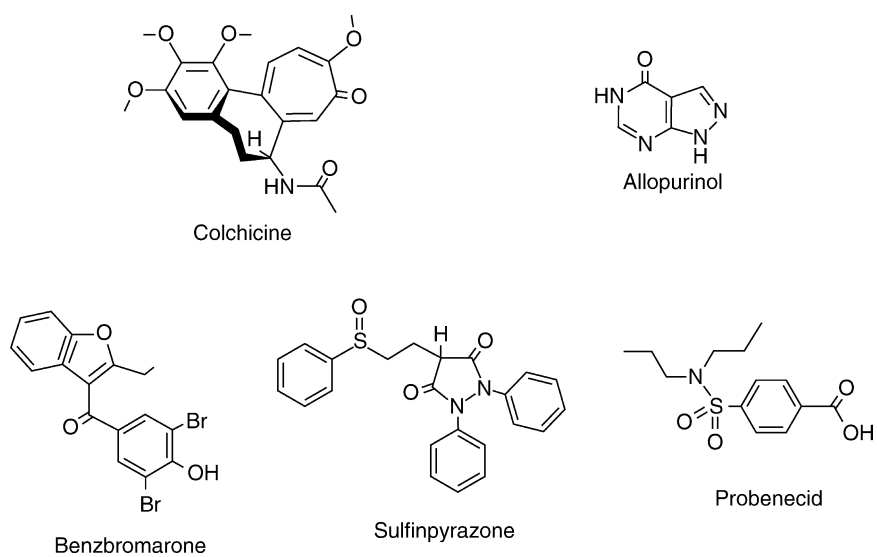
Rasburicase is a recombinant urate oxidase that catalyzes the conversion of uric acid to allantoin which possesses a greater water-solubility than uric acid. In contrast to allopurinol, rasburicase has also an inhibitory effect on existing uric acid pools, and therapy with the enzyme does not require urine alkalinization. Rasburicase is used in the treatment of pediatric patients with leukemias, lymphomas, and solid tumors who are at high risk for chemotherapy-induced hyperuricemia. Hyperuricemia is part of the so-called tumor lysis syndrome. Further studies are required to compare the clinical efficiency of rasburicase in adult patients and its efficiency in comparison to standard treatment with allopurinol. The use of rasburicase can be associated with severe side effects including hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency, methemoglobinemia, kidney failure, and anaphylactic reactions.

Febuxostat (TEI-6720, TMX-67) is a highly potent mixed-type inhibitor of XOD with Ki values in the 0.5–3 nM-range. Febuxostat displays high selectivity relative to a large number of other purine- and pyrimidine-metabolizing enzymes. Febuxostat can be administered orally and is well tolerated, and there is no need for dose-adjustment in patients with kidney failure and mild-to-moderate liver failure. Therapeutic doses are 80–120 mg daily. Clinical trials indicate that febuxostat may be superior to allopurinol at reducing serum urate concentrations but not at reducing the incidence of gouty arthritis and tophus formation.

Clinical Use (including side effects)

Colchicine

Daily doses of 3–8 mg (6–8 times 0.5–1.0 mg) are used for the treatment of acute gouty arthritis. For prophylaxis, daily doses of 0.5–1.5 mg are used, but the use of colchicine for prophylaxis is controversial.



Anti-gout Drugs. Figure 4 Structures of commonly used anti-gout drugs. Colchicine is an alkaloid from the autumn crocus *Colchicum autumnale* and inhibits tubulin polymerization. Allopurinol is an isomer of xanthine and inhibits uric acid formation (uricostatic drug). Benzbromarone, sufinpyrazone and probenecid are uricosuric drugs and inhibit uric acid reabsorption in the proximal tubulus of the kidney.

The side effects of colchicine are very significant. About 80% of the patients experience gastrointestinal problems including nausea, vomiting, and diarrhea. The anti-mitotic effects of colchicine can result in thrombocytopenia, agranulocytosis, hair loss, and azoospermia. In the central nervous system, confusion, ascending paralysis, respiratory failure, and seizures have been reported. These side effects can be explained by the fact that intact microtubules are essential for proper transport functions in neuronal axons. Moreover, colchicine can cause myopathy. Because of the significant side effects, many physicians prefer to treat acute gouty arthritis with NSAIDs or glucocorticoids. Although colchicine is a classic anti-gout drug, colchicine can also be used to treat other inflammatory diseases including amyloidosis, Dupuytren's contracture, Behcet's syndrome, vasculitis, various forms of hepatic cirrhosis, pulmonary fibrosis, pericarditis, and various inflammatory diseases of the skin. Colchicine is extensively metabolized through the hepatic cytochrome CYP 3A4. Accordingly, inhibitors of CYP 3A4 such as diltiazem, gestodene, grapefuit juice, ketoconazole, and macrolide antibiotics prolong and enhance the pharmacological (and toxic) effects of colchicine. Drugs that are inactivated via CYP 3A4 such as steroid hormones, lidocaine, midazolam, quinidine, terfenadine, nifedipine, and verapamil can also prolong colchicine action. Because of its anti-mitotic effects, colchicine should not be used in pregnant women.

Allopurinol

The daily dose of allopurinol is 300–600 mg. In combination with benzbromarone, the daily allopurinol dose is reduced to 100 mg. In general, allopurinol is well tolerated. The incidence of side effects is 2–3%. Exanthems, pruritus, gastrointestinal problems, and dry mouth have been observed. In rare cases, hair loss, fever, leukopenia, toxic epidermolysis (Lyell syndrome), and hepatic dysfunction have been reported. Allopurinol inhibits the metabolic inactivation of the cytostatic drugs azathioprine and 6-mercaptopurine. Accordingly, the administered doses of azathioprine and 6-mercaptopurine must be reduced if allopurinol is given simultaneously.

Uricosuric drugs

Benzbromarone

The daily dose of benzbromarone is 50–200 mg. In combination with allopurinol, the benzbromarone dose is reduced to 20 mg. Benzbromarone is well tolerated. Rare side effects are headaches, gastrointestinal problems, and exanthems.

Probenecid

The daily dose of probenecid is 0.5–3.0 g. Probenecid is well tolerated, and there are few serious side effects. In less than 10% of the treated patients, gastrointestinal disturbances, hypersensitivity, and skin reactions occur.

Sulfinpyrazone

The daily dose of sulfinpyrazone is 200–400 mg. The side effects of sulfinpyrazone are comparable with those of probenecid. A potential therapeutic advantage of sulfinpyrazone in patients with coronary heart disease and thromboembolic diseases is its inhibitory effect on platelet aggregation.

►Inflammation

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Antihistamines

The term antihistamines describes drugs which bind to the H_1-histamine receptor and antagonize (block) the histamine effect in Type I allergic responses.

►Allergy
►Histaminergic System

Antihypertensive Drugs

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Synonyms

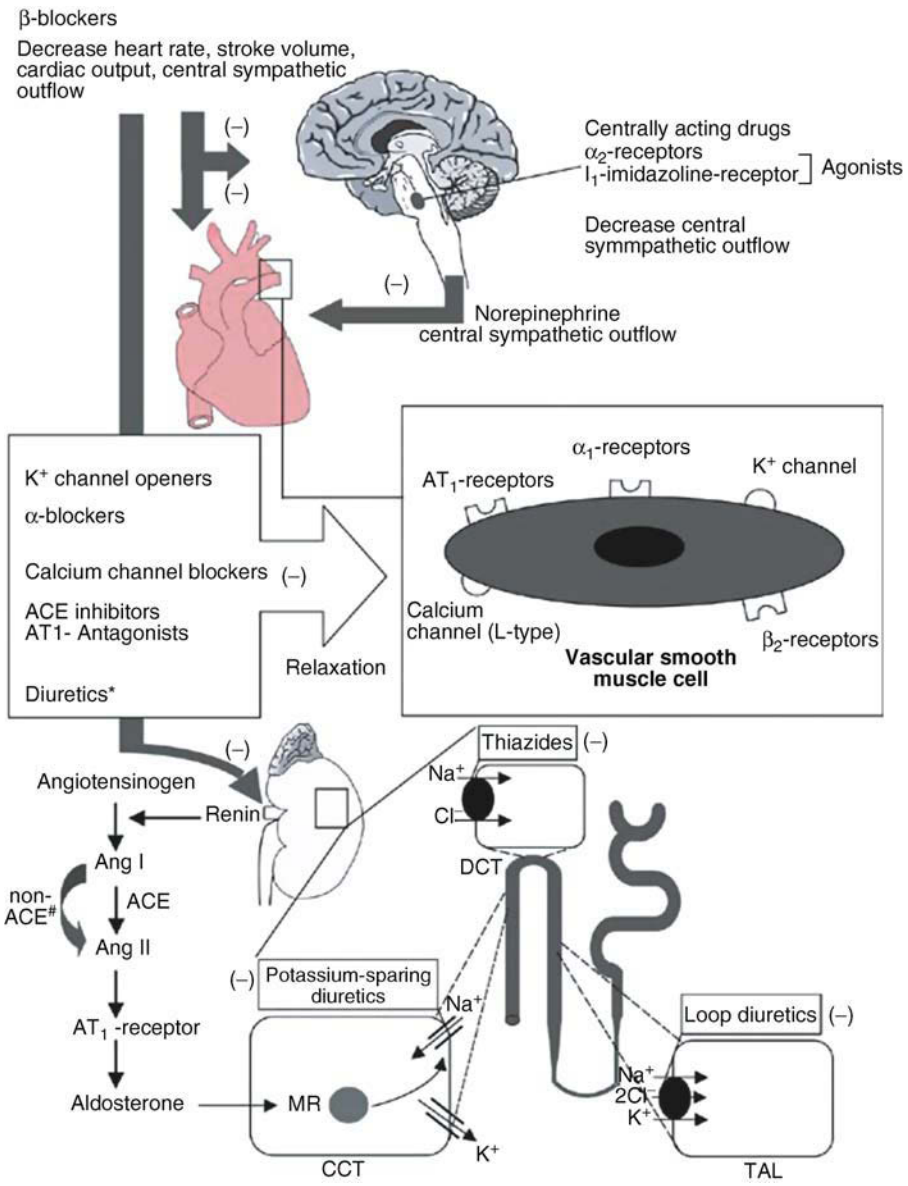
Antihypertensives; Blood pressure lowering drugs

Definition

Reducing blood pressure by pharmacological means reduces cardiovascular morbidity and mortality rates. Benefits include protection from stroke, coronary events, heart failure, progression of renal disease, progression to more severe hypertension, and, most importantly mortality from all causes. Owing to the complexity of the pathogenesis of hypertension, antihypertensive drugs are directed against a variety of pharmacological targets in various cell types in different organs involved in blood pressure control.

Mechanism of Action

The fundamental mechanisms involved in blood pressure control have been outlined in chapter "Blood pressure control." In addition to direct neuronal modulation of arterial pressure two neurohumoral systems, i.e., the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAS) play a pivotal role in blood pressure control. Both systems are always either directly or indirectly affected by treatment with any antihypertensive drug. Antihypertensive agents can be categorized into seven different drug classes (Fig. 1) [1]. Centrally-acting antihypertensive drugs can be classified according to their relative affinities to α_2- and imidazoline (I_1) receptors. Clonidine is considered as a mixed α_2- and I_1-agonist, whereas moxinidine acts as a relative selective I_1-agonist. Methyldopa is a selective α_2-agonist. Administration of clonidine results in decreased cardiac output, a preservation of baroreflexes with a relative reduction in tendency of heart rate to rise, and little change in peripheral resistance. Methyldopa decreases sympathetic outflow predominantly to the α_1-receptors of the arterioles, thereby reducing peripheral resistance with little (but some) effect on the heart. The baroreceptor arc is impaired because of the effect on arterioles [2]. Direct vasodilators such as minoxidil and hydralazine work by opening potassium channels in vascular smooth muscle cells in arterioles, which leads to K^+ efflux and hyperpolarization. Since the heart is not directly affected, direct vasodilators lead when used alone to reflex increases in heart rate and force of contraction; a significant neurohumoral activation of both the sympathetic nervous system and RAS occurs. Because of the activation of counterregulatory systems, the simultaneous use of β-blockers and diuretics is generally required [3]. Selective α_1-blockers inhibit the action of norepinephrine (noradrenaline) at arteriolar receptors, thereby leading also to activation of counterregulatory systems [4]. Calcium channel blockers act primarily as inhibitors of vasoconstriction by blocking L-type calcium channels in vascular smooth muscle cells. However, there are two different main classes (dihydropyridines and non-dihydropyridines), which work on different sites within the L-channel and hence produce different effects in the kidney, heart and vasculature. The nondihydropyridines verapamil and diltiazem blunt increases in heart rate in response to exercise and have both negative inotropic and negative chronotropic (verapamil > diltiazem) effects; most dihydropyridines do not have major cardiodepressant effects because the long-acting agents slightly increase sympathetic nervous system tone, while this negative effect is even more pronounced with short-acting agents. In general, dihydropyridines lead to increases in heart rate and do not blunt the increase in heart rate response to exercise. Calcium channel blockers have a slight (transient) natriuretic effect [5]. The competitive inhibition of β-blockers on β-receptors results in numerous effects on functions that regulate blood pressure, including a reduction in cardiac output, a decrease in renin release, perhaps a decrease in both central sympathetic nervous outflow and peripheral resistance. The view that the primary effect is a reduction in cardiac output as a result from the blockade of cardiac β_1-receptors with a subsequent reduction of heart rate and myocardial contractility has been questioned. Indeed, it seems that although cardiac output usually falls acutely and remains lower chronically, peripheral resistance on the other hand rises acutely but falls towards, if not to, normal with time. Thus, the hemodynamic hallmark of chronic established hypertension, which is an increased peripheral resistance, is also normalized by β-blockers. All currently available β-blockers antagonize cardiac β_1-receptors competitively, but they vary in their degree of β_2-receptor blockade in extra cardiac tissues. However, there seems to be little difference in antihypertensive efficacy among those that are more or less cardio- or β_1-selective. Although the presence of intrinsic sympathomimetic activity (ISA) in some compounds such as pindolol and acebutolol could in theory translate into some beneficial effects, there is little convincing evidence that partial agonism confers significant clinical benefits. The newer compounds carvedilol and nebivolol produce additional vasodilator features that are attributable either to the additional blockade of α-receptors (carvedilol) or endothelial nitric oxide release (nevibolol). The mode of action of diuretics depends on their major site of action within the nephron (Fig. 1). These differences determine their relative efficacy as expressed in the maximal percentage of filtered sodium excreted. Sixty percent of the filtered sodium is reabsorbed in the proximal tubule of the nephron. Thirty percent is reabsorbed in the thick ascending limb of Henle by ►$Na^+/K^+/2Cl^-$ cotransport (►$Na^+/K^+/2Cl^-$ cotransporter), which is inhibited by loop diuretics. Seven percent is reclaimed by Na^+/Cl^- cotransport in the distal convoluted tubule, which is inhibited by thiazide diuretics. The last 2% is reabsorbed via the ►epithelial Na^+ channel (ENaC) in the cortical collecting duct, which is a target either directly (amiloride, triamterene) or indirectly via the mineralocorticoid receptor (spironolactone) for potassium-sparing agents (Fig. 1).



Antihypertensive Drugs. Figure 1 Site of action of different classes of antihypertensive drugs. Antihypertensive drugs are directed against a variety of pharmacological targets in various cell types in different organs involved in blood pressure control. The most important targets in the brain, heart, vasculature (vascular smooth muscle cells), and the kidney (nephron) are shown. *Some diuretics produce some direct vasodilation; # non-ACE, conversion of angiotensin I (Ang I) to angiotensin II (Ang II) may occur independent from ACE due to the activity of other enzymes in different tissues such as chymase in the heart; DCT, distal convoluted tubule; CCT, cortical collecting duct; TAL, thick ascending limb of the loop of Henle; (−), indicates inhibition. Modified according to reference 3.

Agents acting in the proximal tubule are seldom used to treat hypertension. Treatment is usually initiated with a thiazide-type diuretic. Chlorthalidone and indapamide are structurally different from thiazides but are functionally related. If renal function is severely impaired (i.e., serum creatinine above 2.5 mg/dl), a loop diuretic is needed. A potassium-sparing agent may be given with the diuretic to reduce the likelihood of hypokalemia. By themselves, potassium-sparing agents are relatively weak antihypertensives. In general, there are four ways to reduce the activity of the RAS. The first way is the use of β-blockers to reduce renin release from the juxtaglomerular (JG). The second way, the direct inhibition of the activity of renin, although being actively investigated has not been successful in the clinical arena thus far. The third way is to inhibit the activity of the

►angiotensin converting enzyme (ACE), which converts the inactive decapeptide angiotensin I to the potent octapeptide angiotensin II (Ang II), by agents referred to as ACE inhibitors. Thus, these agents inhibit the biosynthesis of Ang II and thereby decrease the availability of Ang II at both angiotensin type 1 (AT_1) and angiotensin type 2 (AT_2) receptors. The fourth way is to use a competitive and selective antagonist at the AT_1 receptor (i.e., AT_1 antagonists) and thereby to inhibit the classical effects mediated by Ang II such as vasoconstriction and aldosterone release. ACE inhibitors exhibit additional effects that are independent from RAS such as on kinins, since ACE is also a kininase. Although the clinical relevance is not fully understood, blood pressure effects mediated via inhibition of breakdown of bradykinin may contribute to the vasodilatory effects of ACE inhibitors. Some of the latter effects could be mediated via kinin stimulation of prostaglandin production. In addition to these effects on vascular tone, multiple other effects may contribute to the antihypertensive effects of ACE inhibitors. The blunting of the expected increase in sympathetic nervous activity typically seen after vasodilation is potentially of greater importance for the documented clinical benefits of ACE inhibitors. As a result, heart rate is not increased as is seen with direct vasodilators, α-blockers and less pronounced with dihydropyridine calcium channel blockers. The presence of the complete RAS within various tissues including the vasculature, kidney, heart and brain has been demonstrated and the activation of the RAS at the tissue level seems to play - beyond its role on blood pressure regulation - an important role for the manifestation and progression of hypertensive target organ damage in these organs. Our understanding of the molecular mechanisms by which Ang II contributes to both structural and functional changes, e.g., due to its growth factor capacity, at the tissue level continues to expand. Consequently, the inhibition of tissue ACE may play an important role for the prevention and regression of hypertensive target organ damage that has been documented for these agents in experimental and clinical studies.

The major obvious difference between AT_1 antagonists and ACE inhibitors is the absence of an increase in kinins that may be responsible for some of the beneficial effects of ACE inhibitors and probably their side effects. Direct comparison between the two types of drugs show little differences in antihypertensive efficacy but cough, a common side effect seen with ACE inhibitors, is not provoked by AT_1 antagonists, although angioedema and ageusia have also been reported for these newer agents.

Clinical Use (Including Side Effects)

Recent consensus committees, including the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) and the World Health Organization-International Society of Hypertension (WHO-ISH) Guidelines Subcommittee, have modified traditional treatment recommendations in several important ways.

Criteria for initiation of drug treatment now take into consideration total cardiovascular risk rather than blood pressure alone, such that treatment is now recommended for persons whose blood pressure is in the normal range but still bear a heavy burden of cardiovascular risk factors. Thus, the role of simultaneous reduction of multiple cardiovascular risk factors in improving prognosis in hypertensive patients is stressed. In addition, more aggressive blood pressure goals are recommended for hypertensive patients with comorbid conditions such as diabetes mellitus or renal insufficiency.

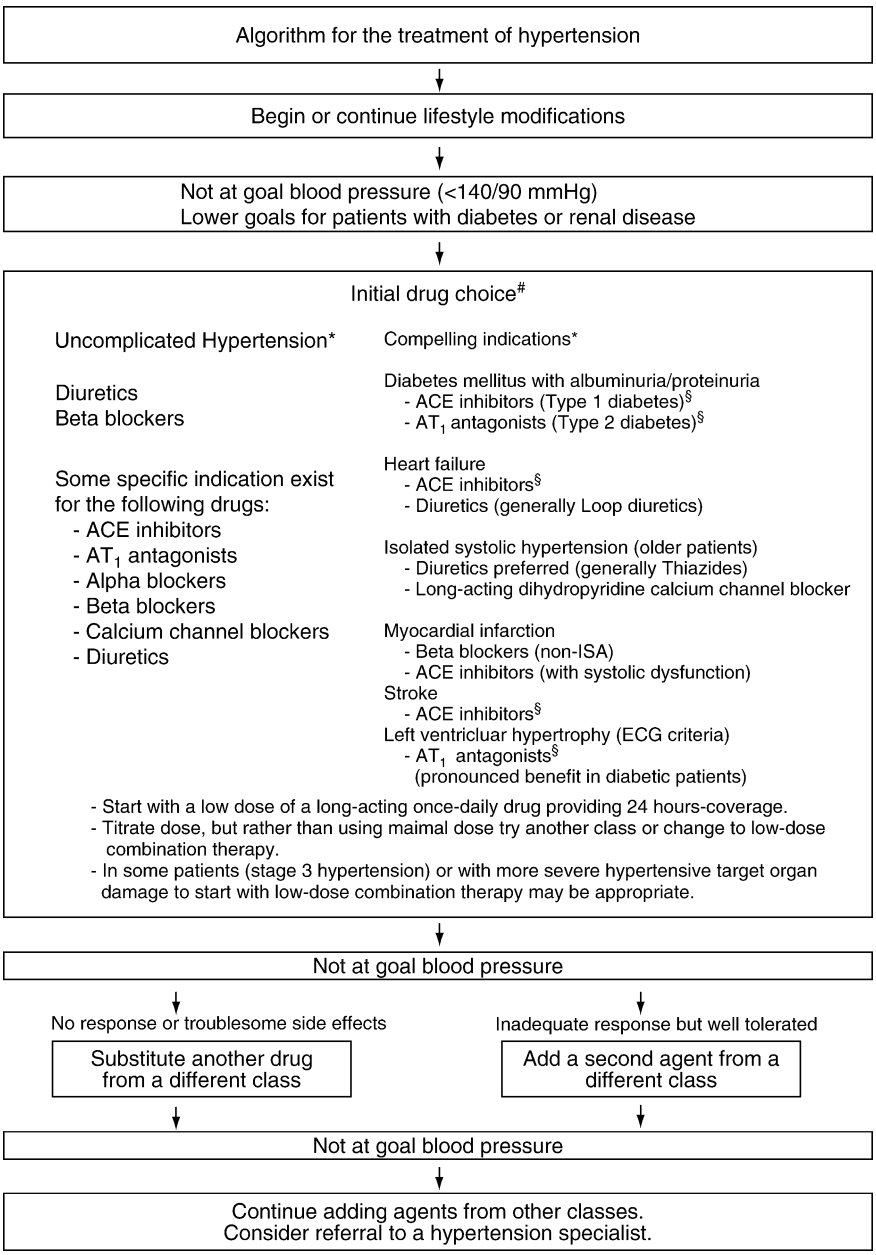
Antihypertensive Drugs. Table 1 Common side effects of antihypertensive drugs

Class of drug		Side effects
ACE inhibitors		Cough, hyperkalemia, skin reactions
AT_1-antagonists		Hyperkalemia (less frequent compared with ACE inhibitors)
Calcium channel blockers	Dihydropyridine	Pedal edema, headache
	Nondiyhdropyridine	Constipation (verapamil); headache (diltiazem)
Diuretics		Frequent urination, hyperuricemia, hyperglycemia, hyperlipidemia
Centrally acting drugs	α2-Receptor agonists	Sedation, dry mouth, rebound hypertension
	Imidazoline-receptor agonists	
Central neuronal blockers (reserpine)		Depression, sedation, nasal congestion
α-Blockers		Orthostatic hypotension, rapid drop of blood pressure after first dose, pedal edema, dizziness
β-Blockers		Fatigue, hyperglycemia, bronchospasm
Potassium channel openers		Hypertrichosis (minoxidil); lupus-like reactions and pedal edema (hydralazine)

Modified according to reference 3.

Finally, drug treatment in the elderly is of great importance and warrants special attention with regard to safety and tolerability, since systolic blood pressure is recognized as an important target for treatment, particularly in older persons. The benefits of antihypertensive treatment in the elderly and in patients with isolated systolic hypertension are greater than in younger persons.

As a consequence for drug treatment, an increasing number of patients will be treated with antihypertensive compounds and the importance of tailoring the choice of antihypertensive drug treatment to the patient's individual profile of concomitant cardiovascular risk factors/comorbid conditions has to be emphasized. Moreover, it is reasonable to individualize antihypertensive treatment on the basis of each patient's personal needs with respect to tolerability, convenience and quality of life. Initiation of treatment with a drug that is expected to be well tolerated and therefore likely to be effective in lowering blood pressure over time is



Antihypertensive Drugs. Figure 2 Algorithm for the treatment of hypertension. # Unless contraindicated; *based on randomized controlled trials; § Evidence suggests that the beneficial effects of ACE inhibitors can be duplicated with AT_1 antagonists (and probably vice versa). Thus, ACE inhibitors could be substituted by AT_1 antagonists in the case of troublesome side effects, such as cough under treatment with ACE inhibitors. Modified according to reference 5.

prudent (common side effects are listed in Table 1). Long-acting agents are preferable because adherence to therapy and consistency of blood pressure control are superior when the drug is taken once a day. Low-dose, fixed-dose combination therapy can be used in place of monotherapy as initial treatment or as an alternative to adding a second agent of a different therapeutic class to unsuccessful monotherapy. The advantage of this approach is that low doses of drugs that act by different mechanisms may have additive or synergistic effects on blood pressure with minimal dose-dependent adverse effects. Giving the patient a single tablet provides an additional benefit. A case in point represents the well-established combination of an ACE inhibitor or AT1-antagonist with low-dose hydrochlorothiazide, which does not produce more side effects than placebo.

Many of the concepts of antihypertensive treatment put forward are adopted from the algorithms recommended by the JNC VI (Fig. 2). Treatment should always include lifestyle modifications. For the minority of hypertensive patients without comorbid conditions, target organ damage, or concomitant cardiovascular disease, the JNC VI recommends starting drug therapy with a diuretic (i.e., thiazides) or β-blocker because these agents had been proven to lower morbidity and mortality compared with placebo in randomized controlled trials. Secondly, they are less costly than newer classes of drugs. Therefore, the era of placebo-controlled trials is past and any new agents can only be compared against the gold standard of diuretics and β-blockers. Overall, these early trials with diuretics and β-blockers established a greater reduction of risk related to stroke (–40%) than the risk related to coronary heart disease (–14%). While diuretics are more effective in preventing stroke than β-blockers the opposite holds true for cardiac risk. A reduction in cardiovascular risk has also been documented for the ACE-inhibitor captopril and in elderly patients with isolated systolic hypertension for the dihydropyridine calcium channel blocker (nitrendipine). However, outcome trials comparing two anti-hypertensives require large groups of patients, because the risk in patients with mild-to-moderate essential hypertension is low, and intervention trials are usually limited to duration of 5 years. Therefore, differences between drug classes have been documented when patients with higher absolute cardiovascular risk and/or comorbid conditions were studied. Compelling indications that have been established in randomized controlled trials are summarized in Fig. 2.

►ACE Inhibitors
►Blood Pressure Control
►Ca^{2+} Channel Blockers
►Diuretics
►Renin–Angiotensin–Aldosterone System

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Anti-inflammatory Drugs

►Non-steroidal Anti-inflammatory Drugs

Anti-integrins

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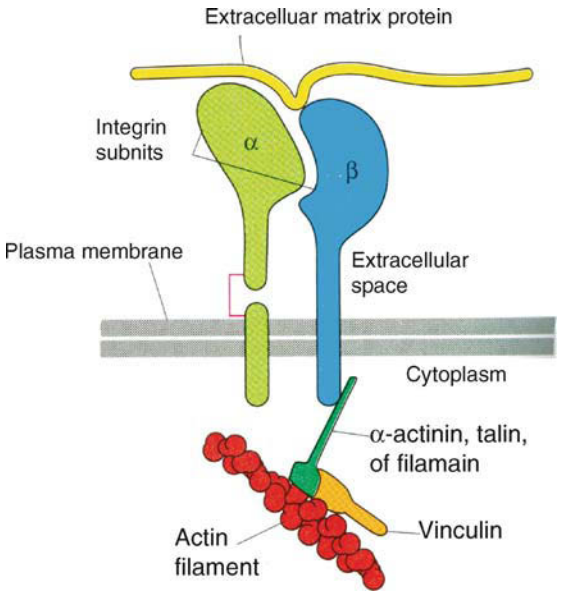
Definition

Integrins are a widely expressed family of cell adhesion receptors via which cells attach to extracellular matrices either to each other or to different cells. All integrins are composed of αβ heterodimeric units (Fig. 1), expressed on a wide variety of cells, and most cells express several integrins. The interaction of integrins with the cytoskeleton and extracellular matrix appears to require the presence of both subunits. The binding of integrins to their ligands is cation-dependent. Integrins appear to recognize specific amino acid sequences in their ligands. The best studied is the RGD sequence found within a number of ►matrix proteins including fibrinogen, vitronectin, fibronectin, thrombospondin, osteopontin and VWF (Fig. 1). However, other integrins bind to ligands via non-RGD binding domain such as the α4β1 integrin receptors that bind and recognize the LDV sequence within the CS-1 region of fibronectin. There are at least 8 known β subunits and 14 α subunits [1]. Although the association of the different β and α subunits



Anti-integrins. Figure 1

could in theory result in more than 100 integrins, the actual diversity is restricted.

Basic Characteristics

Integrin adhesion receptors contain an extracellular face that engages adhesive ligands and a cytoplasmic face that engages with intracellular proteins. The interactions between the cell adhesion molecules and extracellular matrix proteins are critical for cell adhesion and for anchorage-dependent signaling reactions in normal and pathological states. For example, platelet activation induces a conformational change in integrin αIIb/β3, thereby converting it into a high affinity fibrinogen receptor. Fibrinogen binding then triggers a cascade of protein tyrosine kinases, phosphatases and recruitment of numerous other signaling molecules into F-actin-rich cytoskeletal assemblies in proximity to the cytoplasmic tails of αIIb and β3 [2]. These dynamics appear to influence platelet functions by coordinating signals emanating from integrins and G protein-linked receptors. Studies of integrin mutations confirm that the cytoplasmic tails of αIIb/β3 are involved in integrin signaling presumably through direct interactions with cytoskeletal and signaling molecules [2]. Blockade of fibrinogen binding to the extracellular face of αIIb/β3 has been shown to be an effective way to prevent arterial ►thrombosis after coronary angioplasty in myocardial infarction and unstable angina patients [2].

Mechanism of Action and Clinical Use

Pathophysiology and Therapeutic Potential

The role of integrins has been found in various pathological processes, including ►angiogenesis, thrombosis, apoptosis, cell migration and proliferation. These processes lead to both acute and chronic diseases such as ocular diseases, metastasis, unstable angina, myocardial infarction, stroke, ►osteoporosis, and a wide range of inflammatory diseases, vascular remodeling and neurodegenerative disorders. A breakthrough in this field is evident from the role of the platelet αIIbβ3 integrin in the prevention, treatment and diagnosis of various thromboembolic disorders. Additionally, significant progress in the development of leukocyte α4β1 antagonists for various inflammatory indications and αv integrin antagonists for angiogenesis and vascular-related disorders has been achieved.

β1 Integrins

α4β1 Integrin

The largest numbers of integrins are members of the β1 integrins, also known as the very late antigen (VLA) subfamily because of its late appearance after activation. There are at least seven receptors characterized from this subfamily, each with different ligand specificity. Among the most studied include the α4β1 and α5β1 receptors. The leukocyte integrin α4β1 is a cell adhesion receptor that is predominantly expressed on lymphocytes, monocytes and eosinophils.

Potent and Selective Small Molecule Antagonists of α4β1 Integrins

The α4β1 integrins are heterodimeric cell surface molecules central to leukocyte-cell and leukocyte-matrix adhesive interactions. The integrin α4β1, expressed on all leukocytes except neutrophil, interacts with the immunoglobulin superfamily member VCAM-1 and with an alternately spliced form of fibronectin. Additionally, the integrin α4β7 is also restricted to leukocytes and can bind not only to VCAM1 and fibronectin, but also to MAdCAM the mucosal addressin or homing receptor, which contains Ig-like domains related to VCAM-1. *In vivo* studies with α4β1 monoclonal antibodies in several species demonstrate that the interactions between these integrins and their ligands play a key role in immune and ►inflammatory disorders [3] and selected ones are in clinical trials.

α5β1 Integrin in Angiogenesis

In contrast to collagen, expression of the extracellular matrix protein fibronectin in provisional vascular matrices precedes permanent collagen expression and provides signals to vascular cells and fibroblasts during blood clotting and wound healing, atherosclerosis and hypertension. Fibronectin expression is also upregulated on blood vessels in granulation tissues during wound healing. These observations suggest a possible role for this isoform of fibronectin in angiogenesis. Evidence was recently provided that both fibronectin and its receptor integrin α5β1 directly

regulate angiogenesis [4]. Thus, integrin antagonist for α5β1 integrin might be a useful target for the inhibition of angiogenesis associated with human tumor growth; neovascular related ocular and inflammatory diseases.

α5β1 Integrin and Bacterial Invasion

Recent studies suggested a key role for α5β1 integrin in certain bacterial invasion of human host cells leading to antibiotic resistance [5].

β3 Integrins

Intravenous and Oral Platelet IIb/β3 Receptor Antagonists: Potential Clinical Utilities

There is an urgent need for more efficacious antithrombic drugs superior to aspirin or ticlopidine for the prevention and treatment of various cardiovascular and cerebrovascular thromboembolic disorders. The realization that the platelet integrin αIIbβ3 is the final common pathway for platelet aggregation regardless of the mechanism of action prompted the development of several small molecule αIIb/β3 receptor antagonists for intravenous and/or oral antithrombotic utilities. Platelet αIIb/β3 receptor blockade represents a very promising therapeutic and diagnostic strategy of thromboembolic disorders. Clinical experiences (efficacy/safety) gained with injectable but not oral αIIbβ3 antagonists (Abciximab, Eptifibatide, Aggrastat) elucidate the safety and efficacy of this mechanism in combination with other antiplatelet and anticoagulant therapies.

Orally Active GPIIb/IIIa Antagonists

A high level of platelet antagonism has been required when GPIIb/IIIa antagonists have been employed for acute therapy of coronary arterial diseases using intravenous GPIIb/IIIa antagonists with heparin and aspirin. Interaction with aspirin and other antiplatelet and anticoagulant drugs lead to shifts in the dose-response curves for both efficacy and unwanted side effects, such as increased bleeding time. More recently, all oral GPIIb/IIIa antagonists with or without aspirin but not with anticoagulant were withdrawn because of a disappointing outcome (no clinical benefit or increased thrombotic events). This raises a lot of serious questions with regard to the potential of oral GPIIb/IIIa antagonists as compared to the well-documented success of intravenous GPIIb/IIIa antagonists.

GPIIb/IIIa Integrin Receptor Antagonists in the Rapid Diagnosis of Thromboembolic Events

The role of the platelet integrin GPIIb/IIIa receptor and its potential utility as a radio-diagnostic agent in the rapid detection of thromboembolic events has been demonstrated [6]. This approach may be useful for the noninvasive diagnosis of various thromboembolic disorders.

Integrin αvβ3 Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels

Antagonists of integrin αvβ3 inhibit the growth of new blood vessels into tumors cultured on the chick chorioallantoic membrane without affecting adjacent blood vessels, and also induce tumor regression [7]. Antagonists of αvβ3 also inhibit angiogenesis in various ocular models of retinal neovascularization [7].

Integrin αvβ3 in Restenosis

The calcification of atherosclerotic plaques may be induced by osteopontin expression, since osteopontin is a protein with a well-characterized role in bone formation and calcification. Vascular smooth muscle cell migration on osteopontin is dependent on the integrin αvβ3 and antagonists of αvβ3 prevent both smooth muscle cell migration and restenosis in some animal model [8].

Integrin αvβ3 Antagonists Versus Anti- αvβ3 and αvβ5

Since the recognition of at least two αv integrin pathways for cytokine-mediated angiogenesis, αvβ3 and αvβ5 antagonists may be more effective in certain indications as compared to a specific anti- αvβ3. However, further work is needed to document this notion.

Potential Role of αvβ3 Antagonists in Osteoporosis

RGD analogs have been shown to inhibit the attachment of osteoclasts to bone matrix and to reduce bone resorptive activity *in vitro*. The cell surface integrin, αvβ3, appears to play a role in this process. RGD analogs may represent a new approach to modulating osteoclast-mediated bone resorption and may be useful in the treatment of osteoporosis [9].

Integrins αvβ3 Ligands

Therapeutics: A number of potent small molecule antagonists for αvβ3 integrin are under preclinical investigations for various angiogenesis or vascular-mediated disorders [10].

Site directed delivery: This approach of conjugating αvβ3 integrin ligand with a chemotherapeutic agent for optimal efficacy and safety in cancer is under investigation. Earlier work demonstrated the validity of this concept [10].

Diagnostics: Imaging metastatic cancer using technetium-99m labeled RGD-containing synthetic peptide has been demonstrated. Additionally, detection of tumor angiogenesis *in vivo* by αvβ3-targeted magnetic resonance imaging (MRI) was demonstrated [10].

►Angiogenesis and Vascular Morphogenesis
►Antiplatelet Drugs

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Antimalarial Drugs

Antiprotozoal Drugs.

Antimetabolic Agents

►Antimetabolites

Antimetabolites

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Synonyms

Anticancer antimetabolites; Antimetabolic agents; Antineoplastic antimetabolites

Definition

Antimetabolites compete with and possibly oust naturally occurring metabolites required for normal biochemical reactions and lead either to the synthesis of malfunctioning macromolecules and/or blockade of necessary intermediate or final metabolic products that are vital to cell survival. Both processes interfere with DNA synthesis and therefore antimetabolites can be used in cancer treatment, as they inhibit cell division and the growth of tumors.

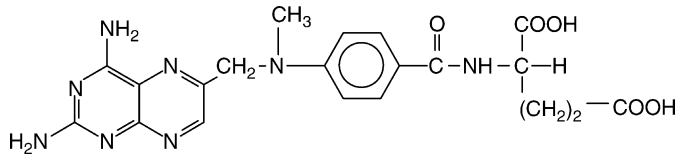
Mechanism of Action

A broad description of antimetabolites would include compounds with structural similarity to precursors of purines or pyrimidines or agents that interfere with purine and pyrimidine synthesis. Antimetabolites can cause either DNA damage indirectly through misincorporation into DNA followed by abnormal timing or progression through DNA synthesis, or altered function of enzymes involved in pyrimidine and purine synthesis. All antimetabolites tend to exert greatest cytotoxicity in the S-phase of cells and the duration of exposure is of great importance. The antimetabolite activity is rather unselective and affects all fast proliferating cells. In contrast to alkylating agents, second malignancies are not associated with their use. The following is a list of commonly used antimetabolites [1].

1. Folate antagonists
   Methotrexate
   Ralitrexed
   Pemetrexed (alimta)
2. Purine antagonists
   Mercaptopurine (6-MP)
   Thioguanine (6-TG)
   Pentostatine
   Fludarabine phosphate (fludara, F-ara-A)
3. Pyrimidine antagonists
   Fluorouracil (5-FU)
   Cytosine arabinoside (cytarabine, Ara C)
   Gemcitabine (gemzar)

Some newly introduced pyrimidine analogs (e.g., azacitidine and decitabine) differ in their mechanism of action to such a degree from the other antimetabolites that they are subgrouped under the heading DNA de- or ►hypomethylating agents (see below).

Methotrexate (MTX, chemical structure shown in Fig. 1.) competitively inhibits the dehyrofolate reductase, an enzyme that plays an essential role in purine synthesis. The dehydrofolate reductase regenerates reduced folates when thymidine monophosphate is formed from deoxyuridine monophosphate. Without reduced folates cells are unable to synthesize thymine. Administration of *N*-5 tetrahydrofolate or *N*-5 formyltetrahydrofolate (folinic acid) can bypass this block and rescue cells from methotrexate activity by serving as antidote.



Antimetabolites. Figure 1 Chemical structure of methotrexate.

MTX and naturally occurring reduced folates are transported into cells by the folate carrier which has a higher affinity to MTX than to the natural folates. Inside of cells, MTX will be polyglutamated and thus enter a deep compartment from which it can be released again only slowly. High extracellular concentrations can bypass this carrier by passive diffusion. MTX is well absorbed orally and at usual dosages half of its level is bound to plasma proteins. After intravenous application the decay is triphasic: distribution phase; initial elimination and a prolonged elimination, the latter corresponding to the slow release from the intracellular polyglutamate compartment.

Clinical Use (including Side-Effects)

This scenario is the background for designing high dose methotrexate regimens with leucovorin rescue of normal hematopoietic and mucosa cells as part of curative therapy schedules for osteosarcoma and hematological neoplasias of children and adults. Methotrexate is cleared from the plasma by the kidney by both glomerular filtration and tubular secretion. Therefore the toxicity of this drug is augmented by renal dysfunction.

MTX is part of curative therapeutic schedules for acute lymphoblastic leukemias (ALL), Burkitt's lymphoma, and choriocarcinoma. It was also used in adjuvant therapy of breast cancer. High dose MTX with leucovorin rescue can induce about 30% remissions in patients with metastatic osteogenic sarcoma. MTX is one of the few antineoplastic drugs that can be safely administered intrathecally for the treatment of meningeal metastases and leukemic infiltrations (routine prophylaxis in ALL). In addition, MTX can be used as an immunosuppressive agent for the treatment of severe rheumatoid arthritis and psoriasis.

Myelosuppression is the major dose-limiting side effect. Gastrointestinal toxicity may appear as ulcerative mucositis and diarrhea. Nausea and vomiting, alopecia, and skin inflammation are common following high-dose MTX treatment. Renal toxicity has major impact for high-dose regimens with MTX and the drug should not be used in patients with renal injury. ►Intrathecal application may be associated with mild arachnoiditis or severe and progressive myelo- or encephalopathy. Chronic low dose MTX may cause cirrhosis of the liver. MTX is a potent ►teratogen and abortifacient.

Ralitrexed is a folate analog with greater selectivity. It easily crosses the cell membrane and undergoes polyglutamation. Within tissues, ralitrexed may be stored up to 29 days. It directly inhibits thymidylate synthase, the key enzyme for synthesizing thymidine triphosphate (TTP). The drug has been described to induce apoptosis in tumor cells. Ralitrexed is used for the treatment of colon carcinomas.

Pemetrexed is an antifolate ►antineoplastic agent that exerts its action by disrupting folate-dependent metabolic processes essential for cell replication. Pemetrexed disodium heptahydrate has the chemical name L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo [2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate. Pemetrexed is an antifolate containing the pyrrolopyrimidine-based nucleus that exerts its antineoplastic activity by disrupting folate-dependent metabolic processes essential for cell replication. In vitro studies have shown that pemetrexed also inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), which are all folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides. Pemetrexed is transported into cells by both the reduced ►folate carrier and the membrane folate binding protein transport systems. Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme folylpolyglutamate synthetase. The polyglutamate forms are retained intracellular and are inhibitors of TS and GARFT. Polyglutamation is a time- and concentration-dependent process that occurs in tumor cells and, to a lesser extent, in normal tissues. Polyglutamated metabolites have an increased intracellular half-life resulting in prolonged drug action in malignant cells. Preclinical studies have shown that pemetrexed inhibits the in vitro growth of mesothelioma cell lines, an effect which was synergistically increased in combination with cisplatin.

The recommended dose of pemetrexed is 500 mg/m^2 administered as an intravenous infusion over 10 min on Day 1 of each 21-day cycle. Pemetrexed is not metabolized to an appreciable extent and is primarily eliminated in the urine, with 70-90% of the dose recovered unchanged within the first 24 h following administration. Pemetrexed has a steady-state volume of distribution of 16.1 L. Pemetrexed is highly bound (approximately 81%) to plasma proteins. Binding is not affected by the degree of renal impairment. Plasma

clearance of pemetrexed in the presence of cisplatin decreases as renal function decreases, with increase in systemic exposure. Patients with creatinine clearances of 45, 50, and 80 mL/min had 65%, 54%, and 13% increases, respectively in pemetrexed total systemic exposure (AUC) compared to patients with creatinine clearance of 100 mL/min. Pemetrexed in combination with cisplatin is used for the treatment of patients with malignant pleural mesothelioma. As a single agent it is used for treating patients with locally advanced or metastatic non-small cell lung cancer after prior ►chemotherapy. The main dose-limiting side effect is myelosuppression. Skin rashes and neurotoxic reactions have been recorded, too.

Mercaptopurine (6-MP, Fig. 2) is an analog of hypoxanthine and was one of the first agents shown to be active against acute leukemias. It is part of maintenance therapy for ALL. Mercaptopurine has to be activated by hypoxantine guanine-phosphoribosyltransferase (HGPRTase) to 6-MP monophosphate. This metabolite inhibits the synthesis of adenine and guanine at the initial aminotransferase step and suppresses the conversion of inosinic acid to adenylate and guanylate. Some part of the drug is also incorporated into DNA in the form of thioguanine. Resistance to 6-MP may be associated with decreased drug activation by HGPRTase or increased inactivation by the alkaline phosphatase. The drug is slightly bound (20%) to plasma proteins and does not cross the blood-brain barrier. Xanthine oxidase is the primary enzyme responsible for its metabolic inactivation.

The plasma half-life of 6-MP after intravenous bolus injection is 21 min in children and is twofold greater in adults. After oral intake peak levels are attained within 2 h. 6-MP is used for the treatment of ALL and has shown certain activity in chronic myelogenous leukemia. The major side effects involve myelosuppression, nausea, vomiting, and hepatic injury.

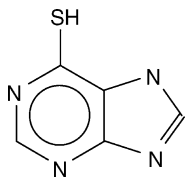
Thioguanine (6-thioguanine, 6-TG; Fig. 3) is an analog of guanine in which a hydroxyl group has been substituted by a sulfhydryl group in position 6 of the purine ring. The mode of action involves two mechanisms: incorporation into DNA or RNA; and feedback inhibition of purine synthesis. Similarly to 6-MP, 6-TG undergoes initial activation by the enzyme HGPRTase. The corresponding monophosphate can be eventually converted to deoxy-6-thioguanosine-triphosphate (dTGTP) and subsequently incorporated into DNA. Resistance to thioguanine has been correlated with decreased activity of HGPRTase and to inactivation by alkaline phosphatase. After oral intake the drug is slowly absorbed and peak levels of metabolites occur only after 6-8 h. Within the first 24 h a part (up to 46%) of the dose administered is excreted via the urine.

Thioguanine is used primarily as part of induction chemotherapy regimens for acute myelogenous leukemia (AML).

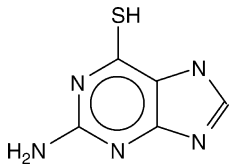
The most common adverse effects are myelosuppression, with leukopenia and thrombocytopenia appearing 7–10 days after treatment, as well as mild nausea. Liver toxicity with jaundice has been reported in rare cases.

Pentostatin (deoxycoformycin; Fig. 4) is a purine isolated from cultures of *Streptomyces antibioticus*. Its mode of action involves inhibition of adenosine deaminase, which plays a key role in purine salvage pathways and DNA synthesis. As a consequence, deoxyadenosine triphosphate (dATP) is accumulated, which is highly toxic to lymphocytes. This is associated with augmented susceptibility to apoptosis, particularly in T cells.

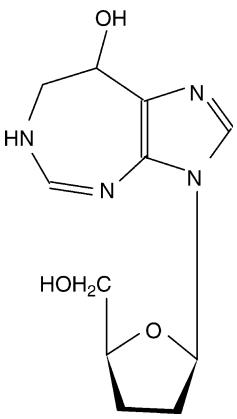
Pentostatin is effective in the treatment of hairy cell leukemia, producing 80-90% remissions (with a complete remission rate of more than 50%). The common side effects of pentostatin include myelosuppression, nausea, and skin rashes. Renal failure,



Antimetabolites. Figure 2 Chemical structure of mercaptopurine.



Antimetabolites. Figure 3 Chemical structure of thioguanine.



Antimetabolites. Figure 4 Chemical structure of pentostatin (nipent).

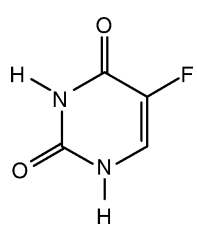
immunosuppression and CNS dysfunction have also been observed.

Cladribine (2-Chlordeoxyadenosine) is a synthetic purine nucleoside that is converted to an active cytotoxic metabolite by the deoxycytidine kinase. The drug is relatively selective for both normal and malignant lymphoid cells.

Cladribine is highly active against hairy cell leukemia (complete remssions were achieved in more than 60% of patients receiving a single 7-day course). Activity has been recorded in other low-grade lymphoid malignancies. The major side effect is myelosuppression.

Fludarabine is a fluorinated purine analog of the antiviral drug vidarabine. The active metabolite, 2-fluoro-adenosine-arabinoside triphosphate (F-ara-A), inhibits various enzymes involved in DNA synthesis such as DNA-polymerase-α, ribonucleotide reductase, and DNA primase. F-ara-A is incorporated into DNA and can cause delayed cytotoxicity even in cells with low growth fraction, e.g., CLL and follicular B cell lymphoma. Unlike typical antimetabolites, it is toxic to nonproliferating cells of lymphoid origin. The drug is highly active against chronic lymphocytic leukemia (CLL) with approximately 40% of patients achieving remission after previous therapy with alkylating agents. Therapeutic responses can also be seen in low-grade lymphomas. Dose-limiting side effect is myelosuppression that contributes to fevers and infections in half of treated patients. Occasional neurotoxicity (agitation, confusion, visual disturbances) has been noted at higher doses. Recently, an oral drug formulation has been developed.

Fluorouracil (5-fluorouracil, 5-FU, Fig. 5) represents an early example of "rational" drug design in that it originated from the observation that tumor cells, especially from gut, incorporate radiolabeled uracil more efficiently into DNA than normal cells. 5-FU is a fluorinated pyrimidine analog that must be activated metabolically. In the cells 5-FU is converted to 5-fluoro-2'deoxyuridine-monophosphate (FdUMP). This metabolite inhibits thymidilate synthase which catalyses the conversion of uridylate (dUMP) to thymidilate (dTMP) whereby methylenetetrahydrofolate plays the role of the carbon-donating cofactor. The reduced folate cofactor occupies an allosteric site of thymidylate synthase, which allows for the covalent binding of 5-FdUMP to the active site of the enzyme. In addition, misincorporation can induce single strand breaks, and RNA can aberrantly incorporate FdUMP. Leucovorin augments the activity of 5-FU by promoting formation of the ternary covalent complex consisting of 5-FU, the reduced folate, and thymidylate synthase. The drug is selectively toxic to proliferating rather than nonproliferating cells and is active in both the G1- and S-phases of the cell cycle. 5-FU is metabolized by dihydropyrimidine dehydrogenase and therefore deficiency of this enzyme can lead to excessive toxicity from 5-FU.



Antimetabolites. Figure 5 Chemical structure of 5-fluorouracil.

Capecitabine is a special prodrug of 5-FU. It is readily absorbed from the gastrointestinal tract. In the liver, a carboxylesterase hydrolyzes much of the compound to 5′-deoxy-5-fluorocytidine (5′-DFCR). Cytidine deaminase, an enzyme found in most tissues, including tumors, subsequently converts 5′-DFCR to 5′-deoxy-5-fluorouridine (5′-DFUR). Finally, the enzyme thymidine phosphorylase (dThdPase or TP), hydrolyzes 5′-DFUR to the active drug 5-FU. Both normal and tumor cells metabolize 5-FU to 5-fluoro-2′-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor N5-10-methylene-tetrahydrofolate bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2′-deoxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division. Second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis. The addition of leucovorin to capecitabine is not recommended since there was no apparent advantage in response rate; however, toxicity was increased.

Capecitabine is used for the treatment of colorectal and breast cancers. It is contraindicated in patients with known hypersensitivity to capecitabine or any of its components or to 5-fluorouracil and in patients with known dihydropyrimidine dehydrogenase (DPD) deficiency. The use of capecitabine is restricted in patients with severe renal impairment. The drug can induce diarrhea, sometimes severe. Other side effects include anemia, hand-foot syndrome, hyperbilirubinemia, nausea, stomatitis, pyrexia, edema, constipation, dyspnea, neutropenia, back pain, and headache. Cardiotoxicity has been observed with capecitabine. A clinically important drug interaction between capecitabine and warfarin has been demonstrated. Care should be exercised when the drug is co-administered with CYP2X9 substrates.

Cytarabine (cytosine arabinoside, ara C, Fig. 6) is an analog of the pyrimidine nucleosides. It is one of the most potent agents available for treating acute myeloid leukemia. The drug must first be activated by pyrimidine nucleoside kinase to the triphosphate nucleotide ara-cytosine triphosphate (ara-CTP). The susceptibility of tumor cells to cytarabine is thought to be a reflection of their ability to activate the drug more rapidly by kinases than to inactivate it by deaminases. Cytarabine is incorporated into DNA and kills cells during the S-phase of the cycle by competitively inhibiting the DNA polymerase.

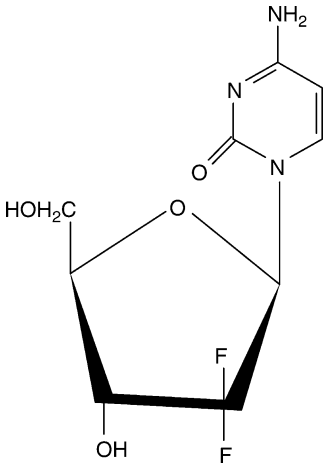
The drug is metabolized rapidly in the liver, kidney, intestinal mucosa, and even red blood cells. Therefore it has a plasma half-life of only 10 min after bolus intravenous application. The major metabolite, uracil arabinoside (ara-U), can be detected in the blood shortly after cytarabine administration. About 80% of the dose is excreted in the urine within 24 h, with less than 10% appearing as cytarabine; the remainder is ara-U. After continuous infusion, cytarabine levels in the liquor (cerebro-spinal fluid) approach 40% of that in plasma. Continuous infusion schedules allow maximal efficiency, with uptake peaks of 5-7 μM. It can be administered intrathecally as an alternative to methotrexate.

Cytarabine is used in the chemotherapy of acute myelogenous leukemia, usually in combination with anthracyclines, thioguanine, or both. It is less useful in acute lymphoblastic leukemia and lymphomas and has marginal activity against other tumors. Myelosuppression is a major toxicity, as is severe bone marrow hypoplasia; nausea and mucositis may also occur.

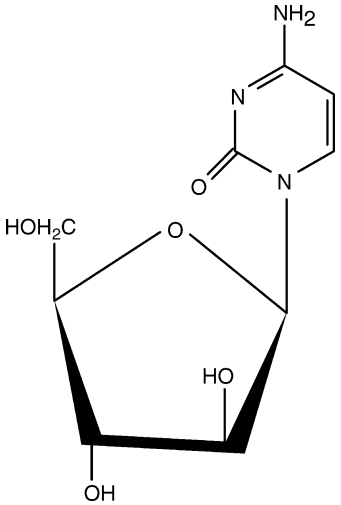
Gemcitabine (Fig. 7) is a cytosine derivative that is very similar to ara C. It undergoes metabolic activation to difluorodeoxycytidine triphosphate, which interferes with DNA synthesis and repair. Gemcitabine causes the so called masked termination of DNA elongation. After incorporation into the DNA strand a normal nucleotide is further added. Therefore, DNA repair enzymes can not recognize and reconstruct the damaged DNA. In contrast to ara C, gemcitabine appears to have useful activity in a variety of solid tumors, with limited nonmyelosuppressive toxicities. Gemcitabine is a well known inducer of apoptosis at micro- to nanomolar concentrations. It is administered by intravenous infusion and has pharmacokinetics similar to ara C.

The usual dose consists of 1000 mg/m^2 i.v. It is the most active single agent for treating pancreatic cancer, and it is used as a first-line treatment for both pancreatic and small cell lung cancers. The dose-limiting toxicity is bone marrow suppression.

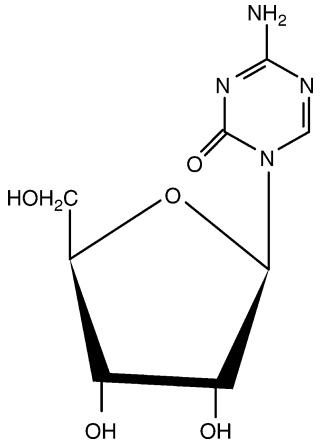
Azacitidine (5-aza-cytidine, Fig. 8) is a pyrimidine nucleoside analog of cytidine. The structural formula is



Antimetabolites. Figure 7 Chemical structure of gemcitabine (gemzar).



Antimetabolites. Figure 6 Chemical structure of cytarabine (ara C).



Antimetabolites. Figure 8 Chemical structure of Azacitidine (4-amino-1-β-D-ribofuranosyl-s-triazin-2 (1*H*)-one).

shown in Fig. 8. Azacitidine is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms. Nonproliferating cells are relatively insensitive to azacitidine.

The pharmacokinetics of azacitidine shows that it is rapidly absorbed after s.c. administration; with the peak plasma concentration occurring after 0.5 h. The bioavailability of s.c. azacitidine relative to i.v. azacitidine is approximately 89%. Urinary excretion is the primary route of elimination of azacitidine and its metabolites. The mean elimination half-lives are about 4 h, regardless of i.v. or s.c. administration.

In vitro studies in human liver fractions indicated that azacitidine may be metabolized by the liver. Azacitidine and its metabolites are known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function.

Azacitidine is used for treating patients with some myelodysplastic syndrome subtypes and chronic myelomonocytic leukemia. The most commonly occurring adverse reactions include nausea, anemia, thrombocytopenia, vomiting, pyrexia, leucopenia, diarrhea, fatigue, neutropenia, and ecchymosis.

Azacitidine is contraindicated in patients with a known hypersensitivity to azacitidine or mannitol as well as in patients with advanced ►malignant hepatic tumors.

Decitabine (5-aza-deoxycytosine) is an analog of the nucleoside 2′-deoxycytidine. It is believed to exert its antineoplastic effects after phosphorylation and direct incorporation into DNA and by inhibition of the enzyme DNA methyltransferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. DNA hypomethylation is achieved at concentrations below those required to significantly inhibit DNA synthesis, which may promote restoration of function to genes associated with control of cellular differentiation and proliferation. Cytotoxicity in rapidly dividing cells may also result from covalent adducts between DNA methyltransferase and decitabine.

Decitabine is specifically indicated for the treatment of multiple types of myelodysplastic syndromes and chronic myelomonocytic leukemia. As anticipated, use of decitabine is associated with bone marrow suppression including neutropenia and thrombocytopenia which are the most frequently observed serious adverse effects.

►Antineoplastic Agents
►Cancer, Molecular Mechanisms of Therapy

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Antimicrobial Agents

Antimicrobial drugs are used for the treatment of diseases caused by microorganisms (bacterial or viral infections).

►β-Lactam Antibiotics
►Ribosomal Protein Synthesis Inhibitors
►Quinolons
►Antiviral Drugs
►Microbial Resistance to Drugs

Antimode

The antimode is the cut-off value separating different functionally defined groups in a bi-modal or multi-modal frequency distribution.

►Pharmacogenetics

Antimycotic Drugs

►Antifungal Drugs

Antineoplastic Agents

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Synonyms

Anticancer drugs; Cytostatic drugs; Cytotoxic drugs; Antitumor drugs

Definition

Cancer or neoplastic disease is a genomic disorder of the body's own cells which start to proliferate and metastasize in an uncontrolled fashion that is ultimately detrimental to the individual. Antineoplastic agents are used in conjunction with surgery and radiotherapy to restrain that growth with curative or palliative intention. The domain of antineoplastic chemotherapy is cancer that is disseminated and therefore not amenable to local treatment modalities such as surgery and radiotherapy.

Development and Characteristics of Cancer Cells

The genesis of cancer cells can be modeled by the formula

$$\text{Cancer} = \text{f}\{\text{Exposure}, \text{Genetic disposition}, \text{Age}\}$$

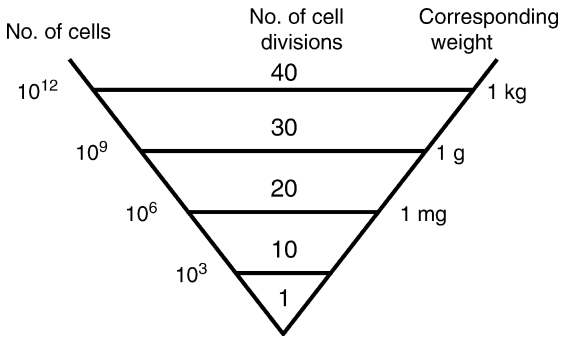
in which "*Exposure*" denotes the impact of exogenous factors that can be of chemical (chemical carcinogens), physical (UV or γ-irradiation) or biological (viruses, bacteria) origin. "*Genetic disposition*" indicates the germline transmission of genes associated with cancer development and "*Age*" points to the fact that certain cellular injuries which cause mutations and lead to cancer development are not reversible but accumulate with time. Thus, a cancer cell is characterized by genetic abnormalities such as chromosomal alterations, changes in its DNA- methylation status as well as activation of cellular protooncogenes (mainly related to cellular growth) to oncogenes and inactivation of tumor suppressor genes. As result of these changes they show autonomous proliferation, dedifferentiation, loss of function, invasiveness, and metastasis formation. Furthermore, drug resistance (primary or acquired in response to treatment with antineoplastic drugs) is a common phenomenon.

Growth of Cancer Cells

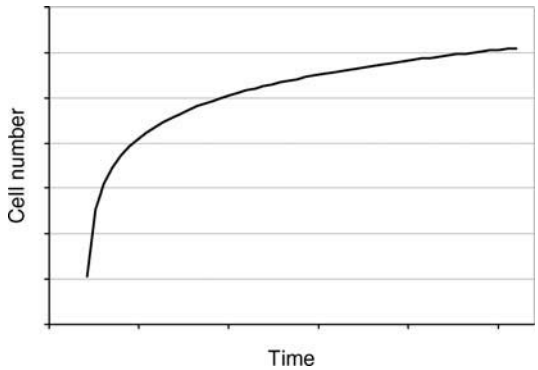
Proliferation of cancer cells is not restricted by ►contact inhibition as for normal cells but rather by the supply of growth factors and nutrients. Once a cell has become malignant and its descendants got the necessary blood supply, the initial growth rate is exponential and follows approximately the pattern shown in Fig. 1. The subsequent loss of logarithmic growth is related to insufficient supply of nutrients which drives cancer cells to either die or exit the cell cycle. As result, larger tumors contain only a certain ratio of dividing cells which is termed growth fraction. The resultant steady state growth is depicted by the so called Gompertz function (Fig. 2).

Mechanism of Action

Anticancer drugs can be grouped into several classes, according to their mechanism of action and origin [1]. These are (i) alkylating agents and related compounds which act by forming covalent bonds with cellular



Antineoplastic Agents. Figure 1 Relationship of cell number, number of cell divisions, and corresponding weight.



Antineoplastic Agents. Figure 2 Growth curve of tumor cells according to Gompertz.

macromolecules such as DNA, (ii) antimetabolites which block metabolic pathways that are vital for cell survival or proliferation, (iii) cytotoxic antibiotics of microbial origin and (iv) plant derivatives which mainly interfere with mammalian cell division, (v) hormonal agents which suppress hormone secretion, block hormone synthesis or antagonize hormone action, (vi) biological response modifiers which enhance the host's response to cancer cells, (vii) antibodies which recognize antigens specific for cancer cells, (viii) tyrosine kinase inhibitors, (xi) antisense oligonucleotides, and (x) miscellaneous agents which do not fit into the classes described above [2].

General effects include cytostatic or cytotoxic effects, the latter being related to killing a constant fraction of cells. The mode of action of antineoplastic agents is not causal for it does not reverse the basal changes that have led to the development of cancer cells but symptomatic since it aims at their destruction, or more recently, on blocking/ablating the result of genetic alterations by inhibiting proteins whose malfunction contributes to the formation and maintenance of cancer cells. However, cytotoxicity is generally not restricted towards cancer cells but for the

majority of currently used, classical antineoplastic drugs (groups 1–4) affects all (quickly) dividing cells, especially those from bone marrow, gastrointestinal tract, hair follicles, gonads, and growing tissues in children (lack of selectivity).

The limited efficacy of classical anticancer drugs can be explained in part by the compartment model of dividing (growth fraction, compartment A) and nondividing (compartment B) cells. The majority of antineoplastic drugs acts upon cycling cells and will hit, therefore, compartment A only.

Alkylating Agents and Related Compounds

Alkylating agents are activated spontaneously or enzymatically to give rise to an electrophilic species that can form covalent bonds with nucleophilic cellular constituents. Reaction of monofunctional agents with DNA bases will lead to single strand breaks, that of bifunctional groups to ►crosslinks between bases of the same strand of DNA (intrastrand crosslink) or two complementary strands (interstrand crosslink). Replicating cells are more susceptible to these drugs since parts of the DNA are unpaired and not protected by proteins. Therefore, although alkylating agents are not cell cycle specific, cells are most susceptible to alkylation in late G1- and S-phases of the cell cycle resulting in block at G2 and subsequent apoptotic cell death.

Cells which survive these damages may have undergone mutations leading themselves to cancer development. This is reason for the carcinogenicitiy of alkylating agents.

The most important subgroup is that of nitrogen mustard derivatives. Nitrogen mustard was developed in relation to sulfur mustard, the "mustard gas" used during World War I which was found to suppress ►leukopoiesis. Nitrogen mustard was the first drug to induce a remission in a lymphoma patient at the end of World War II, but has been abandoned since then. The highly reactive R-N-bis-(2-choroethyl) group, however, is part of many drugs in current use, such as cyclophosphamide, melphalan, and chlorambucil. The activity of cyclophosphamide is dependent on P450 mixed function oxidases mediated activation into active and/or toxic metabolites. One of the toxic metabolites is acrolein which causes hemorrhagic cystitis if not prevented by the antidote mesna. Mesna is a sulfhydryl donor and interacts specifically with acrolein, forming a nontoxic compound.

Other subgroups of alkylating agents are the nitrosoureas (examples: carmustine, BCNU; lomustine, CCNU) and the triazenes (example: dacarbazine, DTIC). Platinum derivatives (cisplatin, carboplatin, oxaliplatin) have an action that is analogous to that of alkylating agents (formation of crosslinks) and therefore are appended to this class, as well.

Alkylating agents are used for treating solid tumors as well as leukemias and lymphomas. Their broad spectrum of activity is reason for their inclusion into many past and current chemotherapy schedules. Platinum derivatives are especially useful in treating testicular and ovarian cancers. All alkylating agents depress bone marrow function and cause gastrointestinal side effects. With prolonged use depression of gametogenesis will result leading to sterility and an increased risk of leukemias as well as other malignancies.

Antimetabolites

Antimetabolites interfere with normal metabolic pathways. They can be grouped into folate antagonists and analogues of purine or pyrimidine bases. Their action is limited to the S-phase of the cell cycle and therefore they target a smaller fraction of cells as compared with alkylating agents.

The main folate antagonist is methotrexate. In structure, folates are based on three elements: a heterobicyclic pteridine, *p*-aminobenzoic acid and glutamic acid. The latter moiety is polyglutamated within cells which causes a prolonged intracellular half life and, as compared with monoglutamate, an increased affinity to dihydrofolate reductase, the target enzyme of antifolates. This enzyme catalyses the reduction of dihydrofolate to tetrahydrofolate and its inhibition interferes with the transfer of monocarbon units that are needed for purine- and thymidylate synthesis and thus blocks the synthesis of DNA, RNA, and Protein. Methotrexate has a higher affinity for dihydrofolate reductase than the normal substrate (dihydrofolate) and inhibits thymidylate synthesis at a tenfold lower concentration (1 nM) than purine synthesis. Methotrexate is toxic to normal tissues (especially the bone marrow) and causes hepatotoxicity following chronic therapy. Acute toxicity following iatrogenic error or high dose therapy can be rescued by using folinic acid, a form of tetrahydrofolate, as antidote.

Purine- and pyrimidine analogs are characterized by modifications of the normal base or sugar moieties. The uridine analog 5-fluorouracil is converted intracellularly into fluorouridine-monophosphate (FUMP) and fluorodeoxyuridine-monophosphate (FdUMP). Further phosphorylation leads to the respective triphosphates FUTP and FdUTP. FdUMP inhibits the enzyme thymidylate synthase and thus blocks the generation of thymidine whereas FUTP and FdUTP are incorporated into RNA and DNA, respectively. Gemcitabine is a cytosine analog in which the pentose moiety contains two fluorine atoms at position 2 of the sugar ring. This drug is converted into the respective diphosphate, which inhibits ribonucleotide reductase, and the triphosphate, which after incorporation into DNA causes masked termination of DNA chain elongation since the altered base sequence cannot be

efficiently repaired. Cytosine arabinoside is a cytosine analog with a "wrong" pentose which after phosphorylation to the respective triphosphate inhibits the DNA polymerase. The purine analogs mercaptopurine and fludarabine are converted into fraudulent nucleotides and inhibit DNA polymerase. The main unwanted effects are gastrointestinal epithelial cell damage and myelotoxicity.

Cytotoxic Antibiotics

Cytotoxic antibiotics affect normal nucleic acid function by intercalating between DNA bases which blocks reading of the DNA template and also stimulate ►topoisomerase II dependent DNA-double strand breaks. In addition, metabolism of the drugs gives rise to free radicals which cause DNA damage and cytotoxicity, and to membrane effects that occur directly or via oxidative damage. Cytotoxic antibiotics are poorly absorbed from the gut and therefore are given intravenously. They have long half-lives (1–2 days), and are eliminated by metabolism.

Anthracyclins (e.g., doxorubicin, epirubicin, and idarubicin) are the most important subgroup. They consist of a four ringed planar quinone structure attached to an amino sugar group. In addition to the general unwanted effects, anthracyclins can cause cumulative, dose-related cardiotoxicity leading to heart failure and hair loss. Epirubicin is less cardiotoxic than doxorubicin.

Mitoxantrone has a three ringed planar quinone structure with amino containing side chains and exerts also dose-related cardiotoxicity and bone marrow depression. Mitomycin C is a nonplanar tricyclic quinone which is activated to give an alkylating metabolite. Bleomycins are metal chelating glycopeptides that degrade DNA, causing chain fragmentation and release of free bases. This subgroup causes little myelosuppression but pulmonary fibrosis, mucocutaneous reactions, and hyperpyrexia. Actinomycin D (dactinomycin) is a chromopeptide which intercalates in the minor groove of DNA between adjacent guanosine–cytosine pairs and interferes with RNA polymerase, thus preventing transcription. Unwanted effects include nausea, vomiting, and myelosuppression.

In general, the mechanisms of action are not cell cycle specific, although some members of the class show greatest activity at certain phases of the cell cycle, such as S-phase (anthracyclins, mitoxantrone), G1- and early S-phases (mitomycin C) and G2- and M-phases (bleomycins).

Plant Derivatives

Plant derivatives comprise several subgroups with diverse mechanisms of action.

Some are mitosis inhibitors which affect microtubule function and hence the formation of the mitotic spindle, others are topoisomerase I and II inhibitors.

Vinca alkaloids (vincristine, vinblastine, vindesine) are derived from the periwinkle plant (*Vinca rosea*), they bind to tubulin and inhibit its polymerization into microtubules and spindle formation, thus producing metaphase arrest. They are cell cycle specific and interfere also with other cellular activities that involve microtubules, such as leukocyte phagocytosis, chemotaxis, and axonal transport in neurons. Vincristine is mainly neurotoxic and mildly hematotoxic, vinblastine is myelosuppressive with very low neurotoxicity whereas vindesine has both, moderate myelotoxicity and neurotoxicity.

Taxanes (paclitaxel, docetaxel) are derivatives of yew tree bark (*Taxus brevifolia*). They stabilize microtubules in the polymerized state leading to nonfunctional microtubular bundles in the cell. Inhibition occurs during G2- and M-phases. Taxanes are also radiosensitizers. Unwanted effects include bone marrow suppression and cumulative neurotoxicity.

Epipodophyllotoxins (etoposide, teniposide) are derived from mandrake root (*Podophyllum peltatum*). They inhibit topoisomerase II thus causing double strand breaks. Cells in S- and G2-phases are most sensitive. Unwanted effects include nausea and vomiting, myelosuppression, and hair loss.

Camptothecins (irinotecan, topotecan) are derived from the bark of the Chinese tree Xi Shu (Camptotheca accuminata). They inhibit topoisomerase I thus effecting double strand breaks. Unwanted effects include diarrhea and reversible bone marrow depression.

Hormonal Agents

Tumors derived from hormone sensitive tissues may remain hormone dependent and are then amenable to therapeutic approaches with hormonal agents. These include hormones with opposing (apoptotic) action, hormone antagonists, and agents that inhibit hormone synthesis.

Glucocorticoids have inhibitory (apoptotic) effects on lymphocyte proliferation and are used to treat leukemias and lymphomas. Estrogens (fosfestrol) are used to block the effect of androgens in prostate cancer. Progestogens (megestrol, medroxyprogesteroneacetate) have been useful for treating endometrial carcinoma, renal tumors, and breast cancer.

Gonadotropin releasing hormone analogs (goserelin, buserelin, leuprorelin, triptorelin) inhibit gonadotropin release and thus lower testosterone or estrogen levels. They are used to treat breast cancer and prostate cancer.

Hormone antagonists (tamoxifen and toremifen bind to the estradiol receptor, flutamide binds to the androgen receptor) are used for treating breast and prostate cancer.

Aromatase inhibitors (aminogluthetimide, formestane, trilostane) block the formation of estrogens from precursor steroids and thus lower estrogen levels. They have been used for treating breast cancer.

Side effects are less prominent in type and extent as compared with cytostatics and include typical hormonal or lack of hormone like effects.

Biological Response Modifiers

Agents which enhance the host's response against neoplasias or force them to differentiate are termed biological response modifiers. Examples include interleukin 2 which is used to treat renal cell carcinoma, interferon α which is active against hematologic neoplasias, and tretinoin (all-trans retinoic acid) which is a powerful inducer of differentiation in certain leukemia cells by acting on retinoid receptors. Side effects include influenza like symptoms, changes in blood pressure and edema.

Antibodies

Recombinant ►humanized monoclonal antibodies have been used recently to target antigens that are preferentially located on cancer cells. Examples include trastuzumab and rituximab which are used to treat HER2 positive breast cancer and B-cell type lymphomas, respectively. Unwanted side effects include anaphylactic reactions.

A more recently developed IgG1 monoclonal antibody is cetuximab which targets the epidermal growth factor receptor (EGFR). This binding inhibits the activation of the receptor and the subsequent signal-transduction pathway, which results in reducing both the invasion of normal tissues by tumor cells and the spread of tumors to new sites. Cetuximab is used to treat colorectal cancer and locally advanced squamous cell carcinoma of the head and neck. The most common side effects include an acne-like skin rash that seems to be correlated with a good response to therapy and hypersensitivity reactions.

Another monoclonal antibody is bevacizumab which binds to and inhibits the activity of vascular endothelial growth factor (VEGF) thus inhibiting the interaction of VEGF with its receptor on endothelial cells. This, in turn, inhibits the proliferation of endothelial cells and the formation of new blood vessels. In essence then, it kills tumors by cutting off its own blood supply. For this activity bevacizumab belongs to a family of drugs termed antiangiogenic agents, or angiogenesis inhibitors. Bevacizumab is used to treat metastatic colorectal cancer and nonsmall cell lung cancer (NSCLC). Side effects include hemorrhage, hypertension, gastrointestinal perforation/wound healing complications, and congestive heart failure.

Tyrosine Kinase Inhibitors/Receptor Associated Tyrosine Kinase Inhibitors (RTK-I)

A number of anticancer drugs have been developed that specifically target kinases known to be oncogenic. The first drug in this area is imatinib mesylate, which targets *ABL*, *KIT*, and *PDGFR*. Imatinib mesylate is the treatment of choice for patients with chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST). In CML, the Philadelphia chromosome (Ph) results from a translocation which codes for the chimaeric fusion protein, BCR–ABL, which is a constitutively activated tyrosine kinase. Imatinib inhibits the normal Abelson tyrosine kinase (ABL) as well as BCR–ABL. The protooncogene *c-KIT* encodes the KIT tyrosine kinase, which serves as a receptor for stem cell factor. KIT is important in cell cycle regulation and critically important in haematopoiesis. GIST tumors contain a mutated *c-kit* gene leading to increased activity of this tyrosine kinase. Imatinib mesylate blocks the activity of c-KIT and thus suppresses tumor cell proliferation. Also, two receptors for platelet-derived growth factor (PDGF) are sensitive to imatinib. PDGF is involved in cell cycle regulation, angiogenesis, and fibroblast proliferation. Side effects of imatinib mesylate are rare.

Other drugs of this class include gefitinib (iressa®) and erlotinib (tarceva®) which attach to the EGF receptor, prevent the receptor from being activated and thus stop the cells from dividing. Both agents have been used in patients with nonsmall cell ►lung cancer. Side effects include diarrhoea, acne-like rash, loss of appetite, nausea and vomiting, tiredness, and change in blood pressure. Sorafenib is a multitargeted kinase inhibitor, which blocks the activity of VEGF. Sorafenib is used to treat ►kidney cancer, side effects include hand/foot skin reaction, effects on the skin, and high blood pressure.

Antisense Oligonucleotides

Oblimersen sodium is a DNA antisense oligonucleotide designed to specifically bind to human bcl-2 mRNA, resulting in catalytic degradation of bcl-2. This results in decreased translation of the protein Bcl-2, which is a cellular antiapoptotic protein. Thus, oblimersen enhances sensitivity to chemotherapy by shifting the intracellular balance to a state in which the cells are more likely to be killed by apoptosis. Currently, it is used in combination chemotherapy for treating advanced melanoma.

Miscellaneous Agents

Antineoplastic agents that cannot be grouped under subheadings 1–9 include miltefosine which is an alkylphosphocholine that is used to treat skin metastasis of breast cancer, and crispantase which breaks down asparagine to aspartic acid and ammonia. It is active against tumor cells that lack the enzyme asparaginase, such as acute lymphoblastic leukemia cells. Side effects include irritation of the skin in the case of miltefosine and anaphylactic reactions in the case of crispantase. Another recent development is the proteasome inhibitor bortezomib which is used to treat multiple ►myeloma.

The ►boron atom in bortezomib binds the catalytic site of the ►26S proteasome with high affinity and specificity. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitinylated proteins, and also cleanses the cell of abnormal or misfolded proteins. While multiple mechanisms are likely to be involved, cancer cells may be especially susceptible to proteasome inhibition since more abnormal or misfolded proteins are likely to be present. Also, bortezomib may prevent degradation of proapoptotic factors, permitting activation of programmed cell death in neoplastic cells dependent upon suppression of proapoptotic pathways. Main side effects include nausea and vomiting, fatigue, and diarrhea.

Clinical Use

Cancer treatment is a multimodality treatment, i.e., surgery is combined with radiotherapy and antineoplastic chemotherapy. The latter treatment mode is used mainly for cancers which have disseminated. Different forms of cancer differ in their sensitivity to chemotherapy with antineoplastic agents. The most responsive include lymphomas, leukemias, choriocarcinoma and testicular carcinoma, while solid tumors such as colorectal, pancreatic and squamous cell bronchial carcinomas generally show a poor response. The clinical use of antineoplastic agents is characterized by the following principles.

1. The therapeutic ratio of antineoplastic agents, which is defined by the dose necessary to cause a significant anticancer effect divided by the dose effecting significant side effects, is generally low (near to one).
2. The intention to treat a cancer patient can vary between curative and palliative, pending on the prognosis. Antineoplastic therapy with curative intention is based on high dosages and takes into account severe side effects that have to be tolerated by patients in order to receive the optimal treatment. Palliative therapy with cytotoxic agents aims at maximum life quality for a patient who can not be cured. This includes palliation of symptoms like pain, fractures, and compression of vital tissues that are caused by cancer growth, but tries to accomplish this aim with dosages of cytostatics that bring about as few side effects as possible.
3. Generally, combination therapy with antineoplastic agents is superior to monotherapy. The reason is that several different mechanisms of action can be combined thus lowering the risk of rapid induction of resistance, and the dosages of the single agents can be reduced. This, in turn, decreases the incidence in side effects caused by the single agents and, in addition, the side effects will not sum up if the respective toxicity profiles differ from each other.
4. To be successful, antineoplastic therapy often has to be applied for considerable periods of time. The initial therapy period is being termed "induction therapy" which is then followed by a "maintenance therapy" and possibly a "reinduction therapy."
5. The therapeutic success is measured by its effect on tumor size and can be described as tumor remission (complete or partial), stable disease, or progression of the tumor. Also, the impact of a therapy is related to time and can be measured as disease free interval, time to progress, or overall survival time.
6. Patients receiving cytotoxic chemotherapy very often need concomitant administrating of antiemetic therapy. Such protocols will start well in advance of administering the cytotoxic, and last for a reasonable time with regard to pharmacokinetics of the antineoplastic agent. In addition, side effects of antineoplastic therapy are made better tolerable by supportive care.
7. Few side effects can be alleviated by the use of antidotes. An example is the prevention of hemorrhagic cystitis caused by cyclophosphamide by the concomitant infusion of mesna.

►Cancer, Molecular Mechanisms of Therapy

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Anti-obesity Drugs

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Synonyms

Weight-loss therapies

Definition

►Obesity results from an energy imbalance, when energy intake exceeds energy expenditure over a prolonged period of time. The excess energy is stored in the form of triglycerides in the adipose tissue.

Obesity is defined by the body mass index (BMI), measured as body weight in kilograms divided by the squared height in metres (kg/m^2). A BMI of 30 or more is a commonly-used criterion for defining obesity and a BMI between 25 and 30 is considered overweight.

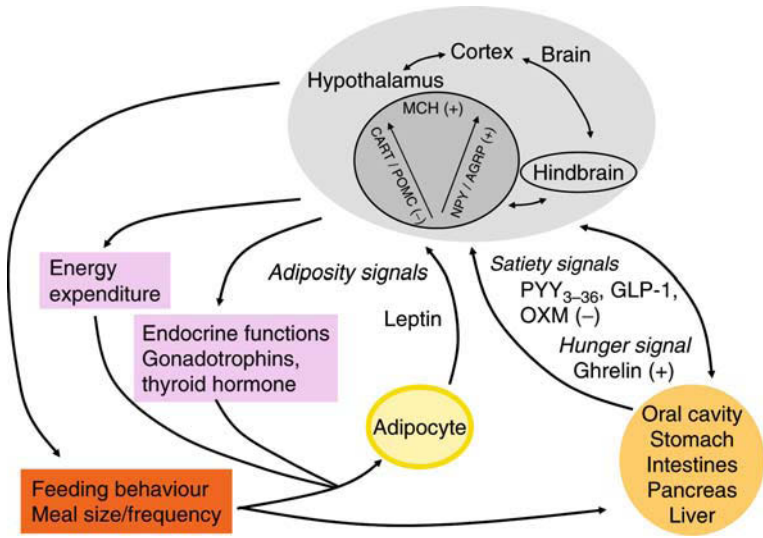
Energy homeostasis depends on energy intake and energy expenditure. The main components determining energy intake are appetite and intestinal absorption, while energy expenditure depends on thermogenesis and metabolism. In theory, anti-obesity therapies could aim to modify energy intake, energy expenditure and/or energy storage. The current anti-obesity drugs on the market and most of those used in clinical development reduce energy intake, either acting centrally (appetite suppressant) or peripherally (reduction of fat absorption). The progress in the field of modulating energy expenditure is not so advanced.

Reduction of energy intake: appetite and satiation
Reduction of energy intake: intestinal absorption
Increase of energy expenditure
Modulation of fat storage

Mechanism of Action

Regulation of Energy Balance and Food Intake

Energy homeostasis, food intake and energy stores are regulated in a complex manner by central and peripheral pathways that are built into short-term and long-term feedback loops (Fig. 1). The hormone leptin plays a central role in the regulation of energy balance. It is an adipocyte-derived factor, or ►adipokine, which is released from adipose tissue into the circulation and informs the brain about the status of energy stores. The blood levels of leptin are positively correlated with adipocyte number and size. Binding of leptin to specific leptin receptors in the ►hypothalamus initiates a cascade that ultimately regulates feeding behaviour, neuroendocrine functions (gonadotrophins, thyroid axes) and energy expenditure (via the sympathetic nervous system). Leptin has thus broad effects on energy balance, which ultimately modulate the size of the energy/fat stores. Adiponectin, another ►adipokine, and the pancreatic hormone ►insulin have been suggested as additional mediators of the energy status between periphery and the CNS.



Anti-obesity Drugs. Figure 1 Regulation of energy/fat stores and feeding behaviour by central and peripheral mechanisms. A central mediator is leptin, which informs the brain about the status of energy reserves in the adipose tissue. Leptin is released in correlation with adipocte number and size and binds to its receptors in the hypothalamus to initiate a cascade that ultimately regulates feeding behaviour, neurondocrine functions (gonadotrophins, thyroid hormone, etc) and energy expenditure. As a result, energy/fat stores are affected and controlled in the long-term. Adiponectin may have a similar role as a mediator between the periphery and CNS. Elevated leptin levels reflecting increased energy stores, downregulate the expression of appetite-stimulating (orexigenic) peptides neuropeptide Y (NPY) and agouti-related protein (AGRP) and stimulate the expression of the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). The activity of these neuropeptides influences the sensitivity to signals from the gastrointestinal (GI) tract. Signals from the GI tract are involved mainly in the short-term regulation, i.e. the meal-to-meal regulation of appetite by inducing hunger signals (e.g. ghrelin) or satiety (e.g. $PYY_{3–36}$, OXM (oxyntomodulin), GLP-1 (Glucagon-like peptide-1)) and include sensors of gastric distention. The short-term and long-term signals are finally integrated by the brain. (+) orexigenic, (−) anorexigenic effects.

Leptin exerts its effects on food intake behaviour via two distinctive neuronal populations in the hypothalamus. Elevated levels of leptin inhibit neurons expressing the appetite-stimulating (►orexigenic) peptides ►neuropeptide Y (NPY) and agouti-related protein (AGRP) and stimulate neurons expressing the appetite-suppressing (►anorexigenic) peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), which are co-localized in a different neuron population. POMC is the precursor of α-melanocyte-stimulating hormone that activates the melanocortin-4 receptor to mediate anorexigenic effects. The neuron populations project further to other brain centres, which ultimately communicate with the cerebral cortex for the final coordination of feeding. Neuropeptides further downstream from leptin include ►galanin, melanin concentrating hormone (MCH) and the ►orexins (also termed hypocretins), which all exert orexigenic effects. The level of food intake will in turn affect fat depots, thus completing the feedback loop between the periphery and CNS.

The ►endocannabinoid system (ECB) system was recently shown to regulate appetite and energy homeostasis. Endocannabinoids are endogenous lipids derived from arachidonic acid that are capable of binding to and activating the two ►G-protein coupled receptors cannabinoid receptor CB1 and CB2. The regulation of food intake and peripheral effects are thought to be mediated via CB1 expressed in the CNS and in various peripheral tissues, including adipose tissue, the gastrointestinal tract, reproductive tissue, muscle and the liver. The 'first-in class' CB1 receptor antagonist rimonabant (Table 1) has recently been approved for anti-obesity treatment.

Sibutramine (Table 1) has been in the market for several years and inhibits the reuptake of serotonin, noradrenaline and, to a lesser extent, dopamine. It acts mainly as an appetite suppressant and may also increase energy expenditure.

Feeding behaviour, defined by frequency and size of meals, is also regulated in a short-term loop. During and after a meal, various signals are generated in the periphery including taste signals from the oral cavity, gastric distension and humoral signals from secretory cells of the gastrointestinal (GI) tract. These afferent signals are transmitted to the hypothalamus and the hindbrain, which communicate with higher brain areas. Several GI hormones were shown not only to regulate appetite and satiety but also to improve the response of the pancreas to absorbed nutrients (►incretin hormones). GI hormones have gained renewed interest, with the most recent example being peptide YY (PYY). $PYY_{3–36}$ is secreted from the distal gut in response to food ingestion. It belongs to the family of peptides including NPY and pancreatic polypeptide (PP), which exert their effects via the ►G-protein-coupled Y receptor family, Y_1–Y_5. Initial studies showed that peripheral administration of $PYY_{3–36}$ reduced food intake, supposedly mediated via Y_2 receptors, however the potential as an anti-obesity drug is as yet unclear. The related peptide PP is released postprandially from the pancreas in a biphasic manner and is also being investigated for its anorexigenic effects. Glucagon-like peptide-1 (GLP-1), which results from processing of the pre-pro-glucagon precursor in the pancreas, intestinal cells and CNS, presumably regulates feeding by both peripheral and central mechanisms by delaying gastric emptying. The satiety peptide oxyntomodulin (OXM) is derived from the same precursor. Cholecystokinin (CCK) has also been proposed as satiety factor. The only gut hormone identified so far with orexigenic effects is ghrelin. This peptide was originally described as a growth hormone segretagogue. Ghrelin is mainly produced in the stomach, released into the blood, and reaches growth hormone segretagogue receptors in the hypothalamus.

Anti-obesity Drugs. Table 1 Profile of drugs approved for long-term treatment of obesity

	Orlistat	Sibutramine	Rimonabant
Site of action	Gut	CNS	CNS/periphery
Molecular target	Gastrointestinal lipases	Serotonin and noradrenaline transporter	Cannabinoid-1 receptor
Mode of action	Enzyme inhibition	Reuptake inhibition	Receptor antagonism
Effect	Reduced fat absorption	Appetite suppression	Appetite suppression, peripheral actions
Additional effects	LDL-C reduction	Increased energy expenditure?	Metabolic effects
Unwanted/side effects	GI effects	CV system	CNS?
Daily dosage	3 × 120 mg with meals	1 × 10 or 15 mg	1 × 20 mg

It was shown to decrease the production of leptin and NPY and reduced food intake when administered to humans and rodents.

Several GI peptides or derivatives are under investigation for their potential use as anti-obesity therapy.

Reduction of Energy Intake: Inhibition of Absorption

Inhibition of the absorption of fat (triglycerides) in the GI lumen represents the most efficient approach for reduction of caloric intake, as triglycerides are the most condensed energy stores. In the intestine, triglycerides are split into free fatty acids (FFAs) and monoglycerides by lipases, the targets for orlistat (Table 1). After hydrolysis, FFAs cross the membranes of the epithelial cells lining the intestinal wall. Once inside the epithelial cell, FFAs are donated to acyl-CoA synthetase in the endoplasmic reticulum by fatty acid-binding proteins (FABPs). Acyl-CoA is then transferred to 2-monoacylglycerol to resynthesize triglycerides. Acyl-CoA:diacylglycerol acyltransferase (DGAT) is a key enzyme responsible for the final step in the glycerol phosphate pathway of triglyceride synthesis. The absorption of dietary fat thus involves several steps catalysed by proteins that might represent promising drug targets.

Increase of Energy Expenditure

Total energy expenditure is the sum of basal metabolism, i.e. the constant obligatory energy expenditure required for cell and organ survival, and a variable portion needed for physical activity and adaptive thermogenesis. The stimulation of thermogenesis has raised much interest as a possible mechanism to treat obesity, especially once the mitochondrial uncoupling proteins (UCPs) were identified. UCP1 is selectively expressed in brown adipose tissue (BAT), which is rich in mitochondria and highly developed in rodents for thermogenesis. Activation of the sympathetic nervous system in response to cold stress or high-fat diet activates β3-adrenoreceptors of the BAT, resulting in increased cAMP levels and stimulation of protein kinase A. This kinase phosphorylates and activates hormone-sensitive lipase, thereby promoting the release of FFAs. These serve both as fuel for mitochondrial respiration and as activators of UCP1. UCP1 dissipates the transmembrane proton gradient coupled to the oxidation of metabolites, releasing energy as heat. In addition, there is a chronic response, i.e. UCP1 is transcriptionally upregulated, and mitochondrial biogenesis is stimulated through mechanisms involving a transcriptional coactivator of the nuclear peroxisome proliferator-activated receptor-γ (►PPARγ), with the acronym PGC-1 (PPARγ coactivator-1).

Despite tremendous efforts, research on energy expenditure did not yet lead to successful therapies. Much attention has been devoted to the stimulation of β3 receptors. The stimulation of these adrenergic receptors should lead to an increased expression of the uncoupling protein UCP1, thereby increasing thermogenesis, and inducing catecholamine-induced lipolysis. The first generation of β3 receptors agonists failed due to species differences between human and rodent receptors. A general problem of this therapeutic approach in human may be the lack of BAT, which is prominent in rodents but disappears in human after birth, while the major thermogenic tissue in man is skeletal muscle. As UCP3 is expressed in skeletal muscle in man, it has been proposed as a promising pharmacological target, but its definite role is still uncertain.

Modulation of Fat Storage

Processes involved in the storage of fat, including adipocyte differentiation, angiogenesis or apoptosis, could also be targeted as a way to reduce fat mass. However, all of these potential approaches to reduce the ability to synthesize or store fat will be safe only if associated with an increase in fat oxidation and/or with a reduction of fat absorption. Otherwise, the inability to deliver excess calories to adipose tissue could have serious secondary consequences as lipids accumulate in the blood or various organs. A safer anti-obesity approach could be the stimulation of BAT formation in man, involving either de novo recruitment from preadipocytes or interconversion of white adipose tissue, which is the major site for triglyceride storage.

Clinical Use (Including Side Effects)

Obesity has reached an epidemic level not only in developed but also in developing countries. In the US, ~65% of the adult population is overweight and 20–25% of these are obese. Obesity is a complex condition frequently associated with other diseases such as type 2 ►diabetes and hypertension, which makes it a major health issue. The treatment of obesity aims at a sustained loss of 5–10% of body weight, which has been shown to reduce the risk of obesity-associated co-morbidities. Anti-obesity therapy should result in a reduction of the fat mass while saving lean body mass, and help to maintain the reduced weight. Furthermore, an anti-obesity drug should not induce counterregulatory mechanisms which limit its efficacy during long-term treatment, and it needs to meet high safety standards.

There are three medications approved for the long-term (>6 months) treatment of obesity: (i) Orlistat, an inhibitor of fat absorption, (ii) Sibutramine, an appetite suppressant and (iii) the recently approved Rimonabant, an appetite-suppressing agent with additional peripheral action. These medications are recommended to be used in conjunction with a reduced caloric diet (or reduced fat diet for orlistat), and increased exercise. Several other drugs are approved for short-term treatment of obesity only and are either catecholaminergic or serotonergic CNS-active (activating the sympathetic nervous system) anorectic agents (e.g. phentermine).

Orlistat (Xenical®, Reductil®) – Lipase Inhibitor

Orlistat inhibits gastrointestinal lipases in the lumen of the GI tract to decrease systemic absorption of dietary fat. It is a hydrogenated derivative of lipstatin, a natural occurring lipase inhibitor of bacterial origin. The drug binds covalently to a serine residue in the active site of gastrointestinal lipases, and thus inhibits the hydrolysis of ingested triglycerides into absorbable FFAs and monoglycerides. At doses of 400–600 mg daily, ~30% of triglycerides are not absorbed by the small intestine and excreted into faeces, thereby contributing to the caloric deficit. Orlistat represents an overall safe treatment for obesity, given that the drug itself is minimally absorbed.

A clinical trial with orlistat in conjunction with a hypocaloric diet showed a weight loss of 8.7 kg in patients receiving orlistat versus 5.8 kg in patients receiving placebo after 52 weeks of treatment. The effect on body weight was sufficient to improve several metabolic parameters, including reduced LDL-C blood levels, improved oral glucose tolerance and blood pressure. The main unwanted effects of orlistat are attributable to its mode of action, as non-digested fat remains in the intestinal lumen and can cause steatorrhoea (fatty stools), flatulence and faecal incontinence. These effects are associated with a high-fat meal, and therefore a low-fat diet is recommended. As absorption of fat-soluble vitamins may be hampered, supplementation of these vitamins is recommended.

Sibutramine (Meridia®) – Serotonin and Noradrenaline Reuptake Inhibitor

Sibutramine is a β-phenylethylamine derivative that inhibits the reuptake of noradrenaline, serotonine, and, to a lesser extent, dopamine in the CNS, resulting in reduced hunger and increased satiety. It may also increase thermogenesis, causing an increase in energy expenditure. Sibutramine treatment is indicated for weight loss and maintenance medication. A meta-analysis of randomized placebo-controlled trials with doses of 10–20 mg per day over 44–54 weeks indicated an average weight loss of 4.45 kg. Several metabolic parameters were also improved. However, there are some safety concerns as increase in systolic and diastolic blood pressure and a rise in heart rate have been observed. These cardiovascular effects of sibutramine may be explained as a consequence of the drug's peripheral effects, i.e. the inhibition of noradrenaline reuptake at sympathetic nerve terminals in the arterioles. Sibutramine should therefore be used with caution in patients with poorly controlled hypertension or with a history of cardiovascular heart disease.

Rimonabant (Acomplia®) – CB1 Receptor Antagonist

Rimonabant is the 'first-in class' CB1 receptor antagonist, which has recently reached the market in Europe (approval in the US is pending). The reduction of weight observed in the clinical trials is of the same order of magnitude as that seen with the other available agents sibutramine and orlistat. In the 'Rimonabant in Obesity'-lipid trial, HDL cholesterol was increased, plasma triglycerides reduced, and glucose tolerance upon oral glucose challenge improved. Rimonabants' beneficial effects on risk factors are supposedly not only attributed to the weight loss induced by its appetite suppressant effect but also mediated through peripheral effects. Observed side effects in the trials consisted mainly of nausea, dizziness and anxiety.

Comment on the First Leptin Trials

Leptin has proved to be an efficient treatment for the rare form of obesity associated with leptin deficiency. By contrast, the results of the first clinical trial with human leptin in obese patients (without leptin deficiency) were less promising. This may be explained by leptin resistance in a high proportion of these patients. However, the mechanisms involved in the development of leptin resistance could become new drug targets.

►Appetite Control
►Adipokines
►Diabetes Mellitus

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Antioncogene

An antioncogene is a gene that suppresses cellular proliferation.

►Targeted Cancer Therapy

Antioxidants

Antioxidants are substances which reduce or prevent the oxidation of other molecules. While oxidation reactions

are important for the basic metabolism of cells, they can also be damaging under certain conditions. One of the major sources for reactive oxygen is the leakage of activated oxygen like superoxide (O_2^-) which is produced via the respiratory chain in mitochondria. Also a variety of enzymes like xanthine oxidase, P450 oxidases, $FADH_2$ oxidase, or NADPH oxidase can produce superoxide. However, under normal conditions most cells maintain a reducing environment due to the activity of various anti-oxidant mechanisms. Several enzymes such as superoxide dismutase, glutathione peroxidase, or catalase can convert superoxide to hydrogen peroxide (H_2O_2) and finally to water. There are also various anti-oxidants such as ascorbic acid (vitamin C), glutathione, or α-tocopherol (vitamin E) which are present in cells and inactivate reactive oxygen species like superoxide or hydrogen peroxide. Under various pathological conditions, however, the balance between the formation of reactive oxygen species and their inactivation by anti-oxidant enzymes and substances can be dysregulated resulting in the increased accumulation of reactive oxygen species which have damaging effects due to the oxidation of DNA, lipids or proteins. These conditions which are described as oxidative stress are often the result of tissue injuries. A pathophysiologically relevant role for reactive oxygen species has been suggested in various neurodegenerative diseases and stroke. Oxidative stress has also been linked to the development of atherosclerosis which requires in its early stages the oxidation of LDL particles for the formation of atherosclerotic plaques. Anti-oxidants have been shown to be able to treat and prevent various diseases to some degree. There are a variety of experimental drugs which can be used as anti-oxidants. Also, several endogenous anti-oxidants can be added to the nutrition like ascorbic acid, lipoic acid, carotenes, α-tocopherol and others. Also, fruits and vegetables are rich sources of natural anti-oxidants like polyphenoles (e.g. resveratrol) and anthocyanins.

►P450 Mono-oxygenase System
►Reactive Oxygene Species
►Oxidative Stress
►Vitamin C
►Vitamin E

Antiparasitic Drugs

Antiparasitic drugs are used for the treatment of parasitic infections caused by pathogenic protozoa or helminths (worms).

►Antihelmintic Drugs
►Antiprotozoal Drugs

Anti-Parkinson Drugs

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Synonyms

Antiparkinsonian drugs

Definition

Parkinsonism is a clinical syndrome comprising ►bradykinesia, muscular ►rigidity, ►resting tremor, and impairment of postural balance. The pathological hallmark of Parkinson's disease is a loss of more than 60–70% of pigmented dopaminergic neurons of the substantia nigra pars compacta with the appearance of intracellular inclusions known as ►Lewy bodies. Without treatment, idiopathic Parkinson's disease progresses over 5–10 years to a rigid, akinetic state, leading to complications of immobility, e.g., pneumonia and pulmonary embolism. The distinction between Parkinson's disease and other causes of parkinsonism is important because parkinsonism arising from other causes is usually more refractory to treatment with antiparkinsonian drugs.

Mechanism of Action

Pathophysiology

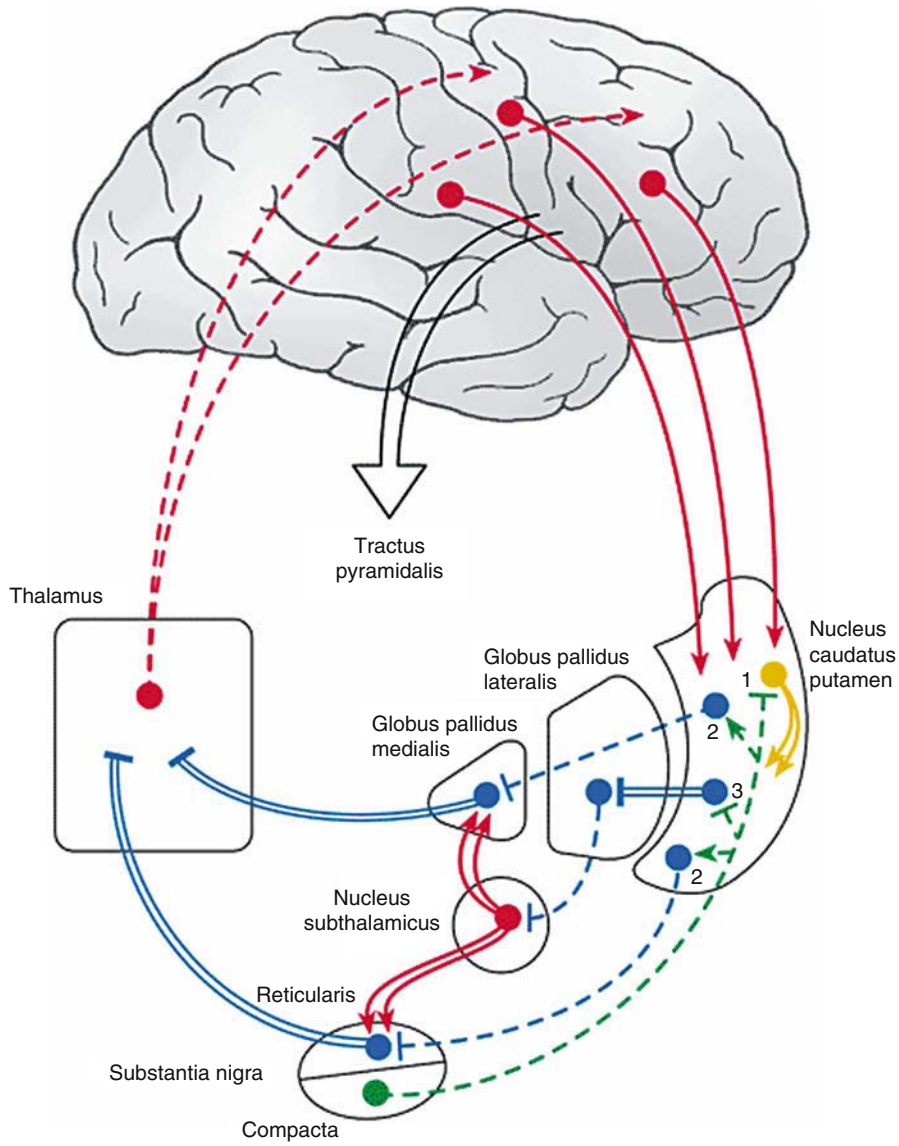
The primary deficit in Parkinson's disease is a loss of dopaminergic neurons in the substantia nigra pars compacta and a corresponding loss of dopaminergic innervation of the caudate nucleus and the putamen (forming the striatum). This suggests that replacement of dopamine could restore function. Physiologically, dopamine is synthesized from tyrosine in terminals of nigrostriatal neurons by the sequential action of the enzymes tyrosine hydroxylase, yielding the intermediary L-dihydroxyphenylalanine (L-DOPA), and aromatic L-amino acid decarboxylase (the corresponding prodrug L-DOPA is the most effective agent in the treatment of Parkinson's disease, see below.). The subsequent uptake and storage of synthesized dopamine in vesicles is blocked by reserpine, an earlier antipsychotic drug and admixture to antihypertensive medicines, which is known to induce parkinsonism. Release of dopamine is triggered by depolarization leading to entry of Ca^{2+} and ►exocytosis. The pre- and postsynaptic actions of dopamine are mediated by two types of dopamine receptors, both of which are seven-transmembrane-region receptors. The D_1-receptor family (consisting of D_1 and D_5 receptors) stimulates the synthesis of intracellular cAMP and phosphatidyl inositol hydrolysis, but

the D_2-receptor family (D_2, D_3 and D_4 receptors) inhibits cAMP synthesis and modulates K^+ and Ca^{2+} channels. D_1 and D_2 proteins are abundant in the striatum; striatal D_3 expression is rather low. Most ►antipsychotic drugs block D_2 receptors and may lead to the adverse event of parkinsonism. As the disease progresses, neuron degeneration continues, involving other systems, including mesocortical dopaminergic cells, and noradrenergic, serotonergic, cholinergic, histaminergic, and peptidergic systems.

The following model of basal ganglia function accounts for the Parkinson syndrome as a result of diminished dopaminergic neurotransmission in the striatum (Fig. 1). The basal ganglia modulate the flow of information from the neocortex to the motoneurons in the spinal cord. The striatum receives excitatory glutamatergic input from the neocortex (red solid arrows). The majority of striatal neurons are projection neurons to other basal ganglia nuclei (blue GABAergic neurons) and a small subgroup is interneurons that interconnect neurons within the striatum (yellow cholinergic neurons). Nigrostriatal dopaminergic neurons (green) innervate GABAergic neurons (blue, *2*, *3*) and cholinergic interneurons (yellow, *1*). The cholinergic interneurons mediate the dopaminergic control of corticostriatal long-term depression in GABAergic neurons. This long-term depression is due to a reduction of the muscarinic M_1 receptor tone at dendrites of GABAergic neurons, which receive glutamatergic input from neocortical, pyramidal neurons. Physiologically, the D_2 receptor-mediated reduction of the innervation of postsynaptic M_1 receptors leads to an enhanced Ca^{2+} influx into the dendrite of GABAergic neurons, resulting in enhanced endocannabinoid production, and a retrograde activation of presynaptic cannabinoid-1 receptors that diminish glutamate release. The outflow of the striatum proceeds as the direct and the indirect pathway. The direct pathway projects directly to the output stages of the basal ganglia, the substantia nigra pars reticulata and the globus pallidus medialis, which contain GABAergic neurons (blue). These in turn relay to the thalamus, which provides excitatory input to the neocortex (red broken arrows). Since two inhibitory GABAergic neurons are arranged successively, the stimulation of the direct pathway at the level of the striatum (by glutamatergic corticostriatal afferents or via *2*) results in an increased excitatory outflow from the thalamus to the neocortex. The opposite effect, i.e., a decreased excitatory outflow from the thalamus, is the result when the stimulation of the first chain link of the direct pathway, GABAergic neurons in the striatum, is abolished.

This is the case when the excitatory D_1 receptors on these striatal GABAergic projection neurons are no longer activated since the transmitter dopamine is reduced (green broken arrows at *2*). The indirect pathway is composed of striatal GABAergic neurons (blue) that project to the globus pallidus lateralis (to blue GABAergic neurons). This inhibitory structure in turn innervates glutamatergic neurons of the subthalamic nucleus (red) to diminish the excitation of subthalamic neurons. The subthalamic nucleus provides excitatory glutamatergic outflow to the output stage, i.e., to GABAergic neurons (blue) of the substantia nigra pars reticulata and the globus pallidus medialis. Thus, the net effect of stimulating the indirect pathway at the level of the striatum is to reduce the excitatory outflow from the thalamus to the neocortex. Striatal neurons forming the indirect pathway express inhibitory D_2 receptors (*3* in the Fig. 1), counteracting the excitation through glutamatergic corticostriatal afferents. Thus, dopamine released in the striatum reduces the activity of the indirect pathway through D_2 receptors, but increases the activity of the direct pathway through D_1 receptors. A reduced dopaminergic neurotransmission in the striatum (depicted as green broken line) ultimately reduces the thalamic excitation of the motor cortex. Note that the view of the striatofugal system as dual (direct/indirect) projections system has been questioned; it was suggested that GABAergic projection neurons project sequentially rather than in parallel to their major target areas, compatible with the repeatedly reported colocalisation of D_1 and D_2 receptors in these neurons.

What is the reason for the rather selective degeneration of nigrostriatal dopaminergic neurons in Parkinson's disease? Apart from their oxidative metabolism, leading to the production of reactive compounds as in every cell (hydrogen peroxide, superoxide anion radical), dopaminergic neurons seem to be additionally compromised by an extra accumulation of hydrogen peroxide due to the metabolic conversion of dopamine to 3,4-dihydroxyphenylacetaldehyde (DOPAL) plus hydrogen peroxide by the enzyme monoamine oxidase (MAO). In the presence of ferrous iron, hydrogen peroxide undergoes spontaneous conversion (Fenton reaction), forming a hydroxyl free radical, one of the most risky species of all reactive compounds. Levels of iron are high in the substantia nigra; it is, however, not clear whether the excess iron exists in a form, capable of participation in redox chemistry. In addition, the increase in iron occurs only in the advanced stages of Parkinson's disease, suggesting that this increase may be a secondary, rather than a primary initiating event. Despite this objection, hydroxyl-free radicals are generated from hydrogen peroxide without the catalytic help of ferrous iron in the presence of DOPAL: Thus, the either MAO product, DOPAL, is a cofactor in the generation of the hydroxyl radical from the other MAO product, hydrogen peroxide, which is also produced enzymatically by superoxide dismutase from the superoxide anion radical. Since MAO is located on the outer mitochondrial membrane, adjacent to the free radical



Anti-Parkinson Drugs. Figure 1 Extrapyramidal wiring diagram of the basal ganglia in Parkinson's disease. Arrow heads: activation; arrow beams: inhibition; solid lines: normal neurotransmission; double lines: increased neurotransmission; broken lines, diminished neurotransmission; red: glutamate excitatory; blue: GABA inhibitory; green: dopamine excitatory (D_1 receptors, *2*) and inhibitory (D_2 receptors, *1*, *3*); yellow: acetylcholine. (from Feuerstein TJ. Antiparkinsonmittel, Pharmakotherapie des Morbus Parkinson. In: 5).

sensitive permeability transition pore, its products, including the hydroxyl free radical, may function as cell death messengers, leading to ►apoptosis. Apart from this local mechanism, reactive oxygen species can lead to DNA damage, peroxydation of membrane lipids, and neuronal death. Because parkinsonian brains are free of pathological signs of necrosis, apoptosis is the likely or predominant mechanism for the death of nigrostriatal dopamine neurons.

Emerging evidence suggests that dysfunction of the ubiquitin-proteasome system may be part of the pathophysiology of sporadic Parkinson's disease, especially the association of parkin mutations with familial forms of the disease. Disease-linked mutations in parkin may cause defects in normal ubiquitin-proteasome system function with subsequent aberrant protein accumulation, resulting in proteolytic stress [1].

Neuromelanin, a dark colored pigment and product of the oxidative metabolism of dopamine, is found in the cytoplasm of dopaminergic neurons of the human substantia nigra pars compacta. Neuromelanin deposits increase with age, matching the age distribution of Parkinson's disease. In the absence of significant quantities of iron, neuromelanin can act as an antioxidant in

that it can interact with and inactivate free radicals. Neuromelanin functions as a redox polymer and may promote the formation of reactive oxygen free radicals, especially in the presence of iron that accumulates in neuromelanin. Thus, in the early stages of the disease, the iron-chelating properties of neuromelanin may act as a powerful protective mechanism, delaying symptom appearance and/or slowing disease progression. Once these protective mechanisms have been exhausted, the pathogenic mechanisms affecting cytoplasmic organelles, other than neuromelanin, destroy neuromelanin-harboring neurons, with consequent pouring out of neuromelanin granules. These, in turn, activate microglia, causing release of nitric oxide, interleukin-6, and tumor necrosis factor-alpha, thus becoming an important determinant of disease aggravation [2].

A genetic defect of complex I of the mitochondrial respiratory chain has been demonstrated specifically for the substantia nigra in Parkinson's disease. This finding matches the observation that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes a Parkinson-like syndrome in humans, acts via inhibition of complex I by its neurotoxic metabolite 1-methyl-4-phenylpyridine (MPP^+), thus destroying dopaminergic neurons in the substantia nigra. Despite this obvious specificity, the question arises whether dopaminergic neurons are more vulnerable to this mitochondrial deficit per se compared with other neurons and whether there is differential vulnerability to complex I inhibition within the dopaminergic substantia nigra population. In addition, which are the death transducers of mitochondrial dysfunction? Apart from increased accumulation of reactive oxygen species and their functional consequences (see above) due to defective mitochondria, dopaminergic neurons may express differentially regulated ►K_{ATP} channels in the plasma membrane. These channels respond to mitochondrial complex I inhibition. There may be a differential opening of K_{ATP} channels owing to a diminished ATP/ADP ratio in dopaminergic midbrain neurons. It has recently been suggested from mice models that K_{ATP} channels are causally linked to the differential degeneration of these dopaminergic neurons: K_{ATP} channel opening due to complex I inhibition was associated with hyperpolarization, functional silencing and, finally, cell death of substantia nigra dopamine neurons; cell death of dopaminergic neurons in the ventral tegmental area was absent, however, since complex I inhibition did not induce the opening of K_{ATP} channels in these neurons [3]. How should this K_{ATP} channel-induced neuronal silencing be linked with the death of dopamine neurons? This question remains open and the possibility that the opening of K_{ATP} channels is a futile effort of cellular self-protection due to another underlying degenerative mechanism should be considered. The fact that genetic inactivation of the K_{ATP} channels under investigation resulted in selective rescue of substantia nigra dopaminergic neurons does not prove a deleterious, neurodegeneration-promoting role of K_{ATP} channels, but may simply mean that the dopamine neurons, lacking K_{ATP} channels, have developed other, undetected self-protection mechanisms.

Symptomatic Drug Therapy and Curative Treatments of the Future

While advances in the symptomatic drug therapy (summarized below) have certainly improved the lives of many Parkinson patients, the goal of current research is to develop treatments that can prevent, retard or reverse the death of dopaminergic neurons in the substantia nigra pars compacta (and of other neurons involved in the pathogenesis of Parkinson's disease not mentioned in this essay).

A large number of molecules have provided experimental evidence of neuroprotection in in vitro and in vivo models of Parkinson's disease and many of these putative neuroprotective substances are now the objects of clinical trials. Recently, a team of experts has identified potential neuroprotective agents to be tested in pilot studies [4]. Twelve compounds have been considered for clinical trials: caffeine, coenzyme Q 10, creatine, estrogen, GPI1485, GM-1 ganglioside, minocycline, nicotine, pramipexole, ropinirol, rasagiline, and selegiline (for individual discussion see [4]).

Clinical Use (Including Side Effects)

Oral L-DOPA is rapidly absorbed by the intestinal active transport system for aromatic amino acids, where dietary amino acids may act as competitors. The same is true at the corresponding aromatic amino acid carrier of the blood–brain barrier. L-DOPA is usually co-administered with a peripherally acting inhibitor of aromatic L-amino acid decarboxylase (benserazide, carbidopa) that prevents (dopamine-induced) nausea and vomiting, cardiac arrhythmias and orthostatic hypotension, and increases the fraction of L-DOPA that remains unmetabolized and available to cross the blood–brain barrier. The therapeutic and adverse effects of L-DOPA result from its intracerebral decarboxylation to dopamine. Entacapone is a selective inhibitor of catechol-*O*-methyltransferase, whose activity is primarily in the peripheral nervous system. Entacapone further increases the fraction of L-DOPA, crossing the blood–brain barrier, and thus prolongs its action and reduces fluctuations in response. In early Parkinson's disease, when some buffering capacity of remaining striatal dopaminergic nerve terminals is still present, the degree of motor improvement due to L-DOPA is highest (9). With time, however, the patient's motor state may fluctuate dramatically with each drug dose. Increasing the frequency of administration can

improve this situation, while increasing the L-DOPA dose may induce dyskinesias, i.e., excessive and abnormal involuntary movements. In view of the above-mentioned dopamine autotoxicity, might L-DOPA accelerate the disease progression? Although no convincing evidence for such an effect has yet been obtained, a pragmatic therapeutic approach may be appropriate, i.e., to use L-DOPA only when required by a functional impairment of the patient, not otherwise treatable.

Alternatives to L-DOPA are direct agonists of striatal dopamine receptors (e.g., pergolide, cabergoline) that are not metabolized in a manner that leads to increased free radical formation. Their use may reduce endogenous release of dopamine and the need for exogenous L-DOPA, possibly with the consequence of a delay in the progression of the disease. At present, however, there are no clinical data to support a neuroprotective effect of dopamine receptor agonists. In contrast to the prodrug L-DOPA, these agonists do not depend on the functional capacities of nigrostriatal nerve terminals, which may be advantageous in the late stages of Parkinson's disease where L-DOPA-induced fluctuations are frequent. In addition, clinically used dopamine agonists have durations of action substantially longer than L-DOPA. However, despite these pharmacokinetic advantages, the clinical efficacy of the currently available agonists that preferentially activate dopamine D_2 receptors is less than that of L-DOPA. Due to their peripheral activity, dopamine receptor agonists may cause orthostatic hypotension and nausea. Typical central adverse events in elderly patients are hallucinosis or confusion, similar to that observed with L-DOPA. In contrast to levodopa, D_2 receptor agonists impaired cognitive function in monkeys, most probably due to "tonic" activation of D_2 autoreceptors, and a similar deterioration was seen with pergolide in humans. In fact, activation of dopamine autoreceptors by drugs like pergolide and quinpirole depresses the neocortical release of dopamine in humans, i.e., phasic dopamine signaling and, thereby, learning performance. This could have deleterious effects especially in Parkinson patients, treated with D_2-receptor agonists, who in part are known to suffer in the first place from disease-related cognitive deficits.

The mode of action of selegiline, which slightly improves parkinsonian symptoms, is unclear. At clinically used doses, it inhibits the MAO-B isoenzyme whereas MAO-A prevails in dopaminergic terminals. Selegiline is metabolized to (−)-desmethyldeprenyl, which seems to be the active principle in its anti-apoptotic effects in animal models, and further to (−)-amphetamine and (−)-methamphetamine. The (−)-amphetamines release biogenic amines, including dopamine from their storage sites in nerve terminals, although with less potency than their (+)-enantiomers. This may partly explain the symptomatic relief seen with selegiline. Developmental drugs, structurally related to selegiline, which exhibit virtually no MAO-B or MAO-A inhibiting properties, and which are not further metabolized to amphetamines, show neurorescuing properties that are qualitatively similar, but obtained with about 100-fold more potency, compared to selegiline. Glyceraldehyde-3-phosphate dehydrogenase, a glycolytic enzyme with multiple other functions, including an involvement in apoptosis, seems to be the molecular target for these neuroprotective selegiline-related drugs of the future.

Adenosine A_{2A} receptors are localized to the indirect striatal output function and control motor behavior. Istradefylline is a novel adenosine A_{2A} receptor antagonist, which demonstrated a clinically meaningful reduction in motor fluctuations in L-DOPA-treated patients with established motor complications, and is safe and well tolerated.

Antagonists of muscarinic acetylcholine receptors had widely been used since 1860 for the treatment of Parkinson's disease, prior to the discovery of L-DOPA. They block receptors that mediate the response to striatal cholinergic interneurons. The antiparkinsonian effects of drugs like benzatropine, trihexyphenidyl and biperiden are moderate; the resting tremor may sometimes respond in a favorable manner. The adverse effects, e.g., constipation, urinary retention, and mental confusion, may be troublesome, especially in the elderly.

Low affinity use-dependent NMDA receptor antagonists meet the criteria for safe administration into patients. Drugs like amantadine and memantine have modest effects on Parkinson's disease and are used as initial therapy or as adjunct to L-DOPA. Their adverse effects include dizziness, lethargy and sleep disturbance.

►Dopamine System
►Monoamine Oxidases

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α-2 Antiplasmin

α-2 antiplasmin, a naturally occurring inhibitor of fibrinolysis, is a single chain glycoprotein that forms a stable, inactive complex within plasmin and thereby prevents plasmin's activity.

►Coagulation/Thrombosis

Antiplatelet Drugs

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Synonyms

Platelet inhibitors; Platelet aggregation inhibitors

Definition

Platelets play a central role in ►primary hemostasis. They are also important in pathological processes leading to ►thrombosis. Antiplatelet drugs are primarily directed against platelets and inhibit platelet activation by a number of different mechanisms. They are used for the prevention and treatment of thrombotic processes, especially in the arterial vascular system.

Mechanism of Action

Antiplatelet therapy is an important means in the prevention and treatment of thromboembolic artery occlusions in cardiovascular diseases. Platelets are discoid cell fragments, derived from megakaryocytes in the bone marrow that circulate freely in the blood. Under normal conditions they neither adhere to each other nor to other cellular surfaces. However, when blood vessels are damaged at their luminal side, platelets adhere to the exposed subendothelium. Adherent platelets release various factors (see below) that activate other nearby platelets resulting in the recruitment of more platelets at the site of vascular injury. The rapid formation of a "platelet plug" at sites of vascular injury is the main mechanism of primary hemostasis. This is followed by a strengthening of the primary thrombus due to the formation of fibrin fibrils by the coagulation cascade. Platelets also play an important role in pathological conditions since they can become activated on ruptured ►atherosclerotic plaques or in regions of disturbed blood flow. This in turn leads to thromboembolic complications that underlie common diseases such as myocardial infarction or thrombotic stroke.

Mechanisms of Platelet Activation

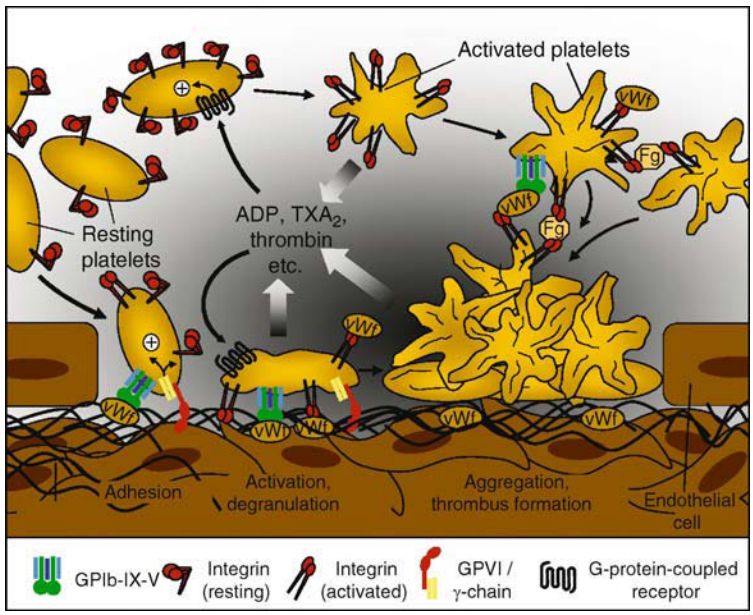
During the first phase of platelet activation, platelets adhere to extracellular matrix proteins of the subendothelium (see Fig. 1). Platelet adhesion is initially mediated by ►von Willebrand factor (vWf) which after binding to subendothelial collagen changes its conformation and interacts with the platelet receptor complex glycoprotein Ib-IX-V (GPIb-IX-V). This interaction brings platelets in contact with the subendothelium but does not result in a stable interaction. A stable adhesion of platelets is induced by the extracellular matrix protein ►collagen which via the platelet-specific receptor glycoprotein VI (GPVI) leads to an activation of platelets. This in turn results in the activation of several ►integrins like integrin α2β1 and αIIbβ3 which then mediate the firm adhesion of platelets to collagen, fibronectin, or laminin exposed at the subendothelial surface.

The formation of a platelet aggregate requires the recruitment of additional platelets from the blood stream to the injured vessel wall. This process is executed through a variety of diffusible mediators which act through ►G-protein-coupled receptors. The main mediators involved in this process are adenosine diphosphate (ADP), thromboxane A_2 (TXA_2), and thrombin (factor IIa). These mediators of the second phase of platelet activation are formed in different ways. While ADP is secreted from platelets by exocytosis, the release of TXA_2 follows its new formation in activated platelets. Thrombin can be formed on the surface of activated platelets (see Fig. 2).

Initially, activated platelets change their shape, an event immediately followed by the secretion of platelet granule contents (including ADP, ►fibrinogen, and serotonin) as well as by platelet aggregation. Aggregation of platelets is mediated by fibrinogen or vWf. They connect platelets by bridging complexes of glycoprotein IIb/IIIa (integrin αIIbβ3) on adjacent platelets, forming a platelet aggregate. Each platelet contains about 50,000–80,000 glycoprotein IIb/IIIa (GPIIb/IIIa) molecules on its surface. In order to bind fibrinogen and vWf, GPIIb/IIIa has to be converted from low affinity/avidity state to a high affinity/avidity state by a process described as inside-out signaling that is initiated during platelet activation (Fig. 2).

Acetylsalicylic Acid (Aspirin)

TXA_2 is produced by activated platelets via the sequential conversion of arachidonic acid by phospholipase A_2, ►cyclooxygenase-1 (COX-1), and thromboxane synthase. Similar to ADP, TXA_2 acts as a



Antiplatelet Drugs. Figure 1 Platelet adhesion, activation, aggregation and thrombus formation on subendothelial surface at an injured blood vessel. After an injury of the vascular endothelium, the processes of primary hemostasis (platelet activation) as well as of secondary hemostasis (fibrin formation via the coagulation cascade) are triggered by a variety of stimuli. Shown are only the mechanisms of primary hemostasis. Platelets adhere via their receptor GPIb-IX-V and GPVI mediated by von-Willebrand factor (vWf) or binding of extracellular matrix (especially collagen) to the subendothelium. Binding of collagen to GPVI initiates platelet activation resulting in integrin activation and the formation of a variety of diffusible mediators like ADP, thromboxane A_2 (TXA_2) as well as thrombin. These mediators initiate the second phase of platelet thrombus formation by recruiting platelets from the blood stream into a growing platelet aggregate. The crosslinking of platelets primarily involves the activated integrin αIIbβ3 (GPIIb/IIIa) which can bind the bivalent ligands vWf and fibrinogen (Fb) resulting in the crosslinking of platelets.

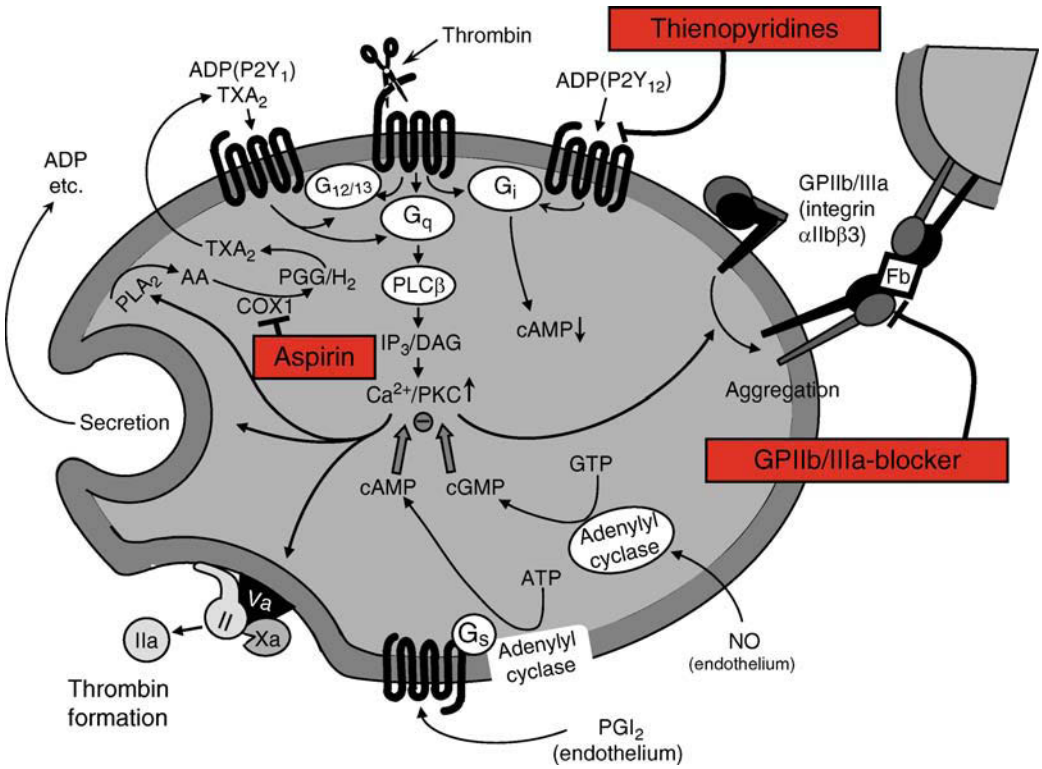
positive feedback mediator. In vascular endothelial cells, COX-1 is involved in the generation of prostacyclin (PGI_2), which inhibits platelet activation and leads to vasodilation. Low doses of acetylsalicylic acid (aspirin) have an antiplatelet effect by inhibiting the TXA_2 production by irreversibly acetylating COX-1 at serine-530 close to the active site of the enzyme which interferes with the binding of the substrate arachidonic acid to the enzyme. This results in impaired platelet function for the rest of its lifespan (7–10 days). Anucleated platelets, in contrast to nucleated cells, are unable to *de novo* synthesize COX-1. The aspirin doses required for this antiplatelet effect are therefore considerably lower than those necessary to achieve inhibition of prostacyclin formation in endothelial cells or analgetic and antipyretic effects elsewhere in the body. Following oral administration of aspirin, platelets are exposed to a relatively high concentration of aspirin in the portal blood. This may further contribute to the relatively high sensitivity of platelets toward the action of aspirin. Most other tissues are partly protected from irreversible COX-1 inhibition by presystemic metabolisation of aspirin to salicylate through esterases in the liver.

Thienopyridines

ADP is released from activated platelets by the secretion of dense granules and acts through at least three receptors. These are the ionotropic purinoceptor $2X_1$ ($P2X_1$) and two G-protein-coupled receptors, the G_q-coupled purinoceptor $2Y_1$ ($P2Y_1$), and the G_i-coupled $P2Y_{12}$ receptor. The latter has also been termed $P2T_{AC}$ or $P2_{cyc}$ and is targeted by a group of antiplatelet agents – the thienopyridines – such as ticlopidine and clopidogrel. To become activated, ticlopidine and clopidogrel require biotransformation by the hepatic CYP3A4 enzyme into active metabolites. The active metabolites irreversibly modify the $P2Y_{12}$ receptor. Due to the requirement of the formation of active metabolites, tienopyridines have a delayed onset of action. Similar to the antiplatelet effects of aspirin, the effects of thienopyridines are long-lasting due to the irreversible inhibition of the $P2Y_{12}$ receptor.

GPIIb/IIIa (Intergrin-IIbβ3) Inhibitors

Most antiplatelet drugs only partially inhibit platelet activation. In contrast, blockers of GPIIb/IIIa interfere at the end of the pathway common to platelet aggregation. They prevent fibrinogen and vWf from



Antiplatelet Drugs. Figure 2 Mechanisms of platelet activation, together with sites of drug action. Most platelet activators function directly or indirectly through G-protein-coupled receptors and induce several intracellular signalling pathways that eventually lead to secretion of granule contents, change of shape, inside-out activation of GPIIb/IIIa (integrin αIIbβ3), the exposure of factor Va and subsequent formation of the prothrombinase complex which forms thrombin (factor IIa) as well as the activation of phospholipase A_2 (PLA_2). Activation of GPIIb/IIIa allows fibrinogen (Fb) or vWf to cross bridge adjacent platelets. The main pathway that leads to platelet activation involves the G_q/phospholipase C-β (PLC-β)-mediated formation of inositol 1,4,5 trisphosphate (IP_3) and diacyl glycerol (DAG). This in turn results in the release of Ca^{2+} from intracellular stores and the activation of protein kinase C (PKC) isoforms. Major inhibitors of platelet activation are the endothelium-derived mediators nitric oxide (NO) and prostacyclin (PGI_2). While NO via activation of guanylyl cyclase increases cGMP levels, PGI_2 via activation of a G_s-coupled receptor increases the levels of cAMP. Both cyclic nucleotides inhibit via different mechanisms signaling processes involved in platelet activation. Aspirin blocks the conversion of arachidonic acid (AA) to prostaglandin G_2 and H_2 (PGG/H_2) by irreversibly inhibiting cyclo-oxygenase-1 (COX-1). Active metabolites of thienopyridines block ADP ($P2Y_{12}$)-receptors on platelets and GPIIb/IIIa-blockers interfere with fibrinogen- and vWf-mediated platelet aggregation. TXA_2 stands for thromboxane A_2.

binding to activated GPIIb/IIIa and can therefore completely inhibit platelet aggregation. The first GPIIb/IIIa antagonist developed was a hybrid human/murine monoclonal antibody. Its Fab fragment, termed abciximab, is clinically used and functions in a noncompetitive manner. An alternative approach to block GPIIb/IIIa involves the use of peptides that mimic short protein sequences of fibrinogen or vWf. Several peptides (e.g. the cyclic heptapeptide eptifibatide) or nonpeptidic, low molecular weight compounds (e.g. tirofiban, lamifiban) have been developed and function as competitive antagonists (Table 1).

Others

The proteolytic enzyme thrombin is known to play a crucial role in the overall thrombotic event leading to both, arterial and venous thrombosis by transforming fibrinogen into fibrin and by serving as a direct platelet activator. Thrombin exerts its effects on platelets via G-protein-coupled protease-activated receptors (PAR-1 and PAR-4 in human platelets). Thrombin-dependent receptor activation is achieved by cleaving an N-terminal extracellular peptide. Exposure of the newly generated N-terminal region functions as a tethered ligand for the receptor. Substances that directly bind to thrombin have been developed. The 65 amino acid long protein hirudin, originally isolated from the medical leech, *Hirudo medicinalis*, as well as related analogs have been recombinantly produced. They bind with the stoichiometry of 1:1 to thrombin and prevent its proteolytic action on fibrinogen as well as its binding to and the activation of PAR. Since they primarily act by

Antiplatelet Drugs. Table 1 Pharmacological properties of GP IIb/IIIa inhibitors

	Abciximab	Eptifibatide	Tirofoban
Molecular weight (Da)	50,000	800	500
Integrin selectivity	αIIbβ3; αVβ3	αIIbβ3	αIIbβ3
Affinity for αIIbβ3 (K_D; nmol/l)	5	120	15
Plasma half life	0.5 h	2–2.5 h	2 h
Duration of action	12–24 h	2–2.5 h	2 h
Elimination	Proteolysis/renal	Mainly renal	Mainly renal

inhibiting thrombin-dependent fibrin formation, they are generally classified as ►anticoagulants.

Since platelets are the major source of TXA_2 production and action, inhibitors of thromboxane synthase and TXA_2 receptor (TP) antagonists are being developed. TXA_2 synthesis inhibitors may have some disadvantages as they lead to the accumulation of cyclic endoperoxides (e.g. PGH_2) that are themselves agonists at the TXA_2 receptor.

The major physiological inhibitors of platelet activation are endothelium-derived mediators like prostacyclin (PGI_2) which via a G_s-coupled receptor activates the formation of cAMP formation by adenylyl cyclase as well as nitric oxide (NO) which stimulates the formation of cGMP by activating guanylyl cyclase. NO-generating drugs like organic nitrates lead to platelet inhibition, however, their main effect is on the vascular smooth muscle. Dipyridamole can inhibit the degradation of cAMP by inhibition of cyclic nucleotide phosphodiesterase and has been used as an antiplatelet agent. However, its clinical usefulness is not clear.

Clinical Use

Due to the pivotal role of platelets in thrombus formation, especially in the arterial system, inhibition of platelet function has become a central pharmacological approach. Antiplatelet drugs are given in order to prevent and treat thromboembolic diseases such as coronary heart disease, peripheral and cerebrovascular disease. They have also revolutionized the procedures of invasive coronary interventions as they reduce the risk of restenosis and thrombosis.

Aspirin leads to maximal anti thrombotic effects at doses much lower than required for other actions of the drug. Clinical trials have demonstrated that aspirin is maximally effective as an antithrombotic drug at daily doses of 75–160 mg. Higher doses have no advantage but increase the frequency of side effects, especially bleeding and upper gastrointestinal symptoms. Despite the development of various other compounds, aspirin has remained the gold standard for antiplatelet drugs due to its relative safety and extremely low cost. Several studies have demonstrated a beneficial role for aspirin as an adjunctive therapy in unstable angina and acute myocardial infarction. Mortality and disease progression were significantly reduced by low dose aspirin treatment. Patients with a history of arterial thromboembolism including myocardial infarction, stroke, transient ischemic attack, or unstable angina were shown to benefit from low dose aspirin treatment in several trials. The overall rate of mortality, as well as the occurrence of further vascular events was reduced in these patients. The results of these studies led to the recommendation to use aspirin for secondary prevention of arterial thromboembolism. However, aspirin is not generally recommended for primary prevention of arterial thromboembolism. A possible beneficial effect, such as a decreased risk of nonfatal myocardial infarction, may outweigh the risk of hemorrhagic complications only in a population already at high risk of cardiovascular diseases but not in a population of average health. Aspirin may also be beneficial as a prophylactic agent to reduce the risk of deep venous thrombosis and pulmonary embolism. However, the effectiveness compared to existing therapies remains to be determined; anticoagulants are still the mainstay of treatment in these conditions.

Thienopyridines are principally suited to treat conditions that respond to aspirin. Ticlopidin, but not clopidogrel, can lead to fatal neutropenia. Gastrointestinal problems and skin rashes can occur with both drugs but are more frequently seen when ticlopidine is used. In various trials, clopidogrel has been shown to be safe and similarly effective as aspirin. In patients at high risk from cerebrovascular events, thienopyridines seem to be somewhat more effective than aspirin in preventing serious vascular complications. Thienopyridines may be used instead of aspirin when the latter is not tolerated. However, aspirin still remains the first choice in most cases due to its low cost, relative safety and well documented efficacy. Studies are under way to test whether clopidogrel, given together with aspirin has advantages under certain clinical conditions.

GPIIb/IIIa antagonists have to be administered parenterally. They are currently used prophylactically during intracoronary interventions such as percutaneous transluminal revascularization with balloon angioplasty or intracoronary stenting, as well as to treat acute coronary syndromes like unstable angina and acute myocardial infarction. The main complications

are bleeding and thrombocytopenia. The bleeding risk appears to increase further with concomittant therapy with heparin at standard doses.

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Antiprogestins

Antiprogestins are progesterone receptor antagonists such as mifepristone (RU 38486), ORG 31710, ZK 137 316, ZK 230 211, ZK98299 (Onapristone).

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
►Selective Sex Steroid Receptor Modulators

Antiproliferative Agents

►Cancer, Molecular Mechanism of Therapy
►Antineoplastic Agents
►Alkylating Agents
►Antimetabolites

Antiprotozoal Drugs

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Synonyms

Protocidal drugs; Antiprotozoan chemotherapeutics

Definition

Protozoa are unicellular eukaryotes and a subregnum of the animal kingdom. Some protozoa exhibit a parasitic life style and are pathogenic to humans, animals and plants. An estimated 1.5 billion people suffer from protozoal infections, with malaria (*Plasmodium* spp.) alone causing an estimated 500 million clinical cases each year. Other examples of important human infectious diseases with protozoan etiology are as follows: toxoplasmosis (*Toxoplasma gondii*), African sleeping sickness (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*), Chagas disease (*Trypanosoma cruzi*); visceral, mucocutaneous, and cutaneous leishmaniasis (*Leishmania* spp.), amoebic colitis and liver abscess (*Entamoeba histolytica*) and lamblic enteritis (*Giardia lamblia*). Although there is a huge demand for antiprotozoal drugs, particularly drugs affordable by people living in developed countries, the incentives to develop such drugs are low. Accordingly, pharmaceutical industry has invested in other areas, such as cardiovascular, where revenues are higher (Table 1). Only recently have private–public partnerships and international institutions, such as Drugs for Neglected Disease Initiative (►http://www.dndi.org/), the Medicines for Malaria Venture (►http://www.mmv.org/papes/page_main.htm), the Institute for One World Health (►http://www.oneworldhealth.org/), the WHO Tropical Disease Research Programme (►http://www.who.int/tdr/), tried to fill this gap. In the following chapter, important antiprotozoal drugs (Fig. 1), including their modes of action, will be discussed (Table 1).

Antimalarial Drugs

Four different protozoa of the genus *Plasmodium* – *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* – can cause malaria. *P. falciparum* is the most virulent, being responsible for virtually all fatal malaria cases. Humans are infected by a feeding female Anopheles mosquito (Fig. 2). The clinical symptoms of malaria are associated with the development of the parasite within human red blood cells, while the liver stages remain asymptomatic. The following drugs (in alphabetical order) are currently in use for the treatment of malaria [5].

Mechanism of Action

Amodiaquine, a Mannich base 4-aminoquinoline, eliminates blood stage parasites. Its mode of action is similar to that of chloroquine (see below) and there is some cross-resistance.

Artemisinin and its derivatives, artesunate and arthemether, kill both asexual and sexual blood stages (Fig. 2). However, artemisinins are quickly eliminated from the body, resulting in parasite recrudescence, and are therefore combined with schizontocides that have a longer biological half-life, such as amodiaquine,

Antiprotozoal Drugs. Table 1 New compounds registered between 1975 and 1999 (with permission Trouiller et al., 2003 Lancet 359: 2188–2194)

Speciality	New compounds (1975–1999)	Proportion (%) of worldwide sales
	n (% of total)	
Cardiovascular	179 (12.8)	19.8
Antiinfectious (including)	224 (16.1)	10.3
Antiparasitic	26 (1.9)	1.5
HIV/AIDS	3 (0.2)	0.2
TB	13 (0.9)	0.2
Tropical diseases	4 (0.3)	0.1
Malaria		

lumefantrine, mefloquine and sulfadoxine/pyrimethamine. The artemisinins have been shown to be potent inhibitors of a plasmodial endoplasmatic Ca^{2+} ATPase. A single-point mutation within the Ca^{2+} ATPase can abolish inhibition by artemisinin, although resistance to artemisinins has not been reported in the field. Artemisinin is a sesquiterpene lactone extracted from the leaves of *Artemisia annua*. Also known as qinghaosu, artemisinin has been used in China for the treatment of fever for more than 1,000 years.

Atovaquone, a hydroxynaphthoquinone, selectively inhibits the respiratory chain of protozoan mitochondria at the cytochrome bc1 complex (complex III) by mimicking the natural substrate, ubiquinone. Inhibition of cytochrome bc1 disrupts the mitochondrial electron transfer chain and leads to a breakdown of the mitochondrial membrane potential. Atovaquone is effective against all parasite stages in humans, including the liver stages.

Chloroquine, a 4-aminoquinoline, targets the intraerythrocytic stages of malarial parasites (Fig. 2). Its mode of action is intricately linked with the plasmodial heme metabolism (Fig. 3). During development within erythrocytes, *Plasmodia* feed on the host cell's haemoglobin, which is digested within an acidic food vacuole. Heme released during haemoglobin proteolysis is highly cytotoxic and perforates cellular membranes. Malarial parasites detoxify heme through biomineralization within their food vacuoles to insoluble and inert ►haemozoin (malaria pigment). Chloroquine, which accumulates in the food vacuole, prevents heme biomineralization by forming complexes with heme, resulting in a build-up of toxic heme–chloroquine complexes that have an even higher affinity for membranes than heme alone and eventually destroy the parasite's cellular membranes (Fig. 3). Chloroquine resistance is linked with polymorphisms within a food vacuolar transporter that, according to current models, mediates efflux of the drug from the food vacuole.

Dapsone, an aromatic sulfone, is administered in combination with a proguanil derivative. Dapsone inhibits the plasmodial dihydropteroate synthase (DHPS) (Fig. 4).

Tetracycline and its derivative doxycycline are antibiotics widely used in the treatment of bacterial infections. They also exert an antimalarial activity. Tetracyclines inhibit the binding of aminoacyl-tRNA to the ribosome during protein synthesis.

Piperaquine, a bisquinoline, is a rapid acting blood schizontocide. The mode of action is unknown.

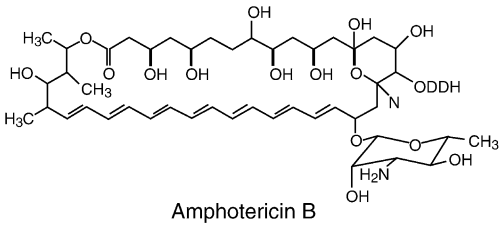
Primaquine, an 8-aminoquinoline, eradicates the dormant stages (hypnozoites) of *P. vivax* and *P. ovale* from the liver. Its mode of action remains obscure.

Proguanil appears to have a dual activity. Part of it is metabolized to cycloguanil, which subsequently inhibits the protozaon dihydrofolate reductase/thymidylate synthase (DHFR/TS) (Fig. 4). In addition, the native form, proguanil itself, exerts a potent antimalarial activity, especially in combination with other antimalarial drugs. The target of proguanil is unknown.

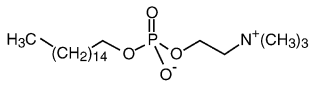
Pyrimethamine, cycloguanil and sulfadoxine (sulfadiazine) are folate antagonists that interfere with the folic acid biosynthesis pathway in malarial parasites and other protozoa, including *T. gondii* (Fig. 4). Folate is an essential precursor of the pyrimidine deoxythymidintriphosphate (dTTP) and the amino acids serine and methionine. Both protozoa and mammalian cells require folate for DNA and protein synthesis. However, protozoa can either synthesize dihydrofolate de novo or salvage folate precursors, whereas mammalian cells have no de novo dihydrofolate synthesis and must rely on dietary sources. By acting as an analogue of *p*-aminobenzoic acid, sulfadoxine (sulfadiazine) inhibits the DHPS, which then fails to convert dihydropteroate to hydroxymethyldihydropterin, resulting in a lack of dihydrofolate in the parasite. This mechanism does not affect the mammalian cells. Pyrimethamine and cycloguanil, the active metabolite of proguanil, act further down in the folic acid pathway by inhibiting the DHFR/TS enzyme complex. In mammalian cells, the DHFR and the TS are two independent

A

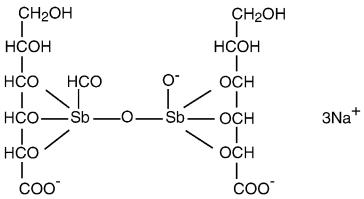
Antileishmania drugs



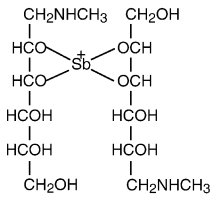
Amphotericin B



Miltefosine

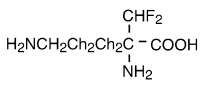


Sodium stibogluconate (Pentostam)

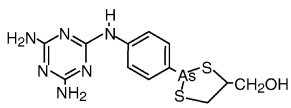


Meglumine antimonate

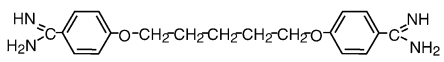
Drugs against sleeping sickness



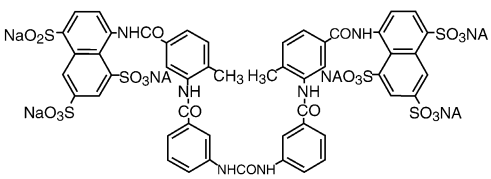
Eflornithine



Melarsoprol

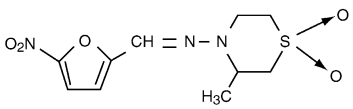


Pentamidine

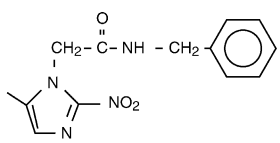


Suramin

Drugs against chagas disease

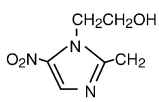


Nifurtimox



Benznidazole

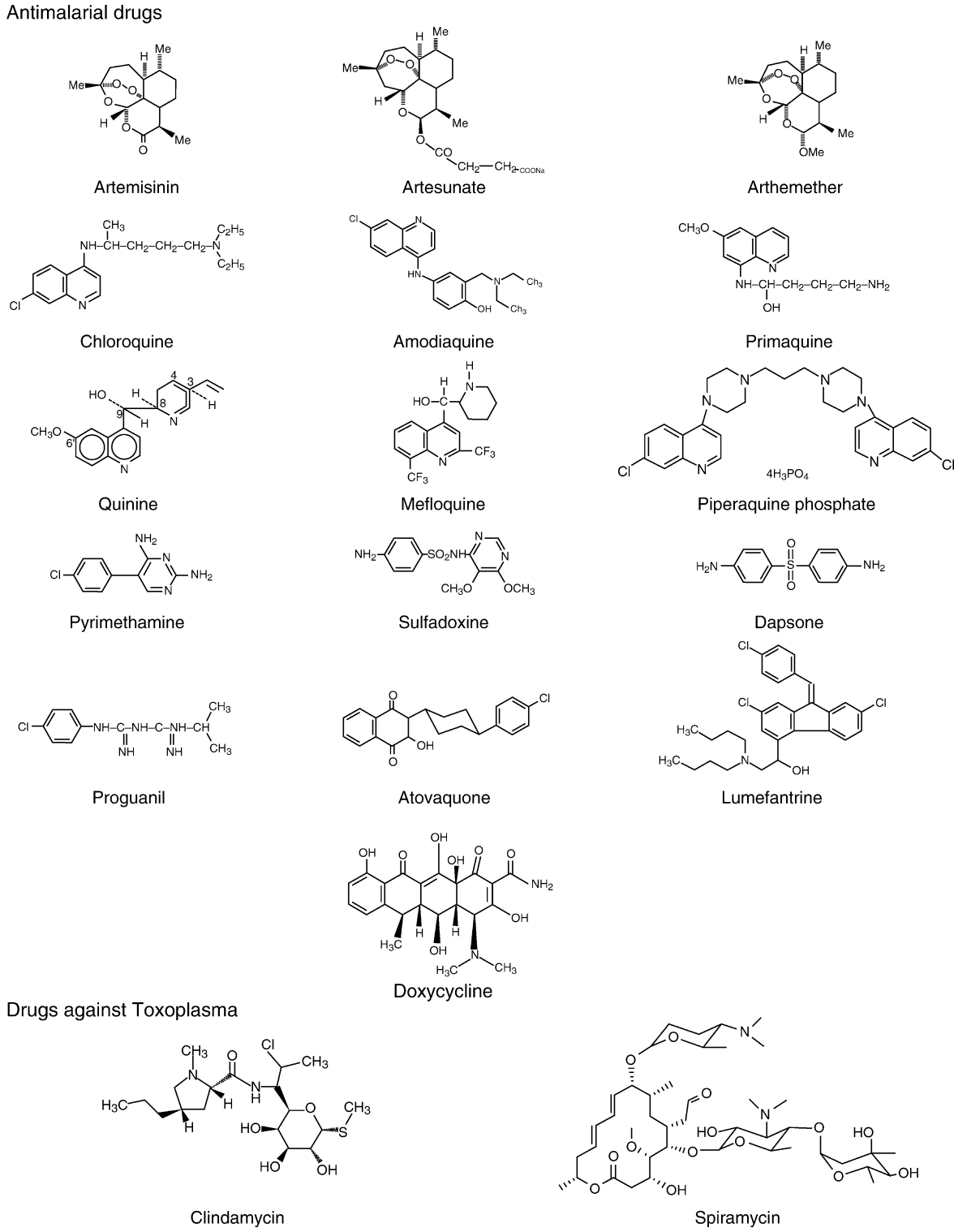
Drugs against anaerobic protozoa



Metronidazole

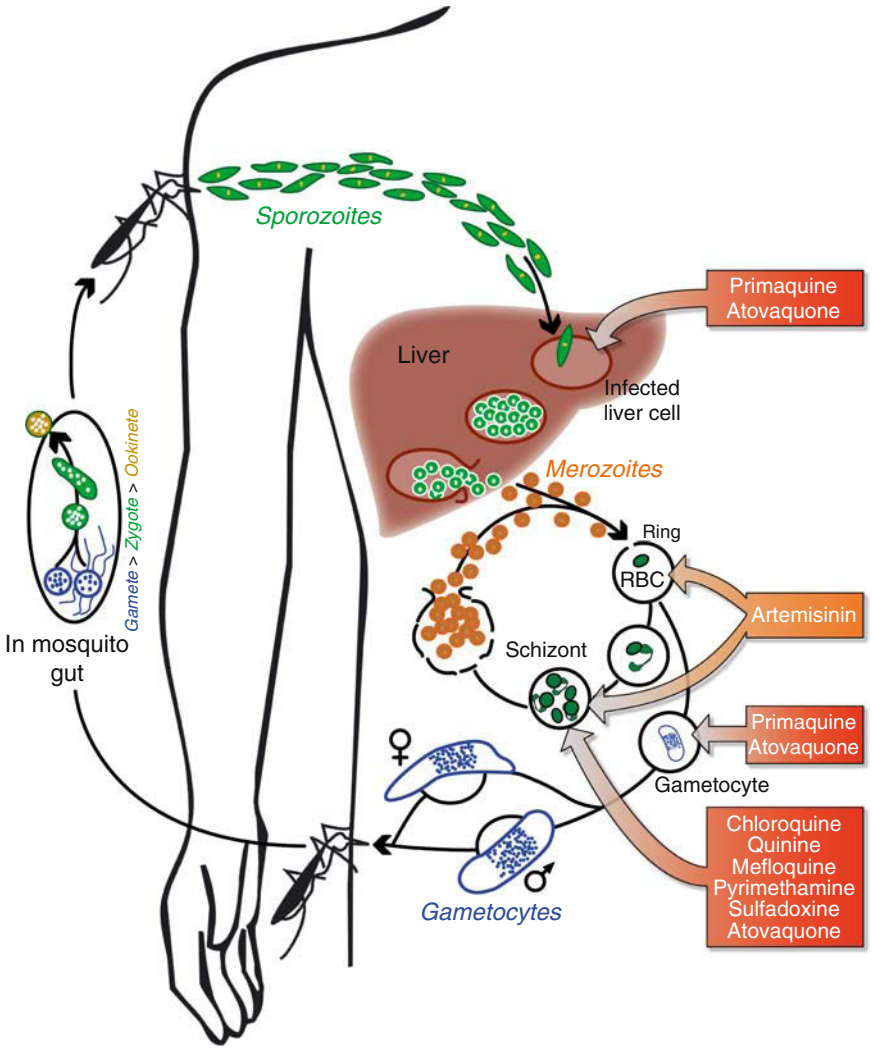
Antiprotozoal Drugs. Figure 1 (Continued)

enzymes. The protozoal DHFR/TS enzyme complex has a higher affinity for pyrimethamine and cycloguanil than does the human DHFR, which explains their high antiprotozoal activity. To avoid deficiency of folic acid in patients treated with antifolate antagonists, folinic acid should be administered. Pyrimethamine is a blood schizontocide and further acts against the liver stages (Fig. 2). Since resistance to pyrimethamine occurs rapidly due to single-point mutations within the DHFR/TS enzyme complex, pyrimethamine is only used in combination with sulfonamides for curative treatment.



Antiprotozoal Drugs. Figure 1 Chemical structure of relevant antiprotozoal drugs.

Quinine, an arylaminoalcohol, was the first antimalarial known to the Western world. It was originally produced from the bark of the cinchona tree and distributed as a powdery substance, which became known as Jesuits powder. Several potent antimalarials are derived from quinine, including lumefantrine, mefloquine, halofantrine and quinidine, the dextrarotatory diastereoisomer of quinine. All arylaminoalcohols

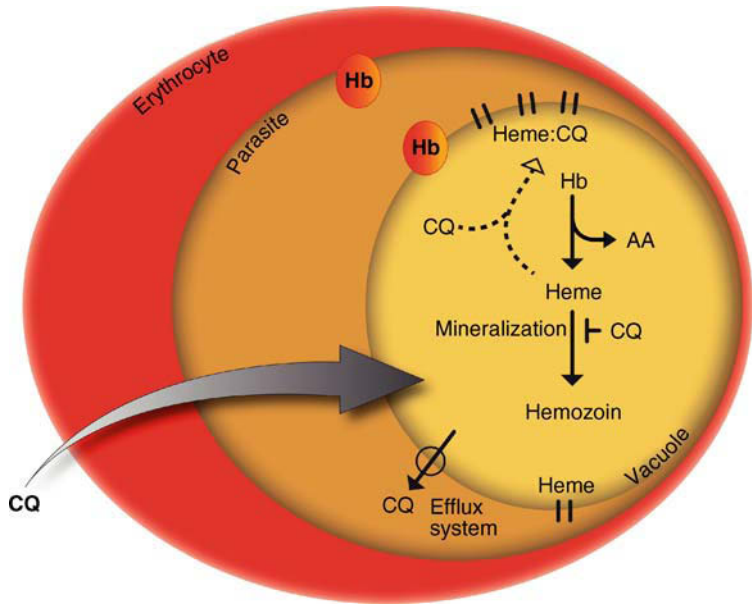


Antiprotozoal Drugs. Figure 2 Life cycle of malarial parasites and site of action of different antimalarial drugs. Malaria is caused by protozoan parasites of the genus *Plasmodium.* Infected *Anopheles* mosquitoes transmitted the parasite to humans during blood feeding. The infective stages are the sporozoites, which invade liver cells where they replicate to form merozoites. Upon rupture of the infected hepatocyte, merozoites are released into the blood stream where they infect erythrocytes. Within the erythrocyte, the parasites develop from ring stages to trophozoits, and then to schizonts. When the infected erythrocyte finally ruptures, merozoites are released, which again invade erythrocytes. Some intraerythocytic ring stages develop to sexual stages (gametocytes). Gametocytes are taken up by feeding *Anophelines.* Within the mosquito, the parasites develop into gametes, zygotes, ookinets and finally sporozoites. Primaquine and atovaquone are effective against liver forms of all *Plasmodia* including dormant stages of *P. vivax* and *P. ovale.* Primaquine also acts against gametocytes. Artemisinin destroys intraerythrocytic ring stage parasites and schizonts. The so-called schizonticidal drugs, chloroquine, quinine, mefloquine, pyrimethamine and sulfadoxine, act against intraerythrocytic schizonts.

are believed to kill asexual blood stages by inhibiting heme detoxification in the parasite's food vacuole (Fig. 3), although the mechanism of action is not well-understood.

Clinical Use

Malaria treatment has relied on a small number of chemically related drugs of the quinoline and the antifolate group for more than 4 decades [5]. Artemisinin derivates have been added only recently as a third group [1]. Various degrees of resistance and geographic distributions of resistance phenotypes have developed for all classes of antimalarials, except the artemisinin derivates, and is limiting their use [1, 5]. Chloroquine now fails in the treatment of falciparum malaria almost everywhere [5]. To prevent, or at least slow down, the



Antiprotozoal Drugs. Figure 3 The antimalarial activity of chloroquine. Chloroquine's mode of action is associated with heme detoxification. During intraerythrocytic development, *P. falciparum* degrades hemoglobin down to amino acids (AA) in its acidic food vacuole. Heme released from hemoglobin (Hb) is toxic and destroys cellular membranes unless it is converted to an inert biomineral, termed hemozoin or malaria pigment. Chloroquine (CQ) binds to heme, thereby preventing biomineralization of heme. The build-up of membrane-lytic heme/chloroquine complexes kill the parasite. Chloroquine resistant parasites have acquired an efflux system that expels the drug from the food vacuole.

emergence of resistance, compounds with different modes of action are being combined. The choice of drug, the route of drug application, and the dose regimen depend on the type of infection and the severity of the disease. The primary objective of treating uncomplicated malaria is to cure the infection, whereas in severe malaria it is to prevent death.

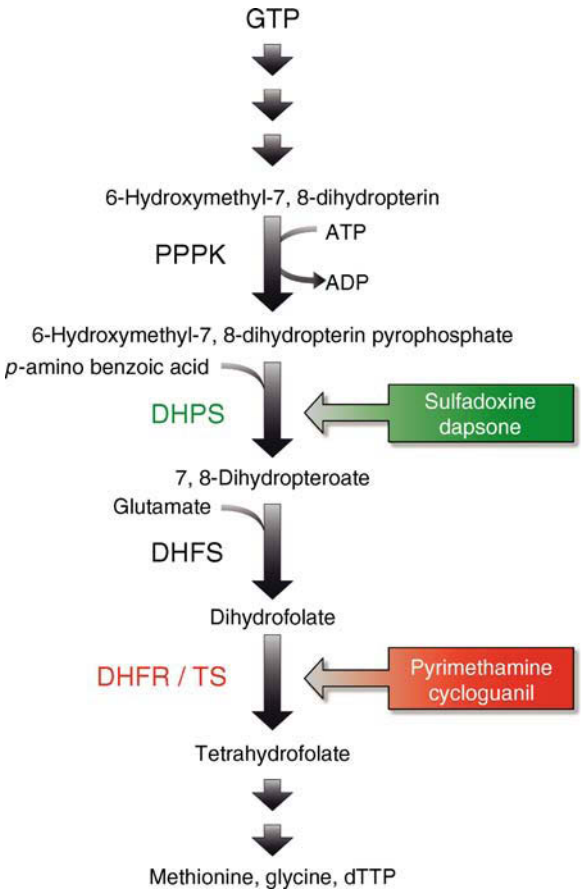
Severe Malaria

In severe malaria, rapid clearance of parasites is vital. This is achieved using an antimalarial compound that rapidly kills the parasites [5]. The compound has to be given intravenously as soon as possible with a loading dose at the start. A loading dose substantially cuts down the time by which therapeutic levels are achieved. Two classes of currently available drugs fulfill these requirements, the quinolines quinine and quinidine and the artesiminin derivates artesunate, artemether, and artemotil. The pharmacokinetic properties of artesunate are superior to those of artemether and artemotil. It is water-soluble and can be given parenterally. Randomized trials in Southeast Asia, comparing artesunate with quinine, have clearly demonstrated the benefits of artesunate in reducing mortality rates by almost 35% as compared with quinine. Based on these results, artesunate has been suggested as the treatment of choice for severe malaria in adults. This recommendation cannot yet be extended to children, particularly from high transmission settings. In African children, no significant difference in mortality rates were found when comparing artemether with quinine.

Uncomplicated Malaria

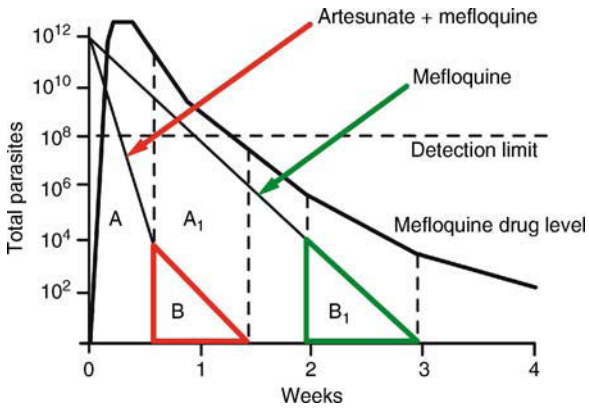
The treatment of uncomplicated malaria seeks to prevent recrudescence and, at the same time, tries to prevent the development of resistance. This is the rational behind artemisinin-based combination therapies (ACT). Artemisinin and its derivates (artesunate, artemether, artemotil, dihydroartemisinin) are clearing both parasitaemia and symptoms rapidly [1]. There is little difference in absorption and bioavailability amongst the different artemisinin derivatives. Due to the extraordinary high parasite clearance rate, artemisinin and its derivates quickly reduce the total burden of parasites to very low levels. Thereafter, the eradication of the remaining parasites relies on the partner drug in ACTs. The partner drug needs to be effective and parasiticidal concentrations have to be sustained until all parasites have been killed. A slowly eliminated drug is ideal. The artemisinin derivates are well tolerated with the exception of type 1 hypersensitivity reactions, which appear to be rare (1:3,000).

ACTs with amodiaquine, atovaquone–proguanil, chloroquine, clindamycin, doxycycline, lumefantrine,



Antiprotozoal Drugs. Figure 4 Mode of action of folate antagonists in protozoa. Protozoa are capable of *de novo* synthesis of dihydrofolate, a precursor of thymidine, serine and methionine. The 6-hydroxymethyl-7,8-dihydropterin pyrophosphate pyrophosphokinase (PPPK) and DHPS form one enzyme complex with two distinct active sites. The PPPK transforms GTP to 6-hydroxymethyl-7,8-dihydropterin and then to 6-hydroxymethyl-7,8-dihydropterin pyrophosphate. The product is linked to p-aminobenzoic acid (PABA) by DHPS, forming 7,8-dihydropteroate. Sulfadoxine exerts its activity by acting as a substrate analogue of PABA in this reaction. Glutamate is added to 7,8-dihydropterate by dihydrofolate synthase (DHFS) resulting in dihydrofolate. Dihydrofolate reductase (DHFR) and thymidylate synthase (TS) form a single enzyme complex with two enzymatically active sites. The catalytic site responsible for DHFR activity converts dihydrofolate into tetrahydrofolate. Pyrimethamine and cycloguanil exert their antimalarial activity by inhibiting the protozoal DHFR. The site of DHFR/TS responsible for TS activity converts tetrahydrofolate into deoxythymidintriphosphate (dTTP).

mefloquine, piperaquine, pyronaridine, proguanil–dapsone, sulfadoxine–pyrimethamine, and tetracycline have been evaluated in trials (Fig. 5) [1]. The following



Antiprotozoal Drugs. Figure 5 Artemisinin combination therapy (ACT): Adding a 3-days artesunate course to mefloquine clears the parasitaemia much more rapidly ($A_1 \rightarrow A$). The remaining parasites are exposed to higher mefloquine levels in ACT (B) compared to mefloquine monotherapy (B_1) (with permission White, 1997 Antimicrob Agents Chemother 41:1413–1422).

ACTs are currently recommended: artemether–lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, artesunate + sulfadoxine–pyrimethamine [1, 5]. In areas with amodiaquine and sulfadoxine–pyrimethamine resistance exceeding 20%, i.e., SE Asia, artesunate + mefloquine or artemether–lumefantrine should be used [1, 5].

Malaria Treatment and Chemoprophylaxis in Nonimmune People

Travelers treated for malaria after returning to nonendemic regions are treated along the same lines as above regarding uncomplicated and severe forms of the disease. Considerations with respect to development of resistance can be neglected. Monotherapy is fully justified and the following drugs are recommended: artemether–lumefantrine, atovaquone–proguanil, mefloquine, and quinine + doxycycline or clindamycin [5]. For the millions of nonimmune travelers to malaria-endemic areas, chemoprophylaxis (high-risk areas) and stand-by treatment (low-risk areas) are the corner stones of malaria prevention [5], along with exposition prophylaxis using repellent and impregnated bed-nets.

Intermittent Preventive Therapy in Pregnant Women and Infants

In endemic areas, chemoprophylaxis has been abandoned for a variety of reasons, mainly due to sustainability problems and the risk of contributing to the development of resistance. Intermittent preventive therapy (IPT), however, appears to be an alternative to protect pregnant women (IPT_p) and children during the first year of their life (IPT_i).

Antimalarial Drugs (*P. vivax, P. ovale, P. malariae*)

Resistance of *P. vivax* to chloroquine occurs, but is geographically limited. *P. vivax* and *P. ovale* produce hypnozoites, parasite stages in the liver that can produce multiple relapses. The drug of choice for blood stage infections to date remains chloroquine for all three species. To achieve radical cure of *P. vivax* and *P. ovale* infection, this must be followed by primaquine.

Antileishmanial Drugs

Leishmania is a disease complex caused by different species of *Leishmania*. The parasite, which is transmitted to humans by the bite of phlebotomine sandflies, multiplies within human macrophages. There are an estimated 1.5 million cases in approximately 88 countries each year, with 0.5 million patients suffering from the visceral leishmaniasis.

Mechanism of Action

Amphotericin B, is a polyene antibiotic, used in the therapy of systemic fungal infections. Its mode of action exploits differences in membrane composition between the pathogen and the human host. Ergosterol, the predominant sterol of fungi, plants, and some protozoan parasites, interacts with Amphotericin B, resulting in an increased ion permeability of the membrane. Humans contain cholesterol, which has a low affinity for amphotericin B.

Miltefosine, an alkylphosphocholine derivative, is a new antileishmanial drug and the first effective oral treatment of visceral leishmaniasis. However, there are concerns regarding teratogenicity, rapid emergence of resistance, and variable cure rates, possibly due to species differences in drug sensitivity. The mechanism of action of miltefosine is not known.

The pentavalent antimonial drugs sodium stibogluconate and meglumine antimonate are prodrugs that require biological reduction to the trivalent form Sb (III) for antileishmanial activity. Sb(III) seems to inhibit the leishmanial trypanothione reductase, which, together with a depletion of thiols, results in a breakdown of the cellular thiol redox potential. *Leishmania* and other kinetoplastidae possess an unusual antioxidant termed trypanothione, which is composed of two molecules of glutathione joined by a spermidine linker. Trypanothione protects the cell from oxidative stress by reducing any disulfide bonds formed within proteins to cysteines. The trypanothione reductase regenerates oxidated trypanothione.

Clinical Use

Pentavalent antimonial drugs have been the cornerstone of antileishmanial therapy for more than 70 years, in spite of their general toxicity causing a wide range of side effects [2]. Pentavalent antimonial drugs have to be administered parenterally, which is a painful procedure. Meanwhile, resistance is widespread. In India, pentavalent antimonial drugs have become almost obsolete because of resistance. They are still used in most other parts of the world where resistance has remained low. Alternative drugs are few and all have significant drawbacks:

1. *Amphotericin B*: This second line drug has moved into the first line in India.
2. *Liposomal Amphotericin B*: This is a highly effective drug against visceral leishmaniasis with remarkably few side effects. There is, however, only one producer and the price per treatment (US $1,500) is beyond the reaches for most communities affected by the disease.
3. *Paromomycin*: This oral aminoglycoside was first shown to be effective as a topical treatment for cutaneous leishmaniasis, and later as a parenteral drug against visceral leishmaniasis. Phase III clinical trials were completed in 2005 in India, 15 years after the potential of this component for treating visceral leishmaniasis was discovered. It is currently not registered for this use.
4. *Miltefosin*: The antileishmanial activity of this anticancer drug was discovered in the mid-1980s. It is the first oral drug available to treat visceral and cutaneous/mucocutaeous leishmaniasis. However, the registration process is slow.

Antitoxoplasma Drugs

Infection with the obligatory intracellular parasite *T. gondii* is mainly acquired by ingestion of contaminated food or water. Approximately a third of the world's human population is infected.

Mechanism of Action

Clindamycin, a lincosamide derivative, inhibits protein biosynthesis within a unique organelle of the parasite, termed apicoplast. Its mode of action is similar to that of spiramycin.

The folate antagonists, pyrimethamine and sulfadiazine, inhibit the parasite's DHFR/TS synthase enzyme complex and the DHPS, respectively (Fig. 4) (see antimalarial drugs). To avoid deficiency of folic acid in patients treated with antifolate antagonists, folinic acid supplementation is recommended to reduce bone-marrow suppression.

Spiramycin is a macrolide that inhibits protein biosynthesis by blocking transfer of the aminoacyl-rRNA along the ribosome in a unique organelle of the parasite, termed apicoplast. The apicoplast is a remnant of a secondary endosymbiosis of a red algae and is only found in the phylum sporozoa.

Clinical Use

Toxoplasmosis remains a challenge to clinicians. *T. gondii* is one of the most prevalent parasites worldwide and it persists in the body for a lifetime.

The infection passes unnoticed, or with little signs and symptoms, in immunocompetent children and adults. The parasite poses a major threat, however, when acquired during pregnancy and transmitted to the fetus, and in immunocompromised patients with or without AIDS, due to reactivation of latent disease or newly acquired infection. The major clinical conditions are congenital toxoplasmosis, ocular toxoplasmosis, and toxoplasmic encephalitis. Current treatment options are confined to the acute stage, but do not eradicate the parasite from the patient. The most commonly used treatment, and currently probably the most effective, is the combination of pyrimethamine and sulfadiazine, supplemented by folinic acid to prevent bone marrow suppression [3]. In maternal infection during pregnancy, the primary goal is to prevent transmission to the fetus, the secondary goal, to treat the infected fetus at the earliest time possible to reduce damage. Due to the specific requirements during pregnancy, spiramycin is recommended for the first and early second trimester, and pyrimethamine/sulfadiazine for the late second and third trimester [3]. In most countries, treatment is continued in the newborn for various lengths of time. The efficacy of this regimen, however, has still to await confirmation by appropriately designed studies, and different drug regimens and strategies need to be tested for different clinical settings. In the immunocompromised patients, recrudescence after successful treatment of acute toxoplasmosis is a problem. If restitution of the immune response cannot be achieved or is, for therapeutic purposes, not desired, chemoprophylaxis needs to be installed.

Drugs Against African Sleeping Sickness

African sleeping sickness is a parasitic disease of increasing importance, with an estimated 300,000–500,000 cases annually. The etiological agents, *T. brucei gambiense* and *T. brucei rhodesiense,* are transmitted to humans by the bite of Tsetse flies.

Mechanism of Action

Eflornithine (difluoromethylornithine, DFMO) inhibits the ornithine decarboxylase of the polyamine pathway, in both the trypanosome and the mammalian cell, by acting as an irreversible competitor of the natural substrate ornithine. Inhibition of ornithine decarboxylase results in depletion of the polyamines, putrescine, spermidine and spermine, which are essential for cell proliferation. Eflornithine selectively harms the parasite and not the mammalian cells, despite acting as an ornithine decarboxylase inhibitor in both cell types. This selectivity is explained by the lower rate of ornithine decarboxylase production in the parasite, as compared to mammalian cells. Due to the high turnover rate, mammalian cells are capable of quickly replenishing inhibited ornithine decarboxylase by newly synthesized enzyme. Eflornithine is only effective against *T. brucei gambiense*.

Melarsoprol, a trivalent organic melaminophenyl arsenic compound, kills intracerebral parasites of both *T. brucei gambiense* and *T. brucei rhodesiense*. Melarsoprol accumulates via an adenosine/adenine transporter in trypanosomes and is believed to inhibit glycolytic enzymes. Melarsoprol leads to a rapid lysis of trypanosomes. Melarsoprol is highly toxic to humans.

Pentamidine is an aromatic diamidine. Pentamidine uptake by the parasite is mediated by several different adenosine transporters. The mode of action is unclear.

Suramin, a symmetrical, polysulfonated naphthylamine, inhibits a number of trypanosomal enzymes; however, the importance of these effects on parasite killing is not clear.

Clinical Use

Despite great advances in the understanding of the biology of the parasite, progress in terms of drug development has been dreadfully slow.

1. Haemolymphatic stage (acute stage): For the last two decades, the first line treatment for the acute stage are Pentamidine and Suramin [2]. One compound, the prodrug DB 289 (a diamidine), is currently in phase III clinical trials.
2. Central nervous system stage (late stage): Melarsoprol has remained the first line treatment for more than 20 years [2]. Melarorpol is very toxic, and it is estimated that, of the patients treated for late-stage disease, an alarmingly high proportion suffers severe side effects or die. A cumbersome dosing schedule, used for decades, has recently been shortened to a 10-days course based on pharmacokinetic studies and controlled clinical trials. This certainly will improve patient compliance and costs. Eflornithine has joined as a second option to treat late-stage *T. gambiense* disease. Compared with melarsoprol, incidence and severity of adverse effects are much lower, however, application and manufacturing problems are the negative trade-offs. Currently, Nifurtimox, in combination with eflornithine, is in phase III clinical trials.

Drugs Against Chagas Disease

Chagas disease is a serious public health problem in Latin America, where an estimated 300,000 new cases occur each year. The causative agent is *T. cruzi*. *T. cruzi,* which is transmitted to humans by reduviid vectors. During acute disease, the parasite develops intracellularly in many tissues, including nervous and muscular tissue. Focal parasite-induced degeneration of infected organs, particularly the heart and the gastrointestinal tract, characterize the chronic form.

Mechanism of Action

Benznidazole, a nitroimidazole derivative, has a mode of action that seems to involve covalent modification of macromolecules by nitroreduction intermediates.

Nifurtimox, a nitrofuran, is a prodrug that is reduced to unstable nitroanion radicals, which react to produce highly toxic oxygen metabolites, such as superoxide and peroxide. Oxidative stress subsequently kills the parasite, which seems to lack effective enzymatic pathways to detoxify oxygen metabolites.

Clinical Use

Benznidazole and nifurtimox, which have been developed in the 1960s and 1970s, have remained the only two drugs available for the acute stage of the disease. There is no proven effect on the chronic stage, as recently reviewed by the Cochrane Collaboration. The specific treatment of the chronic stage has gained renewed interest with recent findings where persisting parasites may play a role in the development of irreversible lesions. The side effect of the two available compounds can be severe. Clinical trials have been carried out with allopurinol for the acute phase in the 1980s (phase II) and are currently in progress with benzimidazole for the indeterminate stage of the disease.

Drugs Against E. Histolytica and G. Lamblia

E. histolytica and *G. lamblia* are waterborne infectious diseases that cause colitis and liver abscess, and enteritis, respectively.

Mechanism of Action

5-Nitroimidazoles derivaties, such as metronidazole, tinidazole, ornidazole and secnidazole, are the drug of choice in the treatment of anaerobic protozoa. All 5-nitroimidazoles share the same mode of action. Anaerobic microorganisms reduce 5-nitroimidazoles to their active forms. This process only occurs under strongly reducing conditions. In some anaerobic protozoa and bacteria, such conditions are achieved when ferrodoxin is reduced by the fermentation enzyme pyruvate ferrodoxin oxidoreductase (POR). Ferrodoxin can transfer one electron to 5-nitroimidazole, resulting in the reduction of the nitro group. POR does not occur in mammalian cells. The corresponding enzyme to POR in mammalian cells is pyruvate decarboxylase, which is not able to establish a reducing potential high enough for the reduction of 5-nitroimidazoles. The reduced products of 5-nitroimidazoles disrupt the DNA structure, thereby interfering with transcription and replication.

Clinical Use

E. histolytica colonization of the large intestine is eradicated using a luminal agent such as diloxanid furoate or paromomycin. Invasive amoebiasis (colitis, liver abscess) is treated with one of the 5-nitroimidazole derivates [4], followed by a luminal agent to prevent relapse from remaining cysts in the intestine.

G. lamblia is treated with 5-nitroimidazole derivates. Paromomycin is a second choice in specific circumstances (e.g. pregnancy). In a Cochrane review, where 34 trials were included and where only one trial was without significant methodological flaws, the authors concluded that a single dose of tinidazole can provide the highest clinical cure rate with relatively few adverse effects. The high recurrence rate of disease after initial drug therapy is a problem.

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Antipsychotic Drugs

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Synonyms

Typical antipsychotic drugs: Neuroleptic drugs; conventional antipsychotic drugs; older antipsychotic drugs;
Atypical antipsychotic drugs: Novel antipsychotic drugs; serotonin/dopamine antagonists; $5HT_{2A}/D_2$ antagonists.

Definition

Drugs that are effective in alleviating psychotic manifestations of a number of neurodegenerative and psychiatric disorders especially ►schizophrenia. Antipsychotic drugs are generally divided into two main groups based on their propensity to cause motor side effects and sustained elevation of plasma ►prolactin levels at clinically effective doses. Older or "typical" antipsychotic drugs (e.g. chlorpromazine and haloperidol) are associated with a high incidence of motor adverse effects and usually cause hyperprolactinemia. Newer antipsychotic drugs (clozapine, risperidone,

olanzapine, quetiapine, and ziprasidone) are called "atypical" antipsychotic drugs because they cause significantly lower motor side effects and usually avoid hyperprolactinemia.

Mechanism of Action

The serendipitous discovery of the antipsychotic effect of chlorpromazine in 1952 brought renewed hope in the treatment of psychotic disorders. The observation of chlorpromazine's antagonism of dopamine receptors heralded the introduction of multiple other antipsychotic drugs based on their common ability to cause catalepsy in laboratory animals leading to the dopamine hypothesis of schizophrenia. Central to the dopamine hypothesis is that all known antipsychotic medications, including atypical antipsychotic medications, bind to dopamine-D_2 receptors (D_2-receptors). With the exception of aripiprazole, a partial agonist at D2-receptors, ►positron emission tomograpghy (PET) studies have shown a direct relationship between central D_2-►receptor occupancy and clinical effects of antipsychotic medications, with clinical response occurring only when at least 60% of central D_2-receptors are occupied while ►extrapyramidal side effects (EPS) occur at D_2-receptor occupancy above 80% [1]. Antipsychotic doses resulting in D_2-receptor occupancy higher than 80% result in more adverse effects with no additional clinical benefit, consistent with earlier observations that increasing the antipsychotic medication beyond this 'neuroleptic threshold' resulted in no additional benefit other than possibly decreasing clinical measures of hostility.

The introduction of clozapine presented a major challenge to the dopamine hypothesis. Not only does it show a modest clinical superiority over older antipsychotic agents (i.e. it is effective in some patients who do not respond to older antipsychotic drugs), but it does so without causing EPS or hyperprolactinemia. Pharmacologically, clozapine has been shown to have a low affinity for dopamine D_2-receptors (resulting in 20–70% occupancy) and a high affinity for serotonin ($5HT_2$) receptors (>80% occupancy). This gave rise to the hypothesis that its atypical nature was related to its high $5HT_2$-receptor (specifically $5HT_{2A}$-receptor) affinity relative to its low D_2-receptor affinity [2]. The role of $5HT_{2A}$ antagonism in the uniqueness of clozapine has been challenged [1]. A number of trials using drugs known to be antagonists of the $5HT_{2A}$-receptor (but not of dopamine receptors) have failed to show clozapine-like efficacy. Similarly, the $5HT_{2A}$-receptors are saturated by clozapine at sub-therapeutic doses, indicating that $5HT2_a$-receptor antagonism is not sufficient to effect an antipsychotic response. There are some case reports suggesting that augmentation of older antipsychotic medications with specific 5HT2-receptor antagonists may potentiate the antipsychotic efficacy, but this remains to be tested in controlled clinical trials.

Recently there has been interest in modulating release of prefrontal dopamine (putative improved cognitive function) using antagonists or inverse agonists at other serotonin receptors including 5HT2C and 5HT6 receptors, though these studies remain at the preclinical stage. In summary, it has been suggested that clozapine's low EPS and avoidance of hyperprolactinemia is attributable to $5HT_2$ antagonism, but some degree of D_2-receptor antagonism may still be necessary for an antipsychotic response.

The $5HT_2/D_2$ hypothesis was influential in the introduction of four new antipsychotic medications (risperidone, olanzapine, quetiapine, and ziprasidone), all having in common a high ratio of $5HT_2/D_2$ receptor affinity and lower incidence of both EPS and hyperprolactinemia [2]. Paliperidone, a recently introduced antipsychotic, is the active metabolite of risperidone (5-hydroxyrisperidone), with a similar binding profile to the parent drug. At least in the case of risperidone and olanzapine (and possibly also ziprasidone), this 'atypical' nature appears to be lost in a dose-dependant manner resulting in the appearance of EPS and sustained hyperprolactinemia at higher doses. Indeed, the relationship between dopamine D_2-receptor occupancy and clinical effects (response and EPS) for risperidone and olanzapine in human subjects studied with PET is very similar to that found with older antipsychotic drugs (i.e. a threshold >60% D_2-receptor occupancy for clinical response and >80% D_2-receptor occupancy for EPS). On the other hand, clozapine and quetiapine are clinically effective at lower D_2-receptor occupancy without showing sustained hyperprolactinemia. Some studies evaluating the ratio of D_2-receptor occupancy in extrastriatal regions relative to the striatum suggested that atypical antipsychotic medications preferentially bind to extrastriatal (i.e. limbic) D_2-receptors when compared with typical antipsychotic drugs. However, data from a study using kinetic analysis (a technique that avoids a number of the limitations of ratio studies) in non-human primates is not consistent with this hypothesis. The dual observation that antagonism of dopamine D2 receptors is associated with both clinical antipsychotic effects as well as adverse motor (EPS) and subjective (anhedonia) effects, partial agonists at D2 receptors have been a focus of drug development, leading to the first drug in this category on the market (aripiprazole). The intrinsic efficacy of the partial agonist at the D2 receptor is thought to be critical to produce adequate antagonism without deleterious stimulation of the D2 receptors leading to worsening of psychotic symptoms. The experience with aripiprazole, with an intrinsic efficacy of circa 30%, has shown this drug to be an effective antipsychotic with minimal EPS notwithstanding very high central D2 occupancy in excess of 90%, consistent with its partial agonist effects.

Recent studies suggest that the apparent low striatal D_2-recptor occupancy may be a result of quetiapine and clozapine's loose binding to D_2-receptors (i.e. high k_{off} resulting in low ▶affinity for D_2-receptor) [1]. Hence endogenous dopamine and low concentrations of radioligands (used in these experiments) may displace an appreciable amount of bound drug resulting in underestimation of D_2-receptor occupancy. In one study, patients treated with quetiapine at doses ranging from 300 to 600 mg/day showed normal prolactin levels and less than 20% D_2-recptor occupancy 12 h after their last dose. However, transiently elevated prolactin levels and appreciable (64%) dopamine D_2-recptor binding were noted 2 h after drug administration. Similarly clozapine (350 mg/day) resulted in 71% D_2-receptor occupancy 1–2 h after administration, declining to 55% and 26% after 12 and 24 h, respectively. While these findings await replication, they raise the possibility that different pharmacodynamic properties of dopamine receptor antagonists may be sufficient to explain their varying degrees of 'atypicality' [3]. The transient dopamine receptor occupancy may also account for clozapine's clinical superiority, since it has been shown that repeated transient dopamine receptor antagonism results in sensitization of the dopamine system, while continuous receptor antagonism results in tolerance and up-regulation of the system.

While all antipsychotic medications have a robust acute effect on delusions, auditory hallucinations, and disorganized behaviour (also known as 'positive symptoms'), and maintenance treatment has been shown to decrease both relapse and hospitalization rates, their effect on negative symptoms (apathy, avolition, alogia, and affective flattening) and related cognitive disturbance (e.g. attentional problems and disrupted working memory) is at best marginal. Newer antipsychotic medications, especially clozapine, have been shown to have some effect on negative symptoms and selected cognitive measures when compared with older antipsychotics such as haloperidol, but this topic remains controversial due to the difficulty in distinguishing primary negative symptoms from secondary (i.e. adverse) effects of the (older) medications [3]. In vivo measurements of extracellular dopamine levels using microdialysis in rodents and primates have shown that while both acute administration of clozapine and haloperidol result in an increase in dopamine levels in the striatum, clozapine results in higher dopamine levels in the prefrontal cortex compared with haloperidol. With chronic administration of clozapine in rodents, the increased dopamine release is maintained only in the prefrontal cortex but not in the striatum. It has been postulated that this modulation of prefrontal dopaminergic transmission may be involved in its effects on cognitive and negative symptoms, which are known to be associated with decreased prefrontral activity in functional neuroimaging studies [4]. Current approaches to drug development are focused on modulation of prefrontal dopaminergic activity directly (e.g. D1 agonism), indirectly via dopaminergic mecanisms (e.g. dopamine reuptake inhibition) or serotinergic mechanisms (e.g. 5HT2C and 5HT6 antagonism) – this focus is part of a growing realization that the 'antipsychotic' effect of currently available antipsychotics is largely limited to positive symptoms, while negative and cognitive symptoms are associated with functional outcome, remain unresponsive to D2 antagonists. Other approaches to cognitive enhancement also involves modulation of muscarinic M1 receptors (e.g. desmethylclozapine, a metabolite of clozapine with M1 agonist properties), nicotinic receptors (in particular alpha-7-nAchR), NMDA receptors (e.g. glycine and other NMDA receptor modulators), and metabotropic glutamate receptors (e.g. mGluR5 and mGluR2). These mechanisms are unlikely to be associated with effects on positive symptoms, though some downstream effects may also be related to prefrontal dopamine release (e.g. M1 agonists and medial prefrontal cortical release of dopamine) so that the effects may well be convergent mechanistically notwithstanding the targeting of different receptors.

It is thought that the mesolimbic dopaminergic projections from ventral tegmental area (VTA) are involved in the clinical response to antipsychotic drugs, and that in contrast to older antipsychotic drugs, newer antipsychotic drugs may act preferentially on these neurons. Acute administration of haloperidol in anaesthetized rodents has been shown to increase firing rate of neurons in the substantia nigra (SN) as well as the VTA. On the other hand daily administration for 3 weeks leads to a decline in the activity in these dopaminergic neurons below that at baseline, an electrophysiological phenomenon known as 'depolarization block'. All antipsychotic medications have the ability to cause depolarization block in the VTA, and their ability to cause depolarization block in the SN is related to their propensity to cause EPS in human subjects. Clozapine causes depolarization block in the VTA but not in the SN, consistent with involvement of the mesolimbic system in its antipsychotic effect [5].

In summary, the mechanism of action of antipsychotic drugs appears to be intricately linked with the normalization of a disrupted state of dopaminergic transmission. Remission of positive symptoms and the emergence of extrapyramidal side effects are associated with specific levels of striatal dopamine D_2-receptor occupancy. An increased ratio of $5HT_2/D_2$-receptor antagonism and/or altered pharmacodynamic properties of atypical antipsychotic drugs resulting in loose binding to D_2-receptors may be involved in the decreased incidence of motor side effects with some newer antipsychotic drugs, while antipsychotic action may involve activity of these drugs on the mesolimbic

ascending dopaminergic neurons. Preferential activity and modulation of prefrontal dopaminergic activity by atypical antipsychotic drugs may be related to their effects on cognitive and negative symptoms of schizophrenia.

Clinical Use (Including Side Effects)

Antipsychotic medications are indicated in the treatment of acute and chronic psychotic disorders. These include schizophrenia, schizoaffective disorder, and manic states occurring as part of a bipolar disorder or schizoaffective disorder. The co-adminstration of antipsychotic medication with antidepressants has also been shown to increase the remission rate of severe depressive episodes that are accompanied by psychotic symptoms. Antipsychotic medications are frequently used in the management of agitation associated with delirium, dementia, and toxic effects of both prescribed medications (e.g. L-dopa used in Parkinson's disease) and illicit drugs (e.g. cocaine, amphetamines, and PCP). They are also indicated in the management of tics that result from Gilles de la Tourette's syndrome, and widely used to control the motor and behavioural manifestations of Huntington's disease.

The choice of antipsychotic medications is largely dependent on considerations related to their individual side effect profile. Older antispychotic medications are generally divided into high, moderate, and low potency drugs, potency being related to their propensity for causing EPS. ▶Tardive dykinesia is the most common and potentially most disabling long-term side effect. The neuroleptic malignant syndrome is the most severe neurological side effect and consists of hyperthermia, autonomic instability, and muscle stiffness that may result in dehydration, renal failure, and death. Early diagnosis and management has resulted in decreased mortality from this condition. In addition to neurological side effects, other systems may also be affected by typical antipsychotic medications. In contrast to the neurological side effects, the incidence of these side effects are generally inversely proportional to the potency of the drug used. These include autonomic effects (e.g. tachicardia, dry mouth, urinary retention, constipation), haematologic effects (e.g. neutropenia and rarely agranulocytois), neurological (sedation, seizures), endocrine (e.g. weight gain, galactorrhoea, obesity, hypercholsterolemia, and hypergylcemia), and dermatological effects (e.g. photosensitivity).

The atypical antipsychotic drugs were introduced with the goal of minimizing neurological adverse effects associated with older antipsychotic medications. However, these medications are not free of serious adverse effects including dose-related parkinsonism (risperidone and olanzapine), dose-related risk of seizures (clozapine), endocrinological manifestations (including diabetes, weight gain, and hypercholesterolemia), and haematological abnormalities (neutropenia and agranulocytosis with clozapine). Nonetheless, while older antipsychotic medications remain the most widely used antipsychotic medications globally, the use of newer antipsychotic medications has largely dominated the market in Western Europe and North America. The principle clinical advantage that has led to this shift in prescribing practice is undoubtedly the decreased incidence of neurological side effects, which are associated with significant morbidity and poor outcome largely secondary to non-compliance. Indeed, they are generally recommended as first line agents in the treatment of psychotic disorders, with typical antipsychotic drugs reserved for patients having previously been successfully maintained on these medications or requiring parenteral antipsychotic drugs (e.g. short-acting intramuscular neuroleptics for agitation, and long-acting 'depot neuroleptics' for patients who are non-compliant with oral medication). While atypical antipsychotic medications are more acceptable to patients, their impact on the long-term outcome of schizophrenia remains to be established. Moreover, continued vigilance for their potentially significant long-term side effects including obesity, hypercholesterolemia, and impaired glucose tolerance is warranted. While all antipsychotic drugs may be associated with obesity and metabolic syndrome, generally the likelihood for these effects are highest for clozapzine and lowest for ziprazidone and aripiprazole in the following order:

$$clozapine > olanzapine > risperidone, > quetiapine > ziprazidoneandaripiprazole.$$

All antipsychotic medications are effective in alleviating positive and negative symptoms of schizophrenia, while atypical antipsychotic agents have been associated with some superior efficacy in reduction of negative symptoms. Following an adequate trial of antipsychotic treatment (8–12 weeks of treatment with adequately dosed antipsychotic drug) in acute schizophrenia, 60% of patients show significant improvement or remission compared with 20% of patients treated with placebo. Of the 40% who do not respond, approximately half respond to subsequent trials with other antipsychotic medications. The acute phase of the illness is treated with an oral antipsychotic medication titrated to the appropriate clinically effective dose. This may be supplemented by short-acting intramuscular antipsychotic agents in patients in whom rapid sedation is clinically indicated (e.g. severe agitation). Patients failing to respond to an adequate trial of an antipsychotic medication should be switched to another antipsychotic drug from a different pharmacological class. Patients who fail to respond or show only partial response to two adequate trials of antipsychotic drugs (including at least one atypical antipsychotic drug) or experience severe neurological side

effects (e.g. tardive dyskinesia) should be considered for a trial of clozapine [6]. Significant inter-individual variability in dose requirements is commonly seen, and this may be influenced by sex, age, and concomitant medications. Increasing the dose beyond which causes extrapyramidal side effects results in no additional clinical advantage. The augmentation with a second antipsychotic (antipsychotic polypharmacy) has not been supported by randomized controlled trials, and while this is very common in clinical practice, the practice should be reserved after other therapeutic strategies have failed and certainly not before a trial of clozapine. Regular monitoring for weight gain, hypercholesterolemia, triglycerides, and fasting blood sugars, as well as extrapyramidal side effects is warranted especially during the first 3 months of treatment, and then every 6 months or following any medication or dose changes. Patients are maintained on the clinically effective dose for the next 3–6 months. Following the resolution of the acute phase, the dose may be decreased to address any adverse effects having significant functional or emotional impact on the patient's well-being.

The duration of treatment is generally considered to be indefinite in patients diagnosed with schizophrenia, though selected patients recovering from a first psychotic episode may be considered for gradual taper of medication after at least 1-year of treatment and with close psychiatric follow up. Patients with a history of multiple psychotic episodes should be stable for at least 5 years before considering a trial off medication. When considering discontinuation of antipsychotic treatment, an individualized approach is recommended with careful consideration of certain aspects of the disease course (e.g. severe occupational impairment when acutely ill, history of suicidal attempts and violence). Dose discontinuation is associated with a very high 2-year relapse rate up to 60% in chronically treated patients, though most relapses occur within the first 3 months of discontinuation. If drug discontinuation is to be attempted, gradual taper and very close monitoring is critical, and in some studies has not been associated with longer duration of hospitalization. Symptom-targeting strategies and drug holidays have been tried in the past, but for most patients this is no longer recommended since it is associated with very high relapse rates. Finally, with the resolution of the acute phase of the psychotic episode, psychosocial, occupational, and cognitive difficulties need to be addressed, since they usually persist with significant impact on the patients' functional status.

In summary, antipsychotic drugs have a significant impact on the acute resolution and the maintenance of remission of symptoms of schizophrenia, enabling focus on rehabilitation efforts directed at residual cognitive, social, and occupational disabilities. The advent of atypical antipsychotic drugs brought lesser motor side effects and renewed hope to patients and families affected by this devastating illness, though these newer agents incur a variable degree of risk of weight gain and metabolic syndrome which needs to be carefully monitored. It is hoped that a better understanding of pharmacological mechanisms underlying the clinical superiority of drugs like clozapine will lead to the development of new treatment strategies with better efficacy and improved side effect profile.

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Antipyretic Agents

Antipyretic agents are used for the treatment of fever. The most commonly used antipyretics are acetylsalicylic acid and paracetamol (synonym acetaminophen).

►Non-steroidal Anti-inflammatory Drugs

Antiretroviral Agents

►Antiviral Drugs

Antirheumatoid Drugs

Antirheumatoid drugs are employed in the treatment of rheumatoid disease (rheumatoid arthritis). The

characteristic feature of this autoimmune disease is a persistent inflammation of peripheral joints. The inflammatory process leads to joint damage and subsequently to marked functional impairment. Inflammatory cytokines play a major role in the pathogenesi of the disease. Drugs used in the therapy of rheumatoid arthritis are non-steroidal anti-inflammatory drugs, glucocorticoids, immunosuppressive agents and disease-motifying antirheumatoid drugs (DMARDs). DMARDs are not analgesic, but they suppress the inflammatory process. DMARDs include drugs with cytotoxic and immunosuppressant activity (azathioprine, cyclosporin, methotrexate), gold compounds (auranofin and sodium aurothiomalate), anti-malarial drugs (chloroquine and hydroxychloroquine) and sulphasalazine. The last is also used for the treatment of chronic inflammatory bowel disease.

►NSAID
►Glucocorticoids
►Immunosuppressive Agents
►Inflammation

Antisense DNA

►Antisense Oligonucleotides (ASON)

Antisense Oligonucleotides

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Synonyms

Antisense DNA; Reverse complementary oligonucleotides; ASON

Definition

Antisense therapy means the selective, sequence-specific inhibition of gene expression by single-stranded DNA oligonucleotides. By hybridizing to the target mRNA, which results in a subsequent double-helix formation, gene expression is blocked. This process can occur at any point between the conclusion of transcription and initiation of translation or even possibly during translation.

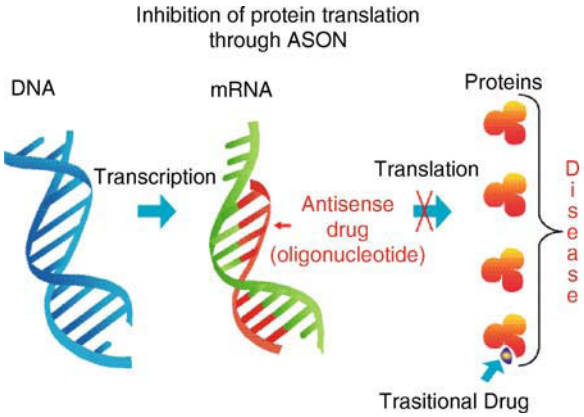
Description

ASON are sequences of usually 17–30 bases of single-stranded DNA that hybridize to specific genes or their mRNA products by Watson–Crick base pairing and disrupt their function. In the case of AS-ODN (antisense oligodeoxyribonucleotides) cellular RNAseH is able to bind to the DNA–RNA duplex and hydrolyze the RNA, resulting in increased transcript turnover. Modifications to the deoxy moiety at the 2′-sugar position prohibits RNAse H action.

In rarer cases the ODNs also prevent normal gene transcription by directly forming triplex-helix structures with target DNA. This does not destroy a gene but prevents its unwinding or its binding to a gene promoter.

The short length of a typical ASON facilitates cell internalization and increases hybridization efficiency by reducing base-mismatch errors. Once hybridization has occurred the ASON–mRNA complex becomes a substrate for intracellular ►RNAses (e.g., RNAse-H) that catalyze mRNA degradation and allow ASON to recycle for another base pairing with the next target mRNA molecule. The net result of this process is a sustained decrease of target mRNA translation and a lower intracellular level of the corresponding protein (Fig. 1).

The therapeutic utility of systemically administered ASON had been limited by their short plasma half life (sometimes even less than 3 min). This is due to their sensitivity to nuclease digestion. When the first-generation ASON were chemically modified, e.g., by replacing the oxygen in the phosphodiester bond with sulfur (phosphorothiorate) they obtained an increased stability in biological fluids while their antisense effect has been maintained. First-generation agents can be delivered via intravitreal injection, parenterally, by topical cream, enema, and inhaled aerosol. These antisense



Antisense Oligonucleotides. Figure 1 Schematic representation of the action of antisense oligonucleotides. They bind to their respective target mRNA preventing protein translation.

drugs have a sulfur chemistry modification that makes the drug more resistant to degradation, increasing stability in the blood stream and in tissues. This specific chemical modification prevents the rapid elimination of the drug from the body.

The second-generation chemistry adds 2′MOE modifications to the sulfur modification of first-generation chemistry. In turn, second-generation drugs have increased target-binding affinity and resistance to degradation. Second-generation drugs are composed of both RNA-like and DNA-like nucleotides, while first-generation drugs are entirely DNA-like. Because RNA hybridizes more tightly to RNA than to DNA, the second-generation drugs have a greater affinity for their RNA targets and, therefore, greater potency.

Another new modification is the 2′-deoxy-2′flouro-Darabinonucleic acid (2′F-ANA), which increases the strength of the oligonucleotide-mRNA hybrids, elicits efficient RNase H-mediated degradation of the target, is more nuclease resistant and reaches high intracellular concentrations for prolonged time. Similar results could be obtained with oxetane modified ASONs.

A problem with employment of ASON in a larger clinical setting is their poor uptake and inappropriate intracellular compartmentalization, e.g., sequestration in endosomal or lysosomal complexes. In addition, there is a need for a very careful selection of the ASON–mRNA pair sequences that would most efficiently hybridize. To date, several computer programs are used to predict the secondary and tertiary structures of the target mRNA and, in turn, which of the mRNA sequences are most accessible to the ASON. However, even with this sophisticated techniques, the choice of base-pairing partners still usually includes a component of empiricism. Despite these principal limitations, it has become clear that ASON can penetrate into cells and mediate their specific inhibitory effect of the protein synthesis in various circumstances.

The basic concept of the use of ASON can be modified in several ways:

1. Antisense RNA that is expressed intracellularly following transfection with antisense genes.
2. ►Ribozymes that are small RNA molecules with endoribonuclease activity and exhibit catalytic sequence-specific cleavage of the target.
3. The ribozymes were widely modified and can be further subdivided according to their structural features in group I ribozymes, hammerhaed ribozymes, hairpin ribozymes, ribonucelase P (RNase P), and hepatitis delta virus ribozymes.

Pharmacological Relevance

Examples of Applied ASON Therapeutics

The number of clinical trials ongoing represents a growing interest in antisense technology.

1. ASON to inhibit angioplasty restenosis. Patients suffering from coronary stenosis can successfully be treated by percutaneous transluminal coronary angioplasty (PTCA). However, in up to 50% of the patients restenoses occurs necessitating a repeated PTCA: ASON emerged as a potentially useful strategy to prevent such restenoses in animal models and fist clinical trials are currently under progress [1].
2. ASON against HIV infection. Once HIV has infected the cell, the genomic RNA of the retrovirus is used to code for a double-stranded cDNA intermediate. This cDNA is integrated into the genome of the host cell by the viral integrase. RNA identical to the genomic RNA of the virus will be transcribed from the DNA of this provirus by the infected cell. In experimental systems, ASON were used to target various parts of the viral life cycle, e.g., genomic RNA reverse transcription, viral mRNA transcription, and viral translation. With this regard, GEM 91, a 25mer ASON against the HIV-1 gag gene has extensively studied. Newer oligonucleotide analogs are now available, which act as strong steric block agents of HIV RNA function. In ongoing studies targeting the HIV-1 *trans*-activation responsive region (TAR) and the viral packaging signal (psi) with steric block oligonucleotides of varying chemistry demonstrate their great potential for steric blocking of viral protein interactions in vitro and in cells and describe the first antiviral studies [2].
3. ASON for targeting the Bcl-2 proto-oncogene in human cancers. The Bcl-2 protein is a major-apoptosis inhibitor originally identified by its involvement of a chromosomal translocation t(14;18) found in follicular Non-Hodgkin Lymphoma. Beside lymphomas, bcl-2 is up-regulated in several other tumors, e.g., leukemia, breast cancer, melanoma, prostate cancer, small and non-small lung carcinoma. In most of these studies, an 18-mer phosphothiorate ASON targeting the first six codons of bcl-2 (ISIS G3139) was used. The bcl-2 antisense therapy was feasible and showed potential antitumor activity. However, the mean inhibition of bcl-2 expression was only moderate and the clinical significance of this small decline was uncertain. Besides bcl-2, a large variety of other ►oncogenes have been targeted in cancer cell models. Table 1 gives an overview of such attempts. An emerging understanding of the most effective treatment setting – to test the addition of oblimersen to other therapies – has come from recent reports of two randomized studies in melanoma and CLL. In the melanoma study, the addition of oblimersen to dacarbazine produced a survival benefit for patients with normal LDH. In the CLL study, the addition of oblimersen to fludarabine and cyclophosphamide significantly increased durable CR and nPRs,

Antisense Oligonucleotides. Table 1 Malignant disorders as potential targets for ribozyme gene therapy

Target gene	Gene product	Ribozyme-induced change of function
bcr-abl	Tyrosine kinase	Inhibition of cell proliferation and colony formation
PML/RAR α	Transcriptional regulator	Inhibition of cell proliferation; induction of apoptosis; increase in sensitivity against ATRA
AML1/MTG8	Transcription factor	Inhibition of cell proliferation; induction of apoptosis
N-ras, H-*ras*, K-*ras*	Signal transduction pathway	Inhibition of cell proliferation and colony formation; change in morphology, enhanced melanin synthesis; decrease of in vivo tumorigenicity
EGFR	Receptor tyrosine kinase	Inhibition of cell proliferation and colony formation; decrease of in vivo tumorigenicity
c-erbB-2 (HER2/*neu*)	Receptor tyrosine kinase	Inhibition of cell proliferation; decrease of in vivo tumorigenicity
c-erbB-4	Receptor tyrosine kinase	Inhibition of mitogenesis and colony formation; decrease of in vivo tumorigenicity
Estrogen receptor	Transcriptional regulator	Inhibition of cell-cycle progression
Androgen receptor	Transcriptional regulator	Inhibition of androgen receptor transcriptional activity
c-*fms*	Growth factor receptor	Inhibition of cell proliferation
RET	Receptor tyrosine kinase	Inhibition of colony formation
Mdr-1	Drug-efflux pump	Reduction in resistance to chemotherapeutic drugs
c-*fos*	Transcriptional regulator	Change in morphology; reduction in resistance to chemotherapeutic drugs
CD44	Cell adhesion molecule	n.d.
VLA-6	Adhesion receptor	Decrease of in vitro invasion and in vivo metastatic ability
MMP-9	Matric metalloproteinase	Decrease of in vivo metastatic ability
CAPL (S100A4)	Calcium-binding protein	Decrease of in vitro invasion; reduction in expression of MMP-2, MT1-MMP, and TIMP-1; decrease of in vivo metastatic ability
Pleiotrophin	Growth factor	Inhibition of colony formation; decrease of in vivo tumor growth, tumor angiogenesis, and metastatic ability
VEGF-R1/ VEGF-R2	Growth factor receptors	Decrease of in vivo tumor growth; decrease of in vivo metastatic ability (VEGF-R2 only)
VEGF	Growth factor	n.d.
bFGF-BP	bFGF-binding protein	Reduction of release of biologically active bFGF; decrease of in vivo tumor growth and tumor angiogenesis
Telomerase	Ribonucleoprotein	Suppression of telomerase activity; inhibition of cell proliferation; change in morphology; induction of apoptosis
Bcl-2	Anti-apoptotic protein	Induction of apoptosis
PKC-α	Anti-apoptotic protein	Induction of apoptosis

with the greatest benefit occurring in patients with chemosensitive disease. Based on these considerations, a future randomized study of GO and oblimersen would be most likely to demonstrate the benefit of oblimersen in the setting of patients who had not previously been exposed to chemotherapy [2–4].

4. Formivirsen to treat cytomegalovirus-induced retinitis in HIV-infected patients. The first antisense drug approved by the US Food and Drugs Administration (FDA) was formivirsen (ISIS 2922) that targets the CMVIE2 protein. Formivirsen was approved for the treatment of cytomegalovirus-induced retinitis in patients with AIDS. One or both eyes can be affected

Antisense Oligonucleotides. Table 2 Viral disorders with malignant complications as potential targets for ribozyme gene therapies

Virus	Malignant complication	Target gene for ribozymes	Ribozyme-induced change of function
Human papilloma virus (HPV)	Cervical cancer, oral cancer	E6, E7	Inhibition of cell proliferation and colony formation
Epstein–Barr virus (EBV)	Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders in immuno-suppressed patients (e.g., AIDS, transplant recipients)	EBNA-1	Inhibition of cell proliferation
Hepatitis B virus (HBV)	Hepatocellular carcinoma	Progenomic RNA	Inhibition of viral gene expression
Hepatitis C virus (HCV)	Hepatocellular carcinoma	5′-untranslated/ cor region	Inhibition of viral gene expression

and it is not unusual for patients to suffer from severe visual impairment of even blindness as a result of untreated infections. However, the conventional treatment of CMV-retinitis also remains problematic, in particular for patients who cannot take, do not respond or become resistant to standard therapy by gancicolovir, foscarnet, and cidofovir. The main drawback of formivirsen is its need for local administration by intravitreal injection. Of note, the inhibitory effect of formivirsen for cytomegalovirus replication in vitro is about 30 times higher than for gangciclovir, the conventional treatment of choice for CMV infection.

5. For ISIS 301012, a second-generation antisense inhibitor of apoB-100 in November 2006. Isis announced results from two Phase 2 clinical trials of ISIS 301012. In the first study reported, patients with high cholesterol on stable doses of statins were treated with ISIS 301012 for 5 weeks. Patients who received 300 mg/week of ISIS 301012 in this study achieved a 51% reduction in LDL-cholesterol (LDL), a 42% reduction in total cholesterol (TC), and a 41% reduction in triglycerides (TG) beyond the levels achieved with statins alone.
6. ASON are able to regulate the sonic hedgehog pathway by down-regulating Gil2 (glioma associated oncogene), which plays a predominant role in the proliferation of HCC (hepatocellular carcinoma) cell lines. The suppression of Gli2 expression may provide a useful therapeutic option for the treatment of HCC [5].

Table 2 summarizes current ASON-mediated therapies against viral infections.

Non-Sequence Specific Activities of ASON

A rather unexpected stimulation of lymphocyte proliferation by ASON was frequently observed. Of note, the phosphorothiorate backbone of a given ASON has immune stimulatory properties itself, which are independent of its DNA sequence. In contrast, the stimulatory effects of unmodified oligonucleotides are dependent of a simple unmethylated ►CpG dinucleotide motif. The increasing number of ►CpG motifs generally increases the level of activation of B-lymphocytes. In addition, ASON may have effects of cytokine or immunoglobulin secretion or may alter the DNA binding activity of transcription factors. These nonantisense immune-enhancing (or sometimes immune-suppressing) effects are generally recognized as an undesirable side-effect. However, they may have therapeutic utility by their own, even though the mechanisms are not yet fully understood.

In general, systemic treatment with ASON is well-tolerated and side effects are dose-dependent. Among those, thrombocytopenia, hypotension, fever, increasing liver enzymes, and complement activation were most frequently seen.

►Cholinesterases
►RNA Interference (RNAi)–SiRNA

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Antithyroid Drugs

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Synonyms

Thionamides

Definition

Antithyroid drugs block thyroid hormone production in the thyroid gland. Based on their mechanism of action antithyroid drugs are defined as: (i) thionamides, which block thyroid hormone production by inhibition of thyroid peroxidase mediated iodination of tyrosine residues of thyroglobuline. (ii) aniones, which inhibit the transfer of iodine into the thyroid gland, e.g. perchlorate. (iii) high-dose (g range) jodine as lugol's solution or as oral radiographic contrast agents that block the release of thyroid hormones for days with subsequent escape. Other drugs utilized to treat hyperthyroidism are β-blocking agents, and seldom lithium.

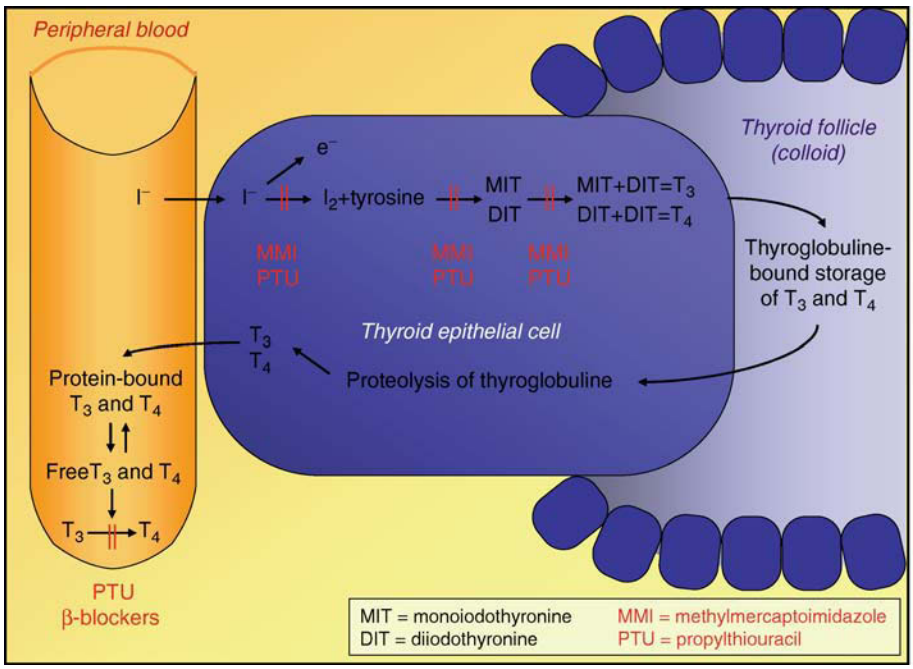
Thionamides are heterocyclic compounds that contain a thiourylene group. Thiouracil was the first widely used antithyroid drug. Further studies led to the introduction of substances with fewer side effects. Three drugs of this type are currently in use: methimazole, carbimazole and propylthiouracil [1]. Thiouracil and propylthiouracil belong to the subgroup of pyrimidines, whereas methimazole and carbimazole belong to the thioglyoxalines. The goitrogenic properties of several substances were first recognized in the 1940s when thyroid enlargement was noticed in rats that had been given sulfaguanine to study the antibiotic effects of sulfaguanidine on the intestinal flora [1]. Others noted the development of goiters in rats fed with phenylthiocarbamide. Later it was concluded that the effect of thiourea and the sulfonamides was due to the inhibition of thyroid hormone synthesis. It was first suggested that the entire thiourylene grouping (NH.CS.NH) would be necessary for antithyroid activity. Further studies revealed that only the thiocarbamide group (S = C–N) is essential for antithyroid activity.

Mechanism of Action

Synthesis of thyroid hormones occurs in several steps. At first, inorganic iodide is actively concentrated by thyroid follicular cells by the sodium iodide symporter. After oxidation to iodine (= iodination) it is bound to tyrosine residues thus forming monoiodothyronine (MIT) or diiodothyronine (DIT). MIT and DIT are coupled to form ►triiodothyronine (T_3) or ►thyroxine (T_4). Both iodination and coupling occur at the apical membrane of the thyroid follicular cell and within the ►thyroglobuline (Tg) molecule, and are catalyzed by the enzyme ►thyroid peroxidase (TPO). TPO is a haemoprotein enzyme with binding sites for both iodine and tyrosine. In model systems, TPO has no catalytic activity in the absence of H_2O_2. Therefore, it is assumed that H_2O_2 production is important also for thyroid hormone formation in vivo. TPO degrades H_2O_2 in a catalase-like reaction releasing O_2. Several iodination intermediates were postulated for this reaction, for instance TPO-bound iodinium (I^+) and TPO-bound hypoiodite (I^-). T3 and T4 are stored in the follicular lumen bound to thyroglobuline. The re-entry of Tg into the thyroid follicular cell involves a macropinocytosis process. Thyroid hormones are released after proteolysis of Tg. Type I and type II 5′ deiodinase generate the active hormone T_3 by reductive deiodination of the phenolic ring of T4 [2] (Fig. 1).

Antithyroid drugs inhibit the thyroid peroxidase-mediated iodination and coupling. Inactivation of TPO by antithyroid drugs involves a reaction between the drugs and the oxidized heme group produced by the interaction between TPO and H_2O_2. Results of several studies suggested that antithyroid drugs bind to the enzyme either at the same site as iodide or at a nearby site, and that the binding interferes with the binding of iodide [1, 2]. The type of inhibition depends on the extent of TPO inactivation and drug oxidation. These rates depend mainly on the iodine to drug concentration ratio. At a high iodine to drug ratio the inhibition of iodination is reversible and TPO is only partially inactivated. Under these conditions extensive drug oxidation occurs. When the iodine to drug ratio is low, iodination is irreversibly inhibited. This is associated with rapid and complete inactivation of TPO [2].

Propylthiouracil (PTU), but not methyl-mercaptoimidazole (MMI), has an additional peripheral effect. It inhibits the monodeiodination of thyroxine to triiodothyronine by blocking the enzyme 5′ monodeiodinase [1]. In humans the potency of MMI is at least 10 times higher than that of PTU, whereas in rats PTU is more potent than MMI. The higher potency of MMI in humans is probably due to differences in uptake into the thyroid gland and subsequent metabolism, because in vitro inhibition of thyroid peroxidase by MMI is not significantly more potent than by PTU [1, 6]. Whether antithyroid drugs have additional immunosuppressive actions is a matter of discussion [1, 2].



Antithyroid Drugs. Figure 1 Synthesis and secretion of thyroid hormones and mechanisms of action of antithyroid drugs. Iodine is actively concentrated by the thyroid gland (sodium iodide transporter). After oxidation it is bound to thyrosine residues thus forming monoiodothyronine (MIT) or diiodothyronine (DIT). MIT and DIT are coupled to triiodothyronine (T_3) or thyroxine (T_4) and are stored in thyroid follicles bound to thyroglobuline. Thyroid hormones are released by proteolysis. In the peripheral blood, T_4 is converted to T_3. Antithyroid drugs act by inhibiting the thyroid peroxidase-mediated formation of T_3 and T_4 and compete with iodothyronine residues for oxidized iodine. Moreover, they inhibit iodine oxidation. Propylthiouracil (PTU), but not methyl-mercaptoimidazole (MMI), inhibits the monodeiodination of thyroxine to triiodothyronine.

Pharmacokinetics

Absorption of MMI from the gastrointestinal tract occurs rapidly and almost completely. Peak serum concentrations increase linearly and are in the range of 300 ng/ml 1–2 h after oral ingestion of 15 mg MMI. In vitro carbimazole is an effective inhibitor of iodination without prior hydrolysis to MMI. In contrast, carbimazole itself is inactive in vivo. During absorption and in serum it is almost completely converted to methimazole. Ten milligram carbimazole is equivalent to 6.7 mg MMI [2]. MMI is virtually not protein bound. The total volume distribution is about 40 L. The serum half life is 4–6 h and remains unchanged in hyperthyroid patients. Patients with hepatic disease have prolonged plasma disappearance, whereas in kidney disease the metabolism is unchanged. Because of its lipophilic character the transplacental passage and excretion in breast milk is high [1]. Little MMI is excreted in urine. Little is known about the products of metabolism of MMI and their way of excretion [1]. MMI can be applied parenteraly.

PTU is also well absorbed from the gastrointestinal tract. Peak serum concentrations are in the range of 3 μg/ml at 1 h after drug ingestion after an oral dose of 150 mg. PTU, 80–90%, is protein bound. The total volume distribution is around 30 L for PTU. The serum half life of PTU is 75 min. It is not altered in patients with thyrotoxicosis, renal disease and, in contrast to MMI, in liver disease. PTU is mostly excreted in the urine after hepatic conjugation with glucuronide [1]. Biotransformation of PTU primarily occurs at the S group. It results in substantial loss of antiperoxidase activity. The metabolites, 6-*n*-propyluracil, *S*-methyl-PTU, PTU disulfide and PTU glucuronide, are only weakly active or completely inactive as thyroid peroxidase inhibitors. Because of its high protein binding and ionization at a physiologic pH, PTU is excreted in breast milk to a lesser degree than MMI. PTU crosses the placenta to an equal extent as MMI.

Both drugs, MMI and PTU, are actively concentrated by the thyroid gland. Intrathyroidal concentrations of MMI are in the range of 5×10^5 M. There is no difference in intrathyroidal concentrations of MMI 3–6 and 17–20 h after ingestion of 10 mg of carbimazole. Little is known about intrathyroidal concentrations of PTU. Eight hours after a single dose of 10 mg of MMI or 100 mg of PTU inhibition of intrathyroidal organification of iodide is about 90% and 60%,

respectively. This may be one reason for the longer effect of MMI compared with PTU. MMI can be administered once daily, whereas PTU should be applied 3 times a day.

Metabolism of the drugs by TPO is largely iodine dependent. Under conditions of reversible inhibition of iodination, the drugs are rapidly metabolized to higher oxidation products such as sulfonate and sulfate, with disulfide as an intermediate. If there is irreversible inhibition of iodination (higher drug to iodide ratio), some of the drug is oxidized only to the disulfide stage, but the TPO is simultaneously inactivated and no iodination is observed [2].

Clinical Use (Including Side Effects)

In patients with a first episode of ►Graves' disease, thionamides are used for long-term treatment to achieve remission of the organ-specific autoimmune disease. The standard therapy in Europe is a 1–1.5 year course of antithyroid drug treatment. In contrast, ►radioiodine is the preferred initial treatment in North America [2, 3, 5]. The relapse rate following antithyroid drug treatment is approximately 50% within 1–2 years. Most relapses occur within the first 12 months [2, 3]. After an unsuccessful course of antithyroid drug treatment there is little chance that a second course will result in permanent remission [2, 3]. Therefore a definite treatment, i.e. surgery or radioiodine, should be performed in these cases. Various parameters have been tested for their ability to predict the outcome of the individual patient after withdrawal of antithyroid drug therapy. A prospective study showed a greater chance of remission in patients with mild hyperthyreoidism, smaller goiters, a lower base-line level of antithyrotropin-receptor antibodies. However, until present no reliable markers with predictive statistical significance for the individual patient's remission or relapse has been identified [2]. In ►thyroid autonomy, which is mainly caused by somatic ►TSH receptor or Gsα mutations [3, 5], a spontaneous remission (e.g. by nodule apoplexia) is very uncommon. Therefore, antithyroid drug treatment is only used to render patients euthyroid before ablative treatment [2, 5].

Initial daily doses of 10–40 and 100–600 mg are recommended in clinical practice for MMI and PTU, respectively [1, 2]. Several studies have shown that treatment of hyperthyroidism with single daily doses of 10–40 mg of MMI is effective in the induction of euthyroidism in 80–90% of patients within 6 weeks [2]. The aim of the further antithyroid therapy is to maintain euthyroidism with the lowest necessary drug dose. Intrathyroidal drug accumulation is one cause for the efficiency of a single daily dose regimen. Moreover, a once daily dose yields better patients' compliance. Single daily doses of PTU have been shown to be less effective in achieving euthyroidism than administration of three divided doses a day. If a once daily dose regimen is considered for the treatment of hyperthyroidism, MMI is preferred to PTU [1, 2]. In the case of antityroid drug therapy before radioiodine therapy MMI should be preferred to PTU, because PTU increases the failure of radioiodine therapy, which may be related to its ability to neutralize iodinated free radicals produced by radiation exposure [1]. This effect can be overcome by increasing the radioiodine dose. Continuous application of MMI during and until 4 weeks after radioiodine therapy with the aim to maintain euthyroidism until the effect of the 131 I therapy sets in was shown to reduce the final cure rate. It should therefore be discontinued a few days before radioiodine therapy and resumed after approximately 1 week.

The response to thionamides depends on the dose and the iodine intake. It occurs faster in subjects living in countries with moderately low iodine intake than in areas with iodine sufficiency [2]. Antithyroid drug doses should be gradually decreased to the minimal maintenance dose as the serum thyroid hormone levels fall. The aim is to restore the euthyroid state within 1–2 months. Relapses of Graves' hyperthyroidism usually occurs within the first 3–6 months after discontinuation of antithyroid drugs. A lifelong follow-up is necessary to prevent spontaneous hypothyroidism, which can develop years after thionamide therapy of Graves' disease [1]. The "block-and-replace regimen" with the simultaneous administration of an antithyroid drug and L-thyroxine is used in case of poor patients compliance or if follow-up is difficult [3]. The titration regimen with low dose therapy showed fewer adverse effects than the high dose "block-and-replace regimen" and was not less effective.

MMI and PTU can lead to methimazole embryopathy with choanal or esophageal atresia. In pregnant women the antithyroid drug dose should be minimized to prevent fetal hypothyroidism by maintaining the maternal free thyroxine serum level slightly above the upper limit of normal.

Side Effects

Antithyroid drugs have several side effects. The most frequent side effects are maculopapular rashes, pruritus, urticaria, fever, arthralgia and swelling of the joints. They occur in 1–5% of patients [1, 2]. Loss of scalp hair, gastrointestinal problems, elevations of bone isoenzyme of alkaline phosphatase and abnormalities of taste and smell are less common. The incidence of all these untoward reactions is similar with MMI and PTU. Side effects of MMI are dose-related, whereas those of PTU are less clearly related to dose [1]. PTU may cause slight transient increases of serum aminotransferase and γ-glutamyl transpeptidase concentrations but also severe hepatotoxicity whereas methimazole or carbimazole can be associated with cholestasis. The side

Antitrypanosomal Drugs. Table 1 Different treatment strategies depending on the cause of hyperthyroidism

	Graves disease	Hot nodule and toxic multinodular goiter Etiology
	Organ specific autoimmune disease	Constitutively activating somatic mutation in TSH receptor or in Gs α
	TSH receptor antibody production	
Remission after antithyroid drug treatment	In 40–50% of patients 1 year after treatment withdrawal	No remission
Antithyroid drug treatment	1 year	Until euthyroid
Treatment with radioiodine or surgery	If relapse after 1 year of antithyroid drug treatment	After euthyroidism is achieved

effects usually appear within the first weeks or months after starting treatment. They occur more frequently with higher drug doses [1, 2]. If they are severe enough to alter treatment, it is possible to change from MMI to PTU or vice versa, although there is an estimated cross sensitivity of about 50% [2]. Most of the minor side effects are considered to be allergic reactions. Serious side effects such as bone marrow-depression, vasculitis, systemic lupus-like syndrome, cholestatic jaundice, hepatitis, hypoglycemia due to antiinsulin antibodies and hypoprothrombinemia are rare. They occur in approximately 0.2–0.5% of patients [1]. Depression of the bone marrow mostly appears as agranulocytosis, but also aplastic anemia and thrombocytopenia can be found. Symptoms of agranulocytosis like sore throat, fever and stomatitis are rare. Because of the sudden onset of agranulocytosis it can mostly not be detected in time by the routine leucocyte cell count. It occurs mostly within the first 3 months of treatment [1, 2]. Therefore, patients should be advised to stop taking the drug immediately if sore throat, pharyngitis or fever occur, and immediately seek medical attention and an urgent blood cell count. Most patients recover from agranulocytosis after discontinuitation of antithyroid drugs. But deaths have been reported in 20% of patients despite treatment with intravenous broad spectrum antibiotics. Granulocyte colony-stimulating factor has been administered, but did not yield a better outcome in the treatment of antithyroid drug-induced agranulocytosis compared with antibiotic therapy only, whereas the time of recovery may be shortened by G-CSF therapy. The risk of agranulocytosis is greater in patients given larger doses and in older patients [1, 2]. Vasculitis and lupus-like syndrome occur much more frequently with PTU than with MMI. Treatment consists of discontinuation of the drug and the use of high doses of glucocorticoids. Major side effects usually occur within the first 3 months after the start of antithyroid drug treatment but can also appear during prolonged treatment and after reinstitution of the drug [1, 2, 3].

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Antitrypanosomal Drugs

The etiological agents of the African sleeping sickness are Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense. Despite the enthusiasm shown by Robert Koch and Paul Ehrlich progress in the control of this disease achieved since the 19th century is sobering. Only four substances have been introduced, in total, in the therapy of African sleeping sickness, although the incidence of this disease has increased tremendously during the past decades with an estimated 50 million people at risk of infection and approximately 20 000 new cases reported each year. Suramin, the oldest of the antitrypanosomal drugs, was discovered by Paul Ehrlich and introduced in 1922. It was followed by pentamidine in 1937. Both drugs are only effective against early stages of the disease. The first and only drug effective against cerebral stages of African trypanosomiasis, of both T. brucei gambiense and T. brucei rhodesiense type, is melarsoprol, which has been in use for more than 50 years. It is still the drug of choice in most endemic areas despite its marked tendency to induce reactive encephalitis. Since then, drug and vaccine development in this field has declined to near zero. The only new antitrypanosomal substance so far has been eflornithine (difluoromethylornithine), which was introduced in 1990. It was originally developed for cancer therapy and proved later to be effective against intracerebral T. b. gambiense.

Nowadays, treatment of African sleeping sickness with the prevailing drugs faces three major problems:

(1) severe side effects, especially of melarsoprol; (2) wide spread drug resistance;and (3) lack of interest in drug development and production due to a low return of investment. To make matters worse, in recent years the producers of melarsoprol as well as eflornithine have tried to stop the production of these drugs entirely. A vaccine against trypanosomiasis is also not in sight, and efforts are complicated by the parasite's ability to constantly change the antigenic properties of its surface coat, a phenomenon called antigenic variation.

►Antiprotozoal Drugs

Antituberculosis Drugs

Antituberculosis drugs or antimycobacterial agents are specifically used for the treatment of tuberculosis (Mycobacterium tuberculosis infections). First-line drugs in tuberculosis therapy are isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin (Ribosomal Protein Synthesis Inhibitors). In order to minimize the development of drug resistance, a compound drug therapy is employed. In a first phase of two months, a combination of three drugs is employed. In a second phase of about four months, a combination of two drugs is used. A major problem is the increasing resistance of Mycobacterium tuberculosis strains against the first-line drugs. Infections caused by resistant strains are treated with combinations of second-line agents (e.g. capreomycin and cycloserine).

Antitumor Drugs

►Antineoplastic Agents

Antitussive Drugs

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Definitions

►Cough is an essential protective reflex response to irritating stimuli in the respiratory tract. It involves the sudden, usually involuntary, expulsion of air from the lungs. It can prevent foreign bodies from entering the lungs, or aid the removal of mucus and irritants from the lungs. Cough is a common symptom in upper respiratory tract infections and more chronically in asthma, chronic obstructive pulmonary disease (COPD) and lung cancer or may also indicate some other underlying disorders such as gastro-oesophageal reflux disease. It is also frequently caused by smoking. Conversely, in certain conditions such as Parkinson's disease, stroke and motorneurone disease, cough may be severely affected and the patient loses this protective function. Cough is useful if it is aiding the expulsion of foreign substances, but chronic cough is an unpleasant, difficult symptom for patients to live with and maybe debilitating.

Basic Mechanisms

Physically, cough results from a series of events starting with the patient inhaling, often to near maximum levels. Secondly, the glottis closes and the breathing muscles cause compression of the air in the lungs, leading to high pressure in the pleural space and alveoli. The expulsive phase occurs when the glottis reopens allowing the compressed air to escape. It is at this point that the cough sound is heard, and also that foreign materials which may have been deposited in the lungs, are removed. Coughing often occurs in bouts, or epochs, rather than as discrete events. *Cough* may be voluntary or involuntary – sub threshold stimuli may evoke the urge to cough, but not the reflex itself.

Airway irritation is detected by ►sensory nerves which are found within the walls of the airways. Various nerve endings are found in this area, but it is thought that the ►rapidly adapting receptors (RARs) are the predominant sensors of *cough*. RARs rapidly adapt to a maintained stimulus, and have thin myelinated Aδ fibres. Slowly adapting receptors and C-fibres (C-fibres are sensory nerves with unmyelinated axons and can have a modulatory role on the cough reflex.) can have a modulatory role on the cough reflex. Several different types of sensor have been found, and more than one type of fibre is involved – it is possible that the combination of fibres and the nature of the discharge regulate *cough* as much as the quantity of action potentials.

These primary afferents project from the airway into the CNS exclusively via the vagus nerve, via the nodose or jugular ganglia. These neurons enter the brainstem and terminate in the nucleus tractus solitarius. Here they synapse with other neurons which determine the various motor components of *cough*. At these central synapses, there are many neurotransmitter receptors present (e.g. tachykinins, glutamate, 5-HT, GABA, NMDA, dopamine, opioids and nociceptin), which may be the site of action of centrally acting antitussive drugs (see below). It is also here that other sensory afferents terminate, and may modulate the synaptic signalling. If there are disorders in other organs which also have

sensory neurons carried in the vagus, it is here that these nerves may interact with airway neurons to cause *cough*. In contrast, in diseases where *cough* is reduced, it is generally due to problems in the efferent part of the reflex loop.

Previously it was thought that there was a central "cough centre" in the CNS, but current research suggests that this is an over-simplification. The ability to coordinate a combination of a variety of motor outputs is essential for *cough*. For example, outputs go to control airway structures such as the glottis and a wide range of breathing muscles such as the diaphragm and intercostal muscles. In addition, synchronisation with the respiratory rhythm is required as it is not possible to *cough* and breathe concurrently. Finally, the CNS is thought to contain a gating mechanism by which the brain determines whether the arriving stimuli are of sufficient magnitude for a cough to occur. The cortex is not necessary for cough, but it can exert descending control on the reflex. (Fig. 1)

The sensory nervous system which governs cough is subject to plasticity (►nerve plasticity) – such that there may be an enhancement of this cough pathway, either by changes in the receptors on ►sensory nerve endings, the ganglia or within the CNS which can increase the activity of these neurons leading to a hypertussive state.

Pharmacological Interventions

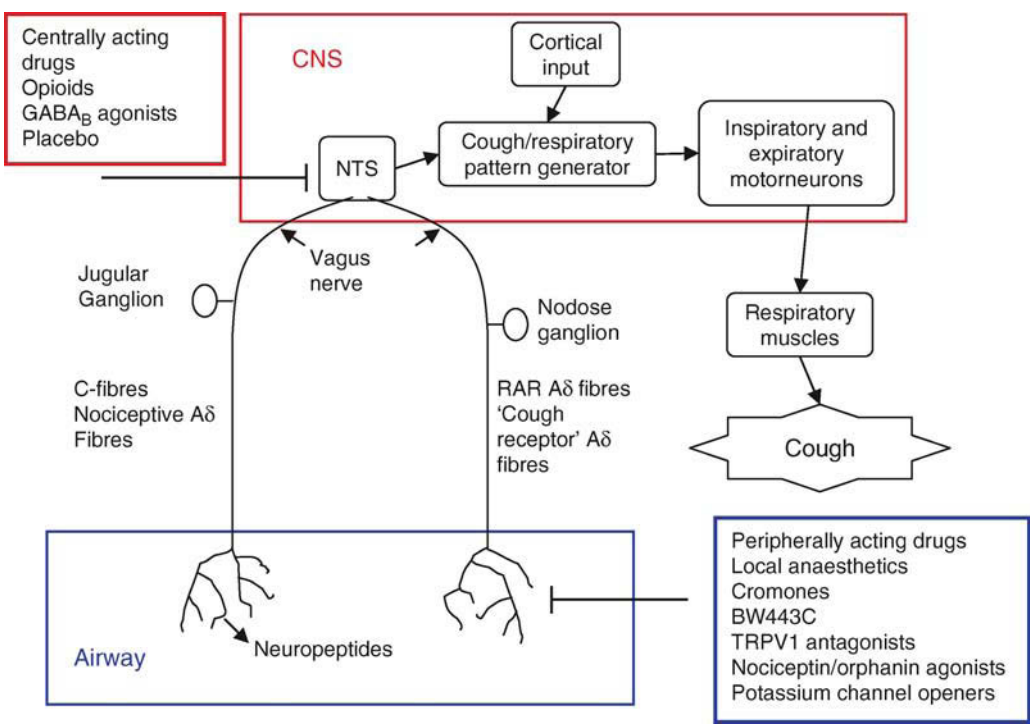
Cough is currently a huge unmet clinical need, as none of the currently available treatments are reliably effective [2]. However, there are many treatments which are currently used, with variable levels of success. In addition, if it is caused by another condition, such as gastro-oesophageal reflux disease, then treatment of that may reduce cough.

Drugs

Centrally Acting Drugs

Opioids

Opioids (►opioid systems) are thought to exert their antitussive effects by acting as agonists at μ- and κ-opioid receptors in the CNS. Activation of these receptors activates various G-proteins and leads to the inhibition of



Antitussive Drugs. Figure 1 Schematic diagram of the cough reflex and sites of action of some tussive agents. Airway sensory nerves activated in response to a tussive stimulus travel though the vagus nerve to the medulla, where they terminate in the nucleus tractus solitarius (nTS). Second order neurons relay the message to the respiratory pattern generator, which modifies the activity of the inspiratory and expiratory motorneurons and leads to cough. Tussive agents can activate a variety of airway receptors to cause cough. Many nerve types (both peripherally and centrally) undergo phenotypic changes and have increased responses to tussive stimuli following inflammation. The sites of action of antitussive drugs are shown. Abbreviations: RAR, rapidly adapting receptor; CNS, central nervous system. Figure adapted from [1].

the activity of most neurons, but the activity of a few are increased. Recent evidence suggests that morphine may also interact with ►TRPV1 receptors to explain some of its antitussive activity.

Centrally acting drugs include dextromethorphan and codeine. However the possibility that there may also be peripheral effects of these drugs has lead to the development of BW443C (see below).

Opioid drugs are often more effective than other non-narcotic treatments, but they are also associated with more side effects making them less suitable for many patients. Higher doses which are more effective are also associated with undesirable effects such as sedation.

Peripherally Acting Drugs

Local Anaesthetics

Local anaesthetics are more consistently effective than other therapies, but their use is controversial. High concentrations are needed for therapeutic benefit, but this also increases the amount crossing the blood brain barrier and entering the brain producing unwanted effects. Topical administration to the airways can reduce this.

Patients who do not obtain sufficient symptomatic relief from other treatments may use lidocaine, benzonatate, bupivacaine topically or mexiletine orally.

Tachykinin Antagonists (►Tachykinins)

►C-fibre afferents from the airways contain peptide tachykinin transmitters such as substance P (SP) and neurokinins A and B (NKA and NKB). Stimulation of these nerves can also cause local release of these mediators at their peripheral terminal, allowing them to enhance the activity of the *RARs*. SP, NKA and NKB act at the tachykinin receptors (NK_1–NK_3), and so understandably, antagonists for NK_2 in particular appear promising in cough.

Cromones

Both disodium cromoglycate and nedocromil sodium have antitussive effects in humans, particularly against ACE inhibitor induced cough. This suggests an effect on bradykinin induced changes in ►sensory nerve function. Antitussive activity of these drugs is thought to occur by increasing the depolarisation of sensory nerves, which increases the threshold for an action potential and therefore inhibits the activity of these neurons.

$GABA_B$ Receptor Agonists

►GABA is the predominant inhibitory neurotransmitter in the CNS. Baclofen acts centrally as an agonist at the $GABA_B$ receptor, which increases inhibition of nerves. 3-Aminopropylphosphinic acid (3-APPi) has been shown experimentally to act as an antitussive at peripheral nerves and preclinical evidence suggests that baclofen indeed has antitussive actions clinically [3].

Novel Drugs

BW443C

BW443C is a novel opioid used for the treatment of cough, but which does not enter the brain and so exerts its effects only on peripheral nerves. It has not been tested as an antitussive in humans due to its rapid metabolism in the lungs but the concept of a peripheral opioid is still possible.

Nociceptin/Orphanin Receptor Agonists

Nociceptin and orphanin are synonyms for the peptide that acts at an opioid-like receptor. Nociceptin may act by inhibiting tachykinin release from sensory ►C-fibres, and a clinical trial has started to test its effects on *cough*.

TRPV1 Receptor Antagonists

Capsaicin, the pungent chemical from chilli peppers, induces cough. Capsaicin is an agonist at the transient receptor potential vanilloid receptor 1 (TRPV1) – a polymodal receptor which integrates several harmful stimuli such as noxious heat, low pH and various possible endogenous mediators to mediate pain and cough. TRPV1 receptors have been demonstrated to be upregulated in patients with chronic cough [4].
Capsaicin is used to cause cough experimentally and clinically and the TRPV1 antagonist capsazepine can inhibit cough elicited by both capsaicin and citric acid. This suggests that other TRPV1 antagonists could be effective treatments for cough (recently reviewed in [5]). However, capsazepine does not block hypertonic saline induced cough, suggesting that other tussive receptors are also important [5]

Potassium Channel Openers

An effect of opening ►K^+ channels is to hyperpolarise the primary sensory neurons. Similarly to local anaesthetics, this makes the cell less likely to produce an action potential because more depolarising stimuli are needed to overcome the block. NS1619 is an example of this type of drug which has initially shown antitussive activity in a variety of experimental systems.

Quaternary Ammonium Salts

Quaternary ammonium salts such as carcainium chloride (RSD 931) have been shown to be antitussive whilst having much reduced local anaesthetic activity. Whilst the molecular mechanisms underlying this antitussive activity is not understood, RSD 931 appears to be Aδ fibre selective and may represent a novel class of antitussive drug. More recently JMF2-1 a lidocaine derivative that blocks Na^+ channels has had beneficial effects in the airways without significant local anaesthetic activity.

►Opioid System
►TRP Channels

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Antiviral Drugs

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Synonyms

Antivirals; Virostatics

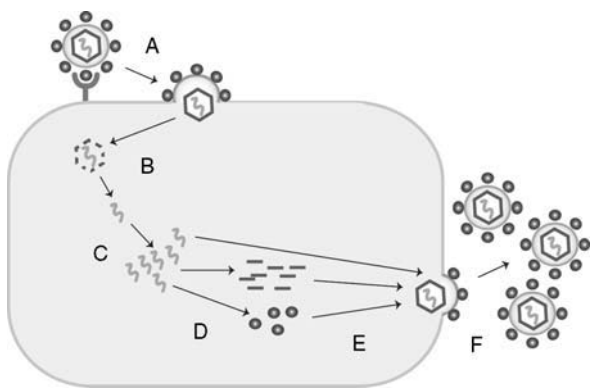
Definition

Viruses are small infectious agents composed of a nucleic acid genome (DNA or RNA) encased by structural proteins and in some cases a lipid ►envelope. They are the causative agents of a number of human infectious diseases, the most important for public health today being acquired immunodeficiency syndrome ►(AIDS), hepatitis, influenza, measles, and viruses causing diarrhoea (e.g., rotavirus). In addition, certain viruses contribute to the development of cancer. Antiviral drugs inhibit viral replication by specifically targeting viral enzymes or functions and are used to treat specific virus-associated diseases.

Mechanism of Action

Basic Principles

Viruses are obligatory intracellular parasites that can only replicate within an appropriate host cell. They rely on host cell-derived factors and mechanisms, and encode only few enzymes and other proteins of their own. Consequently, interfering with virus replication without inflicting damage to the host requires highly specific approaches and there is no general mechanism of action for virostatics. In contrast to antibacterial agents, where fundamental differences between prokaryotic and human cells (e.g., bacterial cell wall synthesis) can be exploited to inhibit a broad range of bacteria without significant toxicity to the patient, antiviral drugs are mostly highly selective for a specific virus or a limited number of related viruses. Furthermore, many human virus infections are acute and are rapidly controlled by the immune system. In these cases, the highest ►viremia – i.e., the time when the patient would benefit the most from causative antiviral treatment – usually precedes the clinical manifestation of symptoms and the diagnosis by serological methods. For these reasons, the number of currently available antiviral drugs is low compared to chemotherapeutics effective against other classes of infectious agents. Most antivirals have been developed to control chronic or recurrent virus infections, and in many cases antiviral treatment does not result in elimination of the virus but rather reduction of virus replication and alleviation of symptoms. In principle, antivirals can be targeted at any of the viral replication steps outlined in Fig. 1. (A) Binding: Viruses attach to their host cells via binding of a viral surface protein to one or several receptor molecule(s) on the plasma membrane. This interaction is highly specific and presents a very attractive target for antiviral intervention. The most advanced compounds in this class are the human immunodeficiency virus (HIV) coreceptor antagonists. These substances bind to and block the cellular chemokine receptors CCR5 or CXCR4, respectively, which are required in addition to the CD4 receptor to mediate HIV entry. The most promising results so far have been obtained with CCR5 antagonists, and one of these compounds (maraviroc) has recently been approved for treatment. (B) Entry of the virus into the cell and release of the viral genome (uncoating): The drug enfuvirtide targets the HIV entry process, by preventing the fusion of the virus with the plasma membrane. Although the uncoating process is not characterized in its entirety for most viruses, random screening has identified amantadine/rimantadine, which



Antiviral Drugs. Figure 1 Basic steps of viral replication: (A) binding, (B) entry, (C) genome replication, (D) gene expression, (E) assembly, (F) release.

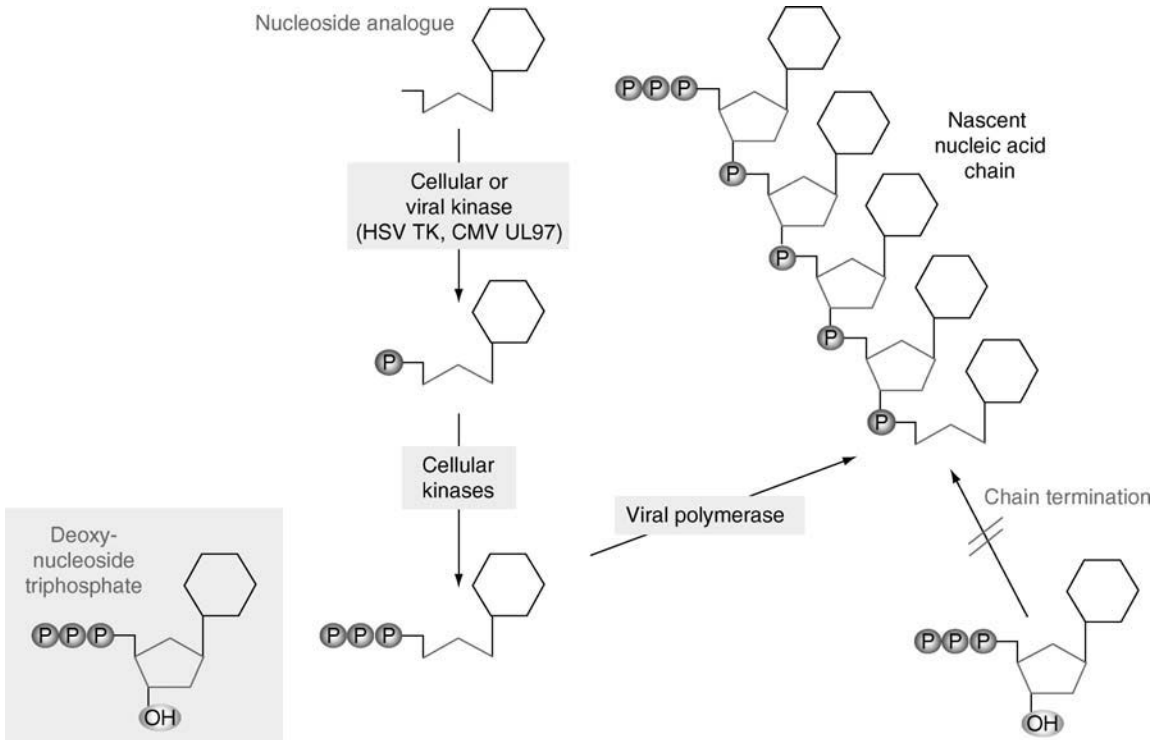
inhibit this step in influenza A replication. In addition, uncoating inhibitors specific for entero- and ►picornaviruses are under development. (C) Replication of the viral genome: Viruses contain either single- or double-stranded DNA or RNA genomes and employ a variety of replication strategies. Thus, there is no general mechanism or replication enzyme common to viruses. However, to accommodate for various replication strategies, many viruses encode their own polymerase(s), the biochemical and structural properties of which differ in some respects from those of the host cell polymerases. This can be exploited to specifically interfere with viral genome replication, and a number of inhibitors targeting viral replicases have been developed as antiviral drugs. (D) Expression of viral genes leads to the production of virus proteins: The basic machineries for transcription of virus genes and translation of mRNA into viral proteins largely rely on cellular factors and are therefore difficult to target. Many viruses encode proteins regulating transcription or mRNA transport and modification (e.g., the HIV Tat and Rev proteins), which are potential candidates for inhibition. However, drugs specifically interfering with viral gene expression have not yet been developed. (E) Virus assembly comprises transport of the virion components to the assembly site, formation of an ordered capsid structure and in some cases morphological maturation of the particle into a fully infectious state. Capsid stability depends on multiple interactions between viral structural proteins, and interfering with only a few interactions should suffice to disturb the ordered capsid architecture essential for infectivity. The subunit interfaces, unlike viral enzymes, do not have correlates in the host cell. This should allow the design of efficient and specific inhibitors, but to date insufficient understanding of the molecular processes involved and the lack of suitable assay systems has prevented the development of assembly inhibitors for antiviral therapy. However, highly effective antiviral drugs targeting the process of virion maturation (►protease inhibitors) have been developed against HIV. (F) Virus release from the producing cell is required for virus spread: It can be accomplished by cell lysis, exocytosis or, in the case of many enveloped viruses, by budding from the plasma membrane. Virus budding is a complex process involving a number of host cell-derived factors, and drugs targeting the budding process itself have not yet been developed. However, the recently introduced influenza neuraminidase inhibitors are effective by blocking the release and spread of influenza viruses.

Interferon (IFN) differs from *bona fide* antiviral drugs since it is a natural defense protein of the host organism and does not directly interfere with the viral replication steps. Interferons are small glycoproteins inducing immune modulatory and antiviral activities. They are secreted by lymphocytes, leukocytes and fibroblasts in response to foreign nucleic acids (dsRNA). IFNs are classified into three groups α, β, and γ, and the different classes are produced from different cell types. Recombinant IFN-α is used in the treatment of chronic hepatitis B and C.

Mechanisms of Action of Currently Used Antiviral Drugs

The initial steps in viral replication are attachment to and entry into the host cell. One drug targeting the fusion event of the virus with its host cell is approved for clinical use against HIV: The peptide derivative enfuvirtide binds to a helix in the viral envelope protein gp41, which is exposed upon binding of HIV to its cellular receptor. This binding of enfuvirtide blocks a conformational rearrangement of gp41 molecules which is required to mediate the fusion of viral and cellular lipid membranes. Similar compounds with improved potency and pharmacokinetics have been generated, but are not approved for clinical use. Several drugs inhibiting the binding of HIV by inhibiting the interaction of the virus with cellular receptor or coreceptors are currently under preclinical and clinical development. One HIV-1 co-receptor antagonist (maraviroc) has recently been approved. Available drugs acting at a step in influenza virus replication are the adamantane derivatives, amantadine and rimantadine. Amantadine has been in clinical use since the early 1970s, but its mechanism of action was elucidated only 20 years later. It blocks the M2 ion channel in the envelope of influenza A virus, thereby inhibiting virus uncoating. Adamantanes are not efficient against influenza B, which lacks the M2 protein. Entry inhibitors specific for entero- and rhinoviruses are drugs based on the substance pleconaril. It binds to a hydrophobic pocket on the surface of picornavirus capsids resulting in conformational changes of the ►capsid that interfere with the release of the viral RNA genome into the host cell. Pleconaril is tested in clinical trials but is currently not approved for therapy.

Polymerase inhibitors with different mechanisms of action account for the largest group of currently available antiviral drugs. The most important class are the chain terminating nucleoside analogues (Fig. 2). The prototype of this class is acyclovir (ACV) which is used against herpes simplex (HSV) and ►varicella zoster viruses (VZV). ACV is an acyclic analogue of the nucleoside thymidine, with carbon atoms C2 and C3 missing from the deoxyribose ring. Phosphorylation by HSV thymidine kinase (TK) inside an infected cell yields ACV-monophosphate, which is further converted into ACV-triphosphate by cellular enzymes and then serves as a substrate for the HSV polymerase. ACV incorporation into DNA results in chain termination due to the lack of the 3′OH group required for further elongation. Since both monophosphorylation and incorporation into DNA are preferably carried out by



Antiviral Drugs. Figure 2 Mechanism of action of chain-terminating nucleoside analogues.

viral enzymes, selectivity on two levels reduces toxicity to uninfected cells. This principle is also exploited by related drugs acting against herpesviruses (brivudin, famciclovir). Efficacy of phosphorylation and incorporation into DNA are not correlated: whereas penciclovir (oral prodrug: famciclovir) is a much better substrate for TK than ACV, penciclovir triphosphate is incorporated less efficiently by the viral polymerase than ACV-triphosphate. An optimal inhibitor should be a good substrate for both viral enzymes. Human cytomegalovirus (HCMV) lacks a *tk* gene and is relatively insensitive to ACV, but a protein encoded by gene *UL97* of HCMV is able to phosphorylate the nucleoside analogue ganciclovir (GCV). This analogue also efficiently interferes with cellular DNA polymerisation, and thus has a higher toxicity than ACV. Resistance against GCV can develop through mutations in either *UL97* (90%) or the viral polymerase gene. The alternative drug cidofovir is a cytosine phosphonate analogue, which only depends on cellular enzymes for its conversion into the active form. Thus, its efficacy is not affected by mutations in *UL97*. A direct inhibitor of viral polymerase, which does not require intracellular activation, is foscarnet (phosphonoformic acid). The chain terminating mechanism is shared by another important group of antivirals, the nucleosidic and nucleotidic ►reverse transcriptase (RT) inhibitors (NRTI and NtRTI), which inhibit the RT of HIV. A number of different NRTIs are available in different formulations, e.g., azidothymidine (AZT), ddI, ddC, and d4T. All of these are nucleoside analogues, in which the 3′OH group is missing or replaced by another functional group, e.g., an azido group in AZT. NRTI are also applied as prodrugs which have to be phosphorylated by cellular kinases into their active triphosphate form. NtRTi (Tenofovir, Adefovir) are monophosphorylated derivates. In this case the first- and often rate-limiting step of activtion is circumvented. Hepatitis B virus (HBV) (►Hepatitis), another important human pathogen, also encodes an RT, and several nucleoside/nucleotide analogues originally developed against HIV (e.g., lamivudine) are also active against HBV. The compounds lamivudine, adefovir and the HBV polymerase selective nucleoside derivative entecavir are available as anti-HBV drugs, and several other drugs from this class are expected to be approved for this use in the future (e.g., telbivudine, clevudine, emtricitabine). As obvious from this example, chain terminators are not exclusively selective for the particular viral polymerase targeted, since nucleoside analogues bind to the relatively conserved active site of polymerases. Thus, a certain degree of inhibition of cellular polymerases also has to be taken into account. Furthermore, soon after the introduction of AZT for the treatment of AIDS patients in 1987, it became apparent that although it was possible to lower the viral load by up to 80% through AZT monotherapy, the therapeutic success was limited by the rapid emergence of drug resistant virus. The

same unfortunately holds true for other NRTI, and resistance development against nucleoside inhibitors is also observed in the case of HBV and herpesviruses. The search for alternative antiHIV drugs led to the discovery of another class of polymerase inhibitors, the so-called non-NRTI (NNRTI). NNRTI in clinical use are nevirapine, delavirdine, and efavirenz. These polycyclic compounds do not mimic nucleosides, but act as allosteric inhibitors inducing conformational changes that lock the polymerase active site in an inactive conformation. Unlike NRTI, NNRTI are highly specific for the RT of HIV-1. The RNA-dependent-RNA polymerase NS5B of Hepatitis C virus (HCV) can not efficiently be targeted by the available polymerase inhibitors. However, novel nucleosidic as well as non-nucleosidic inhibitors of NS5B have been developed and are currently in the drug pipeline against HCV infection.

Another antiviral nucleoside derivative is the guanosine analogue ribavirin, which is active against certain RNA viruses (HCV, respiratory syncytial virus, lassavirus) and is administered in combination with IFN-α for treatment of chronic hepatitis C. Its mechanism of action is not completely elucidated. It is known that ribavirin-monophosphate inhibits cellular inosine monophosphate dehydrogenase and this leads to depletion of the cellular GTP pool, which can interfere with viral genome replication or mRNA capping. An antiviral effect of ribavirin mediated by lethal mutagenesis of the viral genome has also been proposed.

In the case of retroviruses like HIV, reverse transcription of the genome by the viral polymerase is followed by irreversible integration of the genetic information of the virus into the host cell genome. Antivirals which target this replication step are not yet approved for clinical use. However, several HIV integrase inhibitors yielded very promising results in clinical trials and the introduction of anti-HIV drugs belonging to this new class can be expected soon. Antiretroviral drugs interfering with a later replication step are ►protease (PR) inhibitors affecting HIV infectivity. Like other retroviruses, HI-virions are released from the cell as immature, noninfectious particles, in which the capsid is assembled from the structural polyprotein Gag. Concomitant with release, Gag is cleaved into its functional subdomains by the viral ►PR, leading to a structural rearrangement of the capsid essential for virion infectivity. Thus, PR inhibitors are effective anti-HIV drugs. Retroviral PR are aspartyl proteases, and inhibitors of members of this class (renin, pepsin) had been investigated prior the onset of the AIDS epidemic. These inhibitors are peptidomimetics resembling substrates in which the scissile peptide bond is replaced by a noncleavable structural analogue of the substrates ►transition state. Further modifications result in optimized selectivity, stability, and bioavailability of the compounds. Based on this concept and detailed structural and biochemical information about HIV PR, effective inhibitors were designed, several of which are used for the treatment of AIDS patients (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, tipranavir, atazanavir, darunavir; more HIV protease inhibitors can be expected to be available in the future). Promising compounds inhibiting the protease of HCV are currently in clinical development. The step of HIV virion maturation is also targeted by a novel compound, bevirimat. This inhibitor does not act by binding to the viral protease, but to its substrate, the viral structural polyprotein Gag. Thereby bevirimat interferes with a specific proteolytic processing step required for maturation of the viral capsid. This compound is currently tested in clinical trials.

The influenza virus inhibitors, zanamivir, and oseltamivir, act outside the cell after virus particles have been formed. The drugs have been designed to fit into the active site of the viral envelope enzyme neuraminidase, which is required to cleave sialic acid off the surface of the producing cells. When its activity is blocked, new virus particles stay attached to the cell surface through binding of the virus protein hemagglutinin to sialic acid and are prevented from spreading to other cells.

Clinical Use (Including Side Effects)

Only a limited spectrum of viral infections can be currently treated with antiviral drugs; otherwise prevention by vaccination (if possible) and hygiene measures are the only options. Influenza can be treated with amantadine or rimantadine. Both drugs are only effective against influenza A and cause significant side effects including dizziness, lightheadedness, insomnia and nausea. Thus, the newer neuraminidase inhibitors zanamivir (aerosol) and oseltamivir (oral), active against influenza A and B and with lower risk of side effects, are preferable. Since potential side effects include bronchospasm, these drugs are not recommended for patients with chronic pulmonary disease or asthma. In any case, treatment needs to be started within 36–48 h after the manifestation of the first symptoms and only alleviates the course of disease. Amantadine is also approved for prophylactic treatment of exposed persons with particularly high risk of influenza-associated complications. Infections with the herpesviruses ►HSV and ►VZV are treated with ACV (orally available prodrug: valaciclovir), famciclovir or brivudin (indicated for herpes zoster). Intraveneous treatment is indicated for herpes virus encephalitis, neonatal HSV infection, and HSV and VZV reactivation under immunosuppression. Herpes genitalis and herpes zoster are treated orally, and topical ACV treatment is used against herpes labialis. Alternative HSV and VZV

treatment is possible using idoxuridine or vidarabin. Foscarnet can be used against resistant viruses but has a higher risk of side effects (nephrotoxicity). Indications for the use of GCV or valganciclovir are ►CMV chorioretinitis in ►AIDS patients and CMV colitis. It is also used to prevent interstitial pneumonia in immunosuppressed patients. GCV is more toxic than ACV and causes neutropenia in about 40% of patients. Alternatively, CMV infection can be treated with cidofovir or foscarnet, which are also effective against GCV-resistant CMV but have an even higher toxicity. In pharmacologically immunosuppressed transplant recipients, GCV or ACV are administered to prevent CMV or HSV disease, respectively. An anti-CMV drug based on a new mechanism of action is Fomivirsen. It is an antisene RNA which inhibits the synthesis of a viral protein and is approved for intravitreal treatment of CMV retinitis.

A combination therapy of IFN-α ("pegylated", i.e., coupled to polyethylene glycol, which prevents its rapid clearance from the body) and ribavirin over 24–48 weeks is the most effective way to treat chronic hepatitis C. Therapeutic success depends on virus genotype and viral load, but overall this treatment eliminates the virus in about 60% of patients. A major side effect of this combination therapy is hemolytic anemia, attributed to ribavirin. Interferon monotherapy can be used as postexposition prophylaxis after accidental exposure to HCV to prevent chronification. Patients with chronic hepatitis B, characterized by elevated serum alanine aminotransferase levels and detectable HBe antigen for >6 months, are currently treated with pegylated IFN-α alone as the first choice. Clinical improvement can be accomplished in about 30–40% of treated patients. Fatigue, muscle aches, headache, nausea and diarrhea are common side effects of interferon. The most common serious side effect is depression. Treatment with antiviral nucleoside analogues against HBV is also efficient as monotherapy and is used mainly in cases with moderate elevation of ALT levels and when use of interferon is contraindicated (e.g., for patients with liver cirrhosis). Resistance development occurs frequently upon prolonged treatment with lamivudine; in this case, treatment can be switched to adefovir. The newest drug from this class, entecavir, appears to be superior to lamivudine with respect to both efficacy and resistance development. Combination of interferon with nucleoside analogues or combination of two nucleoside analogues has not been found to have advantageous effects. In the case of acute hepatitis B, antiviral treatment is not recommended.

The broadest spectrum of antiviral drugs is available against HIV. However, monotherapy with any of these drugs leads to rapid treatment failure due to selection and further evolution of resistant viruses. Since acquisition of resistance mutations requires virus replication, an efficient therapy regimen and patient compliance are paramount to minimize resistance development. Currently, AIDS patients in industrial countries are treated with a combination therapy known as HAART (highly active antiretroviral therapy) involving at least three different anti-HIV drugs from the classes mentioned above (NRTI, NNRTI, PRI, entry inhibitor). Treatment is currently indicated when the patient shows symptoms of AIDS or has a CD4+ cell count below 200/μl. Treatment decisions in other cases are complex and need to be made on an individual basis. Triple therapy is also recommended as prophylaxis following accidental exposure to the virus. In this case, it is crucial that treatment is started immediately (best within 1–2 h). The introduction of HAART led to a significant decrease in AIDS morbidity and mortality. However, severe side effects (neutropenia, neurological problems, lipodystrophy) can occur especially under prolonged therapy, and even under HAART, resistant and multiresistant viruses emerge and are transmitted. Thus, HAART requires selection of a suitable drug combination by a physician experienced in AIDS therapy, constant monitoring of viral load and individual adjustment in case of treatment failure or intolerable side effects. Successful treatment in the case of HIV infection does not mean eradication of the virus and therapy has to be continued over many years, probably life-long. For this reason, future goals do not only include the discovery of new anti-HIV drugs, but also the improvement of existing drugs in terms of galenics, side effects and possible combination formulations that make it easier to follow the therapy scheme. Finally, it should be noted that HAART is generally not available in developing countries, where most of the millions of HIV-infected people live.

High replication rates, error-prone polymerase as well as replication strategies favoring genetic recombination result in rapid virus evolution. Thus, the emergence of drug resistant variants is a general problem for antiviral therapy. Successful antiviral strategies will probably have to be combination therapies employing different drugs. Experiences indicate that cotreatment with two or even three drugs does not prevent viral resistance development, and broadening of the antiviral arsenal is a key issue. Future additions to this arsenal will result from different approaches: First, alternative and improved drugs based on the same mechanisms of action or on the same principles (e.g., inhibition of viral enzymes by substrate analogues) will be developed. Second, as the molecular understanding of viral biology increases and suitable assay systems for *in vitro* screening can be established, other steps of viral replication that are currently not accessible (assembly, transcriptional and posttranscriptional regulation or virus release) could become targets of chemotherapeutic intervention. Third, there are attempts to include new

and experimental therapeutic approaches, such as the specific shutdown of viral gene expression by antisense RNA or siRNA (small interfering RNA) or gene therapy, into antiviral strategies. As an alternative antiviral approach, all these strategies are currently considered mainly as potential inhibitors of HIV replication, but also discussed for development of novel treatments of chronic hepatitis B and C. While some encouraging results have been obtained in experimental settings and in small-scale clinical trials, many problems and questions regarding, e.g., specificity and durability of therapeutic effects, safe and targeted delivery of the antiviral principle, and viral escape by mutation remain to be solved before these can translate into clinical applications.

►Interferons
►Viral Proteases

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Anxiety

Anxiety is a normal reaction. Pathological anxiety interferes with daily-life activities and may be accompanied by autonomic symptoms (chest pain, dyspnoea and palpitations). Severe forms include phobic anxiety and panic disorder.

►Benzodiazepines

Anxiolytics

Anxiolytics are drugs used for the treatment of anxiety disorders. Apart from benzodiazpines, a frequently used anxiolytic is the $5HT1_A$ (serotonin) receptor agonist buspiron, which has no sedative, amnestic or muscle-relaxant side effects, but whose action takes about a week to develop. Furthermore, it is less efficaceous than the benzodiazepines. Buspiron's mechanism of action is not fully understood.

►Anxiety
►Benzodiazepines

APCs

Antigen-presenting Cells.

►Immune Defense

Apelins and the Apelin Receptor

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Synonyms

Apelin receptor; APJ receptor; Angiotensin II receptor-like 1

Definition

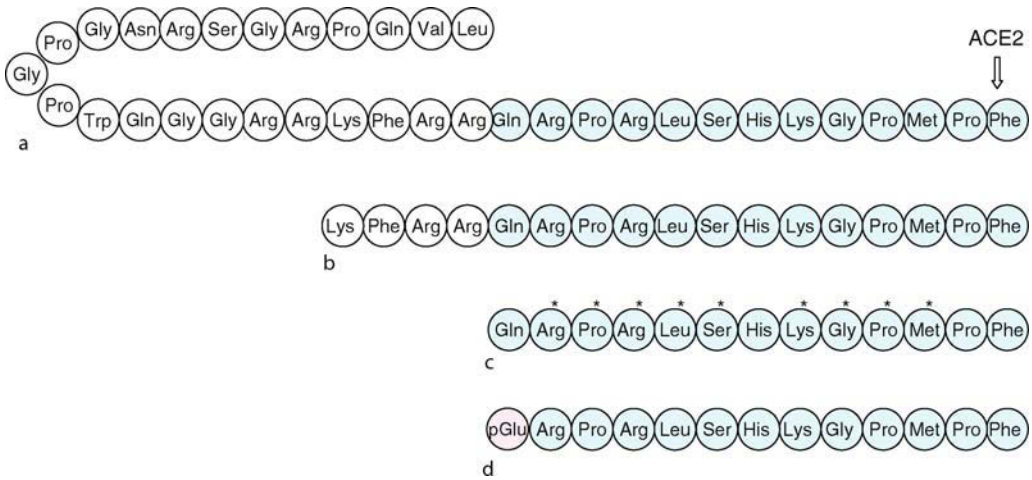
The family of apelin peptides is derived from a single gene, activate a single G-protein-coupled receptor and are substrates for the ►angiotensin converting enzyme-2 (ACE2). Apelins regulate cardiovascular function and fluid homeostasis. The apelin receptor also functions as a co-receptor for infection of CD4-positive cells by human immunodeficiency virus (►HIV).

Basic Characteristics

The human gene encoding the putative APJ receptor was identified in 1993 by O'Dowd and colleagues using a degenerate PCR strategy as having a high degree of sequence similarity with the gene encoding the angiotensin AT_1 receptor (54% in the transmembrane spanning regions) [1]. However, angiotensin II did not activate APJ and it was therefore designated as an ►'orphan' receptor. In 1988, apelin-36, (*APJ endogenous ligand*), a 36 amino acid peptide, was isolated from bovine stomach. Using the technique of reverse pharmacology, Tatemoto and colleagues found that the peptide bound and activated APJ receptors artificially expressed in cell lines [2]. APJ is now classified as the apelin receptor, in Family A of the superfamily of seven transmembrane spanning ►G-protein-coupled receptors.

Apelin Peptides, Synthesis and Metabolism

Cloning of corresponding human and bovine cDNA led to the identification of a gene encoding a 77 amino acid precursor peptide, preproapelin, in a number of species (human, bovine, rat and mouse). This precursor contains a number of basic amino acid pairs (Arg–Arg or Arg–Lys) thought to be proteolytic cleavage sites for endopeptidases, resulting in a family of C-terminal peptides of varying sizes that can all activate the receptor (Fig. 1). Thus far, no specific enzymatic pathway has been discovered which is responsible for cleaving preproapelin into shorter mature peptides. Apelin-36 is highly conserved between species; the last 22 residues of the C-terminus are identical in mammals (Fig. 2). The endogenous forms detected in tissues and biological fluids (usually by chromatographic separation or gel filtration followed by immunoassay) comprise predominantly apelin-36, apelin-17, apelin-13 and following ►posttranslational modification by enzymatic conversion of the N-terminal glutamate to pyroglutamate, [Pyr^{1}]apelin-13 (Fig. 1) [3, 4]. This modification is common to a number of biologically active peptides, which confers resistance to degradation by peptidases. The relative abundance of these molecular forms varies according to tissue examined. For example, in the rat, peptides of a size close to apelin-36 were the major components in the lung, testis, and uterus, but both apelin-13 and apelin-36 were detected in the mammary gland. Apelin-13 predominated in plasma and brain (hypothalamus) with apelin-17 being detected. Apelin-12 also activates apelin receptors in a number of preparations being more potent than aplelin-13 in reducing blood pressure in vivo in rats but apelin-12 has not been identified as an endogenous peptide. Apelin-16 has also been used in preparations. Apelin-11 and shorter sequences are devoid of binding activity to apelin receptors expressed in cells. Stepwise substitution of alanine for residues



Apelins and the Apelin Receptor. Figure 1 Amino acid sequences of (a) apelin-36 (b) apelin-17 (c) apelin-13 and (d), [Pyr1]apelin-13. Amino acids identical in all peptides are shown in blue. The posttranslational modification of the N-terminal glutamate in apelin-13 to pyroglutamate, [Pyr1] is shown in pink. ACE2 hydrolyses apelin-36 and apelin-13 resulting in the removal of the C-terminal residue. * indicates the residues found to be important for binding and activation of the apelin receptor by apelin-13].

Apelins and the Apelin Receptor. Figure 2 Sequence alignment of mammalian and amphibian apelin-36 amino-acid sequences. *Indicates residues conserved across all the species shown. Residues which differ from the human sequence are highlighted in red.

Arg-2, Pro-3, Arg-4, Leu-5, Ser-6, Lys-8 Gly-9, Pro-10 and Met-11 into apelin-13 reduced [^{125}I]-(Pyr^{1})apelin-13 binding to apelin receptors expressed in cell lines, suggesting they are important residues for receptor interaction. Structure activity studies using fragments of apelin-17 also identified Gln-1, Pro-12 and Phe-13 not essential for apelin binding.

Metabolism of apelin peptides in vivo is not yet clear. ACE2, the closest human homologue of angiotensin converting enzyme, cleaves the C-terminal Phe of apelin-13 with high catalytic efficiency in vitro (Fig. 1). The enzyme is expressed in ►endothelial cells particularly in the heart and kidney. ACE2 also hydrolyses apelin-36 as well as other biologically active peptides including angiotensin II to angiotensin 1–7 (a functional antagonist of angiotensin-II which acts predominantly as a vasodilator) suggesting the apelin peptides may be part of the enzymatic cascade of the ►renin–angiotensin–aldosterone system in cardiovascular regulation. ACE2 is also a receptor for severe acute respiratory syndrome coronavirus (SARS CoV) [4].

Distribution of Apelin mRNA and Peptides

Apelin mRNA (Table 1) is present in a range of peripheral rat tissues including stomach, intestine, heart, liver, kidney, testis, ovary and adipose tissues with highest levels in the lung and in particular the mammary gland (Table 1). Highest mRNA expression in the rat brain included the spinal cord, olfactory tubercle, hippocampus, hypothalamus, pineal gland, pituitary and the cerebral cortex. Apelin-like immunoreactivity was detected in rat tissues including white adipose tissue, epithelial cells of the oxyntic stomach mucosa as well as in endothelial cells of small arteries and veins in mesenterium, omentum, heart, lung, gastrointestinal tract, spleen, pancreas and liver. In rat brain, immunoreactivity is present in neurones of the pons, medulla oblongata and arcuate nucleus and also in the supraoptic and paraventricular nucleus of the hypothalamus, where apelin positive neurones also expressed vasopressin mRNA. In humans, apelin-like immunoreactivity was detected in endocardial endothelial cells lining the atria and ventricles of the heart and endothelial

Apelins and the Apelin Receptor. Table 1 Distribution of mRNA encoding apelin peptides and receptor [4]

	Apelin receptor			Apelin peptides		
	Rat	Mouse	Human	Rat	Mouse	Human
Brain	++	+	+++	+	+++	++
Cerebellum	+		+	+		+
Pituitary	+		+	+		++
Spinal cord	+++	++	+	++		++
Adrenal gland	+			+		
Thyroid	++					
Spleen	–	+	+++	–	+	
Thymus		+	+		–	
Heart	++	+++	+	++	++	+
Endothelial cells	++					
Lung	+++	++	++	+++	++	+
Stomach	+		+	+		–
Small intestine	+		++	+		–
Large intestine	+		++	+		
Liver	+	+		–	–	–
Pancreas	–		+	–		+
Kidney	+	+	+	+	+	+
Testis	+	+	+	+	++	+
Prostate			+			+
Ovary	+	+	+	+	+	
Uterus	+	+	+	+	–	–
Placenta	++		++			+++
Mammary gland	+			++		
Skeletal muscle	++	++	+	+	+	–
Adipose tissue	++			+		
Cartilage	++					

cells lining large conduit vessels, small arteries and veins (<500 μm diameter) from lung, kidney and adrenal gland. Within endothelial cells, apelin-like immunoreactivity is present in secretory vesicles of the constitutive secretory pathway but not Weibel-Palade bodies of the regulated stimulated pathway, suggesting the peptide may be released from the former [4].

Apelin-like immunoreactivity has been quantified in tissues using antisera that mainly cross reacts with all apelin peptides. Levels in human atria were 650 pg/mg with lower but detectable levels in the ventricle (3 pg/mg). Apelin-like immunoreactivity in human plasma measured following extraction and radioimmunoassay ranges from 24–89 pg/ml, levels comparable to other locally acting endothelium derived peptides. This may reflect overspill from the vascular and endocardial endothelial cells, consistent with the proposed role as a locally released mediator. Whether there is any contribution from other sources such as epithelial cells of the gut, is unclear. Apelin levels rise in early heart failure and fall with severe disease although other studies reported no change in patients with dilated cardiomyopathy. Apelin is expressed and secreted by human and mouse adipocytes; with plasma apelin levels increasing with obesity and correlating with body mass index, suggesting a role as an adipokine.

In agreement with abundant mRNA in mammary gland, high levels of apelin are present in bovine colostrum; oral intake of apelin might modulate immune responses in neonates [4].

Distribution and Characterisation of Apelin Receptor

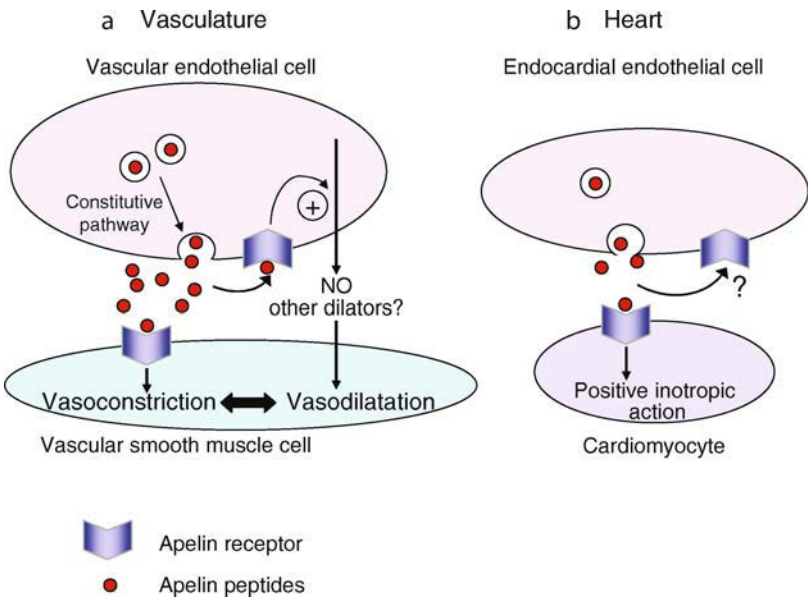
In the rat (Table 1), apelin receptor mRNA is widely distributed in almost all peripheral tissues (consistent with a vascular expression) with highest levels in lung and heart and lower levels in kidney, pituitary gland, ovary and skeletal muscle. In the rat CNS, apelin receptor is expressed in brain including the cerebral cortex, hypothalamus (particularly in neurones of the paraventricular and supraoptic nucleus), hippocampus, thalamus, striatum and pituitary gland. In humans, receptor mRNA is detectable in many peripheral tissues, also consistent with a vascular expression including spleen, thymus, prostate, testis, ovary, intestine. In human brain, mRNA is present in neurones, oligodendrocytes and astrocytes, but was not detected in macrophages or microglia. Apelin receptor-like immunoreactivity is expressed in endothelial cells lining small intramyocardial, renal, pulmonary and adrenal vessels, small coronary arteries, large conduit vessels and in endocardial endothelial cells. Lower levels of receptor are also present in cardiomyocytes and vascular smooth muscle cells of blood vessels from different vascular beds [4].

[^{125}I]-(Pyr^{1})apelin-13 used to characterise native apelin receptors in human heart (atria and ventricles) bound with a single high affinity (►K_D = 0.4 nM), comparable to other vasoactive peptides. ►Hill slopes were close to unity, with no evidence for a biphasic curve that might indicate further receptor sub-types. Binding was time dependent with an association rate constant (K_{obs}) of 0.115 min^{-1} and dissociation rate constant of 0.01 min^{-1}. Receptor density in human heart was comparatively low, ~4 fmol mg^{-1} protein. In rat tissues, [^{125}I]-(Pyr^{1})apelin-13 bound in the CNS (brain) and periphery (lung) with similar high affinities (K_D = 0.6 nM) [4]. Disruption of the gene encoding the apelin receptor in a ***knock-out*** mouse abolished, as expected, all apelin responses, supporting the concept that apelins mediate their actions via a single receptor.

Apelin receptors activate several signalling pathways including coupling through inhibitory ***G-proteins*** (G_i) and Ras-independent activation of extracellular-regulated kinases (ERKs) via protein kinase C (PKC). The apelin receptor is one of number of G-protein-coupled receptors that can act as an alternative co-receptor for entry into cells of HIV and simian immunodeficiency virus (SIV) strains in human U87 cells expressing CD4 in vitro. Apelin peptides blocks entry of HIV but display different potencies, with apelin-36 being more effective than shorter sequences [3].

Role in the Cardiovascular System

In the cardiovascular system, apelins act directly on smooth muscle cells of the vasculature to cause ►vasoconstriction, on apelin receptors on the endothelium to indirectly release ►vasodilators to cause relaxation and on myocyte receptors to increase cardiac contractility (Fig. 3). In animals, short apelin peptides play a role in the regulation of cardiovascular homeostasis. Apelin-13 (~3 μg/kg) transiently lowered ►blood pressure by ~10 mm Hg for 3–4 min in anaesthetised rats in vivo following intravenous injection in a nitric oxide dependent manner. This action was more pronounced in spontaneously hypertensive rats, with apelin-13 (15 μg/kg) lowering blood pressure by about 60%. Concomitant administration of apelin-13(F13A), a synthetic peptide with Ala substituted at residue 13, blocked hypotensive effects of apelin-13 suggesting that apelin-13(F13A) behaved as a functional antagonist [5]. Although apelin receptor knockout mice displayed no significant changes in baseline blood pressure compared to wild type controls, infusion of apelin transiently decreased the blood pressure of wild-type mice, which was abolished in the knock-out. Apelin receptor deficient mice had an increased pressor response to angiotensin II, and the baseline blood pressure of double mutant mice homozygous for both apelin and angiotensin-type 1a receptor was significantly elevated compared with the control. Following removal of the endothelium, [Pyr^{1}] apelin-13 apelin was a potent vasoconstrictor in human vessels (saphenous vein) in vitro, by a direct action on



Apelins and the Apelin Receptor. Figure 3 Scheme illustrating the hypothesised mechanisms of control of human (a) vascular tone and (b) cardiac contractility by apelin peptides (•). In the vasculature, apelins (released via the small vesicles of the constitutive pathway) may act directly to activate apelin receptors on the underlying smooth muscle to produce vasoconstriction. This response may be modified by apelin peptides feeding back onto apelin receptors on endothelial cells to stimulate the release of dilators, such as nitric oxide. In heart, apelin peptides, released from endocardial endothelial cells, activate apelin receptors on cardiomyocytes to elicit positive inotropic actions.

vascular smooth muscle, suggesting in pathophysiological conditions of endothelial cell dysfunction, vasoconstriction may be the predominant action. A potential role for the apelin receptor system in the pathogenesis of high blood pressure has been proposed

Apelin-16 is a potent positive inotropic agent in rats and in animal models treatment with apelin improves heart function. In isolated perfused rat hearts, infusion of apelin-16 (0.01–10 nM) induced a dose-dependent positive inotropic effect with an EC_{50} value of 33 pM. Continuous infusion of apelin-16 at a rate of 0.01 μg/min for 20 min significantly increased contractility in rats in heart failure, 6 weeks after left anterior descending coronary artery ligation. These animal studies suggest apelin may have use as an acute inotropic agent in patients with ischemic heart failure. In humans, mRNA microarrays identified the apelin receptor gene as the only one of 12,000 genes tested that showed significant increase in expression levels in heart failure after implantation of a ventricular assist device, suggesting that apelin may play a compensatory role in the early stages of this condition. Apelin mRNA is increased in human myocardium in heart failure and mRNA encoding the receptor is significantly decreased.

Fluid Homeostasis

Apelin receptor and peptides are co-expressed in two nuclei of the hypothalamus, the supraopticus and paraventricular nucleus which play a major role in the physiological regulation of fluid homeostasis by production of ▶vasopressin (ADH). Axonal transport translocates ADH to the posterior pituitary, where it is released in response to osmotic stimuli sensed by hypothalamic neurones in order to regulate water and sodium uptake in the kidney as well as vascular tone. Messenger RNA encoding apelin co-localised in neurones expressing ADH mRNA, suggesting a role in the regulation of fluid homeostasis. Circulating plasma ADH levels decreased (−47%) after intracerebroventricular administration of apelin-13. In mice deprived of water, intracerebroventricular administration of apelin-13 significantly reduced the water intake in the initial 30 min after re-exposure to drinking water, and apelin-17 lowered circulating ADH levels (−43%). Increased water intake was also observed in the first 60 min after intracerebroventricular administration of apelin-13 in rats [3, 4].

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Apical Membrane

Refers to the aspect of epithelial cells facing the mucosal (as opposed to serosal) side of the cells.

►Na^+/H^+-Exchangers

APJ Receptor

►Apelins

Apolipoproteins

Proteins embedded in the shell of lipoproteins. They serve as scaffold for assembly of the lipoprotein particle in the endoplasmic reticulum. In addition, they control metabolism of lipoproteins in the circulation by interaction with enzymes such as lipases. Finally, apolipoproteins determine cellular uptake of the particles by interaction with specific lipoprotein receptors expressed on the surface of target cells.

►Low-Density Lipoprotein Receptor Gene Family
►Lipoprotein Metabolism Lipid Transfer Protein

Apoptosis

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Synonyms

Programmed cell death

Definition

Apoptosis is the most common form of programmed cell death. Apoptosis is characterized morphologically by cell shrinkage, membrane blebbing (zeiosis), ►DNA fragmentation, chromatin condensation, and nuclear fragmentation [1]. Biochemically, these morphological alterations in most cases require the activation of enzymes called caspases. Apoptosis plays a role during normal development where it facilitates the shaping of organs and their plasticity, e.g. in the developing central nervous system [2]. However, apoptosis also takes place in most tissues throughout life. Older or damaged cells undergo apoptosis and they are replaced by new cells which are constantly formed by proliferation of precursor cells. Thus, there is equilibrium between apoptosis and proliferation in the different tissues. A dysbalance in this equilibrium, e.g. by external stress or by a mutation or an epigenetic alteration of an apoptosis pathway constituent which hampers normal execution of the cell death program, is often causative for a disease or a pathological condition. Hence, the interest in understanding the underlying biochemical mechanisms of cell death induction by apoptosis has increased immensely ever since the importance of apoptosis as one part of this equation has been realized.

Basic Mechanisms

Two main apoptosis pathway exist. The first one is the death receptor pathway which can be triggered from the outside of the cell when a so-called death ligand which belongs to the ►tumor necrosis factor (TNF) family of cytokines crosslinks its cognate receptor on the surface of a cell. Besides TNF also TRAIL and the CD95 (Fas/APO-1) ligand (CD95L) can directly induce apoptosis and form part of the death ligand subfamily. Cross-linking of a death receptor by its ligand or by an agonistic antibody to the receptor results in the formation of the death-inducing signaling complex (DISC). The formation of this multi-protein complex leads to the activation of the initiator caspases 8 and 10 which both form part of this signaling complex.

The other pathway is triggered when so-called BH3-only proteins interact with other members of the Bcl-2 family on mitochondria. The bcl-2 homology domain 3 (BH3 domain) of the BH3-only proteins binds to other Bcl-2 family members thereby influencing their conformation. This interaction facilitates the release of cytochrome C and other mitochondrial proteins from the intermembrane space of mitochondria. Despite much effort the exact biochemical mechanism which governs this release is not yet fully understood. The release of cytochrome C facilitates the formation of the apoptosome, the second platform for apoptosis initiation besides the DISC. At the apoptosome which is also a multi-protein complex the initiator caspase-9 is activated. At this point the two pathways converge.

Active caspases 8, 9 and 10 can convert caspase-3, the most abundant effector caspase from its pro-form to its active cleaved form. Cleavage of a number of different substrates by caspase-3 and also by caspase-6 and -7 which are two other executioner caspases besides caspase-3 then results in the typical morphology which is characteristic of apoptosis. Yet, the activation of caspase-3 and also of caspase-9 can be counteracted by IAPs, so called inhibitor of apoptosis proteins. However, concomitantly with cytochrome C also other proteins are released from mitochondria, including Smac/DIABLO. Smac/DIABLO and potentially other factors can interact with IAPs and thereby neutralize their caspase-inhibitory activity. This releases the breaks on the cell death program and allows apoptosis to ensue.

There is also crosstalk between the two pathways above the mitochondria. The BH3-only protein BID is cleaved by caspase-8 and -10 which yields truncated BID (tBID), the active pro-apoptotic fragment of BID. Thereby, even in cells in which the direct apoptosis pathway which result from death receptor crosslinking is blocked, e.g. by high expression levels of the x-linked IAP (XIAP), the activity of tBID on mitochondria can result in the activation of caspase-3 because the IAP-imposed block on full caspase-3 activation and caspase-9 activity at the apoptosome is released by Smac/DIABLO.

Pharmacological Intervention

Apoptosis Induction

Cancer cells often have a survival advantage over normal cells which is usually established during the transformation process. This is often facilitated by loss of pro-apoptotic factors or the acquisition of anti-apoptotic proteins. One such acquisition led to the identification of ►Bcl-2. High expression of anti-apoptotic proteins like Bcl-2 interferes with release of cytochrome C and Smac/DIABLO by BID or other BH3-only proteins, thus hampering the activation of caspase-9 and caspase-3, thereby interfering with the induction of apoptosis. Intriguingly, however, the cancer cells are often dependent on these changes acquired during transformation. Thus, if it were possible to interfere with the activity of a given factor the transformed cells have become dependent on this would be a very suitable therapeutic target. It now appears that in many types of cancer IAPs and the anti-apoptotic ►Bcl-2 family members fulfill this criterion. In addition, the triggering of apoptosis from the outside of the cells by TRAIL receptor agonists has shown to be effective in killing tumor cells and to be non-toxic. Importantly, also in combination with conventional chemotherapeutics, novel targeted therapeutics, or radiation therapy these drugs which specifically target TRAIL-R1 (DR4) and/or TRAIL-R2 (DR5) have so far shown no or only very few dose-limiting toxicities in a number of phase I and II clinical trials. Taken together, there are three main protein families which have been identified in the apoptosis pathways as cancer drug targets. i.e. the apoptosis-inducing TRAIL receptors [3], the anti-apoptotic ►Bcl-2 family members [4] and the IAPs [5]. As a consequence there are three novel classes of cancer drugs: TRAIL receptor agonists, BH3 mimetics and IAP antagonists. These three classes of novel drugs have recently entered clinical trials and it will be interesting to see how these trials will develop, especially considering combinatorial therapies.

Besides direct apoptosis effectors, there are a number of other drugs which influence the above explained apoptosis pathways more indirectly. This class of drugs includes molecules which inhibit survival pathways like e.g. the Ras/Raf kinase pathway, the NF-κB pathway and many others. Also inhibitors of survival cytokines which are sometimes produced by cancer cells in an autocrine fashion can render cells susceptible to apoptosis and, hence, effective cancer therapy. These include, but are not limited to, ligands for dependence receptors and cytokines like e.g. interleukin-4.

The combinations of conventional cancer therapeutics with novel targeted drugs, whether they directly or indirectly target the cell's apoptosis pathways will open a plethora of novel intervention strategies for cancer treatment in the future. The results of the preclinical work as well as the first results from clinical trials are very encouraging, perhaps promising. It seems that some of the new combinations which are now possible may finally allow the breaking of tolerance of cancer to most currently used therapies. The results of clinical studies with these new multi-target therapeutic strategies will teach us whether we will have managed to outmaneuver the cancer by depriving it of its capacity to generate a viable therapy-resistant variant.

Apoptosis Prevention

Often cells undergo apoptosis at a stage when they were not yet supposed to die, at least under normal physiological conditions. One such condition is reached when cells are deprived of oxygen. This is the case in stroke and acute myocardial infarction. At the core of the lesion the cells die by ►necrosis in both, the ischemic part of the heart and the oxygen-deprived part of the brain, as a consequence of complete deprivation of oxygen for too long a time period. Yet, in the penmubra, i.e. the region surrounding this central necrotic lesion, the cells are only deprived of oxygen for a limited period of time. However, if no intervention takes place, many of the cells in the penumbra die within the next days by apoptosis. Therefore pharmacological intervention has aimed at blocking the death of these cells by interfering with apoptosis. For quite some time caspase inhibitors were thought to be drugs

which are potentially useful in these diseases because of their apoptosis-inhibitory capacity *in vitro*. Currently caspase inhibitors are still being evaluated in the context of acute liver failure and first results are encouraging. However, it was discovered that in order to efficiently block cell death with caspase inhibitors it is necessary to very efficiently block caspase activity (i.e. by more than 98%) at any given time in a cell which has been triggered to undergo apoptosis. Since this is very difficult to achieve *in vivo* the attention has shifted towards inhibitors of the initial triggers of the cascade. Death ligands are one class of such triggers. Inhibition of death ligands can be achieved already outside the cell, i.e. before the apoptosis signal is transmitted to the inside of the cell. Hence, interference with death ligand binding to its cognate receptor(s) should allow for efficient inhibition of apoptosis. Consequently, in cases in which a given death receptor-ligand system has been implicated in a specific pathological conditions it should be possible to intervene therapeutically with a blocker of a given death ligand. In the case of TNF the concept of using TNF blockers, i.e. soluble TNF receptor Fc fusion proteins or anti-TNF antibodies, has been demonstrated to be very efficient in blocking the action of TNF in diseases such as rheumatoid arthritis and psoriasis, amongst many other diseases. Also for CD95L this concept has recently been shown to be very promising: in animal models of e.g. myocardial infarction, stroke, spinal cord injury, acute liver failure and graft-versus-host disease as inhibition of CD95L by CD95 blockers, i.e. soluble CD95 receptor Fc fusion proteins or antibodies to CD95L, has resulted in a therapeutic effect. Consequently, efforts are under way to test whether inhibition of CD95L can interfere with the tissue damage in these different disease situations in humans.

►Monoamine Oxidases
►Caspases
►Matrix Metalloproteinases
►Cell Cycle Control
►Insulin Receptor
►MAP Kinase Cascades
►Neurodegeneration
►Cancer, Molecular Mechanism of Therapy

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Apoptotic Executioner Caspases

Apoptotic executioner caspases (caspase-3, -6, -7) constitute a subgroup of the caspase family. These proteases are the workhorses of the apoptotic process as they are responsible for cleaving many down-stream substrates important for cellular morphology, organelle homeostasis, cell cycle arrest, and regulation of transcription and translation.

►Caspases
►Apoptosis

Apoptotic Initiator Caspases

Apoptotic initiator caspases (caspase-2, -8, -9 and -10) constitute a subgroup of the caspase family. These caspases are the first to become proteolytically active in the apoptotic cascade. Their activation takes place in multiprotein complexes initiated by pro-apoptotic stimuli, such as TNFα, α-Fas, staurosporine. Once activated, they can process their substrates, which include the apoptotic executioner caspases.

►Caspases
►Apoptosis

Appetite

Mood and hedonic value associated with feeding, food intake, foraging, consummatory behaviors, and craving in addiction; complex regulation by food entrainable oscillators in the brain and periphery, neuropeptides (including orexins) and biogenic amines.

►Orexins
►Appetite Control
►Anti-obesity Drugs

A

Appetite Control

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Synonyms

Control of food intake; Regulation of ingestive behaviour

Definition

Appetite control is a complex function of the brain that regulates feeding behaviour. This function integrates cognitive and emotional factors with a complex array of signals from the gastrointestinal tract and from adipose tissue.

Basic Mechanisms

Feeding behaviour is subjected to both short-term regulation during a single-meal and long-term regulation related to the maintenance of body weight and fat content. As a complex function of the brain, ingestive behaviour is controlled by psychological and cognitive factors such as sociocultural context (e.g. eating habits), experience (sensory preferences) or emotional status (mood). Appetite control also integrates information about the status of peripheral organs, particularly the gastrointestinal tract and adipose tissue [1]. Two main groups of signals can be distinguished: (i) satiety signals secreted from gastrointestinal organs, and (ii) adiposity signals that are proportionate to body fat stores. A key factor of this control system is that energy intake is primarily controlled by adjustment of meal size rather than meal onset, allowing the organism to initiate meals at times that are convenient and to adapt eating patterns to individual constraints (food availability) and activities (circadian rhythm).

Satiety Signals

Satiety-inducing signals are conveyed to the brain by afferent nerve fibers that are sensitive to mechanical or chemical stimulation of the stomach and small intestine during food ingestion. In addition, humoral signals such as ►cholecystokinin (CCK) are released upon nutrient stimulation of neuroendocrine cells located in the gastrointestinal wall. These satiety signals converge in the nucleus tractus solitarii in the brainstem and induce meal termination in the absence of hypothalamic control, as demonstrated in decerebrated rats. ►CCK is the paradigmatic humoral satiety signal, and its action has been studied extensively in multiple species including human. Exogenous administration of ►CCK dose-dependently reduces meal size. This effect is synergistically enhanced by other factors that limit meal size, such as gastric distension. Specific CCK-A receptor antagonists stimulate food intake in rats, indicating that endogenous ►CCK contributes to the termination of meals. However, repeated administration of ►CCK before each meal does not reduce caloric intake of free-feeding mice or rats, because the animals compensate the reduced meal size by increasing the number of meals.

Adiposity Signals

Insulin as a Satiety/Adiposity Signal

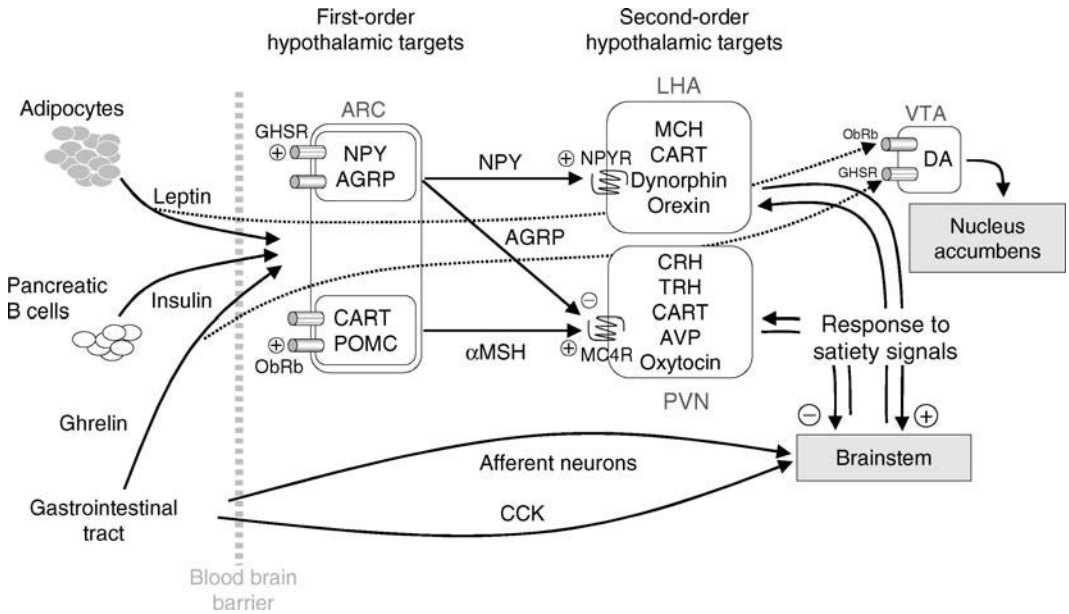
The first hormonal signal found to comply with the characteristics of both a satiety and an adiposity signal was insulin [1]. Insulin levels reflect substrate (carbohydrate) intake and stores, as they rise with blood glucose levels and fall with starvation. In addition, they may reflect the size of adipose stores, because a fatter person secretes more insulin than a lean individual in response to a given increase of blood glucose. This increased insulin secretion in obesity can be explained by the reduced insulin sensitivity of liver, muscle, and adipose tissue. Insulin is known to enter the brain, and direct administration of insulin to the brain reduces food intake. The adipostatic role of insulin is supported by the observation that mutant mice lacking the neuronal insulin receptor (NIRKO mice) develop obesity.

Leptin as an Adiposity Signal

►Leptin is a cytokine produced and secreted by adipose tissue in proportion to the body fat content [3]. Mice and humans lacking leptin or its receptor develop a severe hyperphagia and a dramatic degree of obesity which is considerably more pronounced than that of the NIRKO mouse. Thus, leptin is the key adiposity signal in rodents and humans. Leptin secretion appears to reflect the metabolic status of the adipocyte rather than the sheer size of triglyceride deposits, and leptin levels may transiently be dissociated from total body fat. Nonetheless, over the course of a day with unrestricted food supply, plasma leptin levels reliably reflect the amount of total body fat. Local administration of leptin into the brain results in reduced food intake. The vast majority of patients with obesity have elevated serum levels of leptin. Thus, it is believed that the polygenic obesity is due to leptin resistance rather than to inadequate leptin secretion, or to a reduced blood/brain transport of the cytokine.

Appetite-Regulating Pathways in the Arcuate Nucleus of the Hypothalamus

Two distinct populations of neurons in the arcuate nucleus have been identified as the most relevant target cells of leptin (Fig. 1, [2, 4]). Leptin inhibits expression



Appetite Control. Figure 1 Diagram of pathways integrating appetite control. Satiety is the net output from brainstem centres that leads to the termination of an individual meal. Satiety is primarily determined by neural and humoral inputs from the gastrointestinal tract (satiety signals). Response to satiety signals is modulated by descending anabolic or catabolic pathways originating in the hypothalamus. Appetite regulating hormones are released in proportion to total body fat (leptin), glucose levels (insulin), or changes in food intake (ghrelin). Leptin and insulin stimulate secretion of anorexigenic peptides (αMSH, CART), and inhibit expression of orexigenic ones (NPY, AgRP) in the arcuate nucleus (ARC), whereas ghrelin exerts opposite effects. Secondary target neurons in the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) integrate signals from neurons in the ARC, and connect them with satiety centers in the brainstem. In addition, recent evidence shows that afferent signals such as leptin and ghrelin also directly target brain centres known to play a key role in reward and addiction. Areas where such neurocircuitry is located include the ventral tegmental area (VTA) or the nucleus accumbens and appear to control mostly the hedonic components of feeding behaviour.

of the orexigenic peptides NPY (►neuropeptide Y) and AgRP (►agouti-related protein) in one subset of neurons, and stimulates production of the anorexigenic peptides αMSH ►(α-melanocyte-stimulating hormone) and ►CART (cocaine- and amphetamine-regulated transcript) in the other. Insulin receptors are also highly concentrated in the arcuate nucleus, and insulin appears to elicit similar changes in these neuropeptides as leptin.

The Melanocortin Signalling System

Considerable evidence indicates that the molecules of the melanocortin system are key mediators of the response to leptin. AgRP and αMSH are antagonistic ligands for a common receptor, the melanocortin-4 receptor (MC4R). αMSH is an anorexigenic neuropeptide that activates MC4R and thereby reduces appetite, whereas AgRP is an orexigen that acts as an endogenous antagonist of the receptor and suppresses its activation by αMSH. The critical role of the melanocortin system in appetite regulation is supported by the effects of spontaneous and experimental mutations of AgRP, αMSH, and MC4R in mice. Moreover, patients with complete loss of ►proopiomelanocortin (POMC), the precursor molecule of αMSH, develop severe hyperphagia and overweight, and 4–5% of all cases of severe human obesity appear to be due to mutations in the *MC4R* gene.

Role of NPY/AGRP Neurons

NPY has long been known to be a potent orexigen when directly injected into the hypothalamus. Hyperphagia of the leptin-deficient ob/ob mice is attenuated by knockout of NPY, supporting the role of NPY as a downstream effector of leptin. The effects of NPY on appetite regulation appears to be mediated by different receptor subtypes (NPY1R, NPY2R, and NPY5R). However, the neuropeptide is not an indispensable transmitter of adiposity signals, since lean mice which lack NPY show a normal feeding behaviour. On the other hand, it has recently been shown that the NPY/AGRP neurons play an essential role for basal orexigenic drive. If these neurons are completely ablated from the arcuate nucleus, adult mice stop feeding almost completely and loose substnantial amounts of body fat. In conclusion, the NPY/AGRP neuron likely plays an essential role in the control of feeding behaviour, but their function can not be easily explained solely based on the expression of the neuropeptides NPY and AGRP.

Second-Order Hypothalamic Targets in Adiposity Signalling

Lesions of the ►lateral hypothalamic area (LHA) cause anorexia, whereas ablation of the ►paraventricular nucleus (PVN) cause a hyperphagic obesity syndrome. Consistent with these results, LHA neurons express the orexigenic neuropeptides MCH and ►orexin. PVN neurons produce several neuropeptides that are anorexigenic when administered directly into the brain (CRH, TRH, oxytocin), in addition to their better known roles as endocrine regulators. LHA and PVN receive rich inputs from axons of NPY/AgRP and αMSH/CART-producing neurons in the arcuate nucleus.

Other Hormones, Peptides, and Neurotransmitters Involved in Appetite Control

Many other peptides including galanin, ghrelin, and ►glucagon-like peptide-1 and 2 (GLP-1/GLP-2) have been described to participate in appetite control (Table 1). In addition, the neurotransmitters norepinephrine, dopamine, and serotonin are known to be involved in appetite regulation. The role of the monoamines in energy homeostasis is illustrated by effects of drugs (see below). Agonists of α_1 adrenoceptors, 5-HT_{2C} serotonin receptors, and dopamin receptors (D1 and/or D2) suppress appetite. However, the relevant neural circuits that use these transmitters are not very well defined. A control system mediating appetite-stimulating effects is the cannabinoid signalling. Recently, endocannabinoids have been added to the list of signals that act downstream of leptin. Leptin reduces levels of the endocannabinoid anandamide in the hypothalamus of normal rats, and mice that lack the cannabinoid receptor 1 (CB1) showed reduced food intake under conditions of low leptin levels (after fasting).

Pharmacological Intervention

Appetite-Suppressing Drugs

The increasing prevalence of obesity and its consequences has stimulated the search for appetite-suppressing drugs as anti-obesity agents [5]. Therapy based on nutritional and behavioural counselling produces almost always only a temporary weight loss. The existing drugs that target adrenergic and serotonergic pathways (e.g. metamphetamine, phentermine, fenfluramine, sibutramine) have a negative reputation of toxicity and limited efficacy. The recent insights in appetite control as outlined above have provided new candidate targets for the search of appetite suppressing drugs. Since obesity is usually a chronic disorder which requires life-long therapy, anti-obesity drugs need to meet high safety standards.

β-Phenylethylamine Drugs

The appetite-suppressing effect of β-phenylethylamine drugs is either related to their sympathomimetic effect (metamphetamine, phentermine, diethylpropion), to increased serotonergic transmission (fenfluramine), or both (sibutramine). Compared with metamphetamine, phentermine and diethylpropion appear to have little abuse potential but exhibit the typical side effects of sympathomimetic drugs (insomnia, hypertension). Use of fenfluramine was terminated after a high incidence of valvular heart disease was reported in patients treated with a combination of phentermine and fenfluramine. The same rationale to combine serotonergic and noradrenergic action underlies the therapy with sibutramine, a serotonin-norepinephrine reuptake inhibitor. Weight reduction by 5–10% was achieved over 24 weeks of treatment with sibutramine in doses from 10–15 mg/d. Weight was regained when the drug was stopped, indicating that a continuous therapy would be necessary to achieve the useful, but limited therapeutic effect. This general limitation is likely to apply for any novel drug that targets central noradrenergic and/or serotonergic pathways, e.g. agonists of the 5-HT_{2C} serotonin receptor.

Cannabinoid-1 Receptor Antagonists

These compounds are a novel class of anti-obesity agents which block the cannabinoid-1 (CB-1) receptor. Rimonabant, the only CB-1 receptor antagonist currently on the market, produces a weight loss of 3.4 or 6.6 kg with daily doses of 5 and 20 mg, respectively. In addition, the agent reduces plasma triglycerides and increases HDL cholesterol. The beneficial effects of rimonabant are probably mediated by both central and peripheral CB-1 receptors. Adverse effects were nausea, diarrhoea, dizziness, anxiety and depression. A second CB-1 receptor antagonist presently being tested in clinical trials is CP 945598.

Incretin-Mimetic Agents

Exenatide, a 39-amino acid peptide from the Gila monster (*Heloderma suspectum*), is a functional analog of human glucagon-like peptide-1 (GLP-1). Because of its resistance to degradation, in-vivo potency of exenatide is much greater than that of GLP-1. Exenatide improves glycemic control through glucose-dependent secretion of insulin, suppression of high glucagon levels in patients with type 2 diabetes, delay of gastric emptying, and reduction of food intake. Exanatide is administered at doses of 5 and 10 ug twice daily; its most frequent adverse effects were nausea and hypoglycaemia.

Leptin

Leptin has been shown to markedly reduce appetite and weight in the extremely rare individuals who lack leptin. In contrast, in the first clinical study of patients with polygenic obesity and elevated leptin levels, weight loss was variable and relatively small. This disappointing result may be explained by the leptin resistance consistently observed in obese humans and rodents. However, it cannot be excluded that a small

Appetite Control. Table 1 Hormones, peptides and neurotransmitters implicated in appetite control

Hormones, peptides and neurotransmitters	Effect of ICV injection on food intake	Effect of gene deletion on food intake	Response to adiposity signals	Receptor	Effect of receptor defect on food intake
Satiety signals					
cholecystokinin (CCK)	↓		-	CCK-A	↑(↔[a])
Adiposity signals					
Leptin	↓	↑↑	–	LEPRb	↑↑
insulin	↓		–	IR	↑[b]
Orexigenic					
Neuropeptide Y (NPY)	↑	↔(↓[c,d])	↓	NPY2R	↑
				NPY5R[e]	↑
Agouti-related peptide (AgRP)	↑	↔(↓[d])	↓	MC4R[e,f]	↑
Melanin-concentrating hormone (MCH)	↑	↓	↓	MCHR1	↑
				MCHR2	
Orexin A and B (hypocretins)	↑	↔[g]	↓	HCRTR1	
				HCRTR2	
Galanin	↑	↔		GALR1–3	
Ghrelin	↑	↔	↓	GHSR	↓[h]
Endocannabinoids	↑			CB1	↔(↓[i])
Anorexigenic					
α-Melanocyte-stimulating hormone (αMSH)	↓	↑[j]	↑	MC4R	↑
Cocaine- and amphetamine-regulated transcript (CART)	↓	↔	↑	?	
Corticotropin-releasing hormone (CRH)	↓	↔versus↓	↑	CRHR1	↔(↓[k])
				CRHR2	↔
Urocortin	↓		↑	CRHR1	↔s(↓[k])
				CRHR2	↔
Thyrotropin-releasing hormone (TRH)	↓		↑	THRH	
Glucagon-like peptide (GLP-1,2)	↓			GLPR	↔
Serotonin	↓			5-HT_{1B}	↑
				5-HT_{2C}	
Noradrenaline	↓(↑)			α_1,(α_2)	

[a]Normal basal food intake in knockout mice, but stimulatory response to CCK is abolished.
[b]Neuron-specific insulin receptor knockout.
[c]Reduction of hyperphagia was observed in leptin-deficient mice that also lack NPY.
[d]NPY/AgRP neuronal ablation.
[e]Other receptor isoforms may also be relevant.
[f]AgRP acts antagonistic on MC4 receptors.
[g]Knockout mice exhibit narcolepsy.
[h]Mice under chronic high fat diet.
[i]Reduced feeding response to fasting in CB1 knockout mice.
[j]αMSH deficiency in patients with mutations in the precursor, proopiomelanocortin (POMC).
[k]Normal basal food intake in knockout mice, but inhibitory response to urocortin is attenuated.

subpopulation of obese patients is susceptible to the cytokine. Major efforts are currently underway to develop new drugs that target hypothalamic pathways downstream of leptin (e.g., NPY receptor antagonists, MC4R agonists, CRH agonists).

Drugs with Appetite-Stimulating Effects

Psychotropic Drugs

Stimulation of appetite and weight gain has frequently been observed as a side effect of long-term therapy with various psychoactive drugs. Prominent examples not

only are the tricyclic (e.g. imipramine) and heterocyclic (e.g. mirtazepine) antidepressants but also selective serotonin reuptake inhibitors (e.g. paroxetine), neuroleptic drugs (e.g. olanzapine), and lithium. Although it is reasonable to assume that these drugs interfere with central serotonergic and/or adrenergic signalling, the exact mechanism of their appetite-stimulating effect and the receptors involved are unknown. Stimulation of appetite by cyproheptadine, an antihistamin/antiserotonin agent, is believed to reflect antagonism of serotonin receptors.

Treatment of Cachexia and Anorexia

In the palliative treatment of cachexia and anorexia in advanced cancer and AIDS patients, modest relief can be achieved with appetite-stimulating drugs. Various pharmacologic strategies have been tested, including corticosteroids, anabolic steroids, megestrol acetate, cyproheptadine, melatonin, and dronabinol (delta-9-tetrahydrocannabinol). The cannabinoid receptor agonist dronabinol is approved in the US for stimulation of appetite in AIDS patients. Thalidomide also improves appetite and progressive weight gain in AIDS patients. Megestrol is so far the only agent associated with increased appetite and weight gain in patients with cancer.

►Neuropeptide Y
►Endocannabinoids
►Orexins
►Anti-obesity Drug
►Incretin Hormones

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Aptamers

Oligonucleic acid or peptide molecules that bind a specific target molecule. Nucleic acid aptamer species can be engineered through repeated rounds of in vitro selection to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers offer molecular recognition properties, can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications.

►Tyrosine Kinase Inhibitors
►Antisense Oligonucleotides

aPTT

Activated partial thromboplastin time.

►Anticoagulants

Aquaporins

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Synonyms

Water channels

Definition

Aquaporins (AQP) are a family of integral membrane proteins expressed in all organisms and play a fundamental role in the regulation of water transport into and out of cells.

Basic Mechanisms

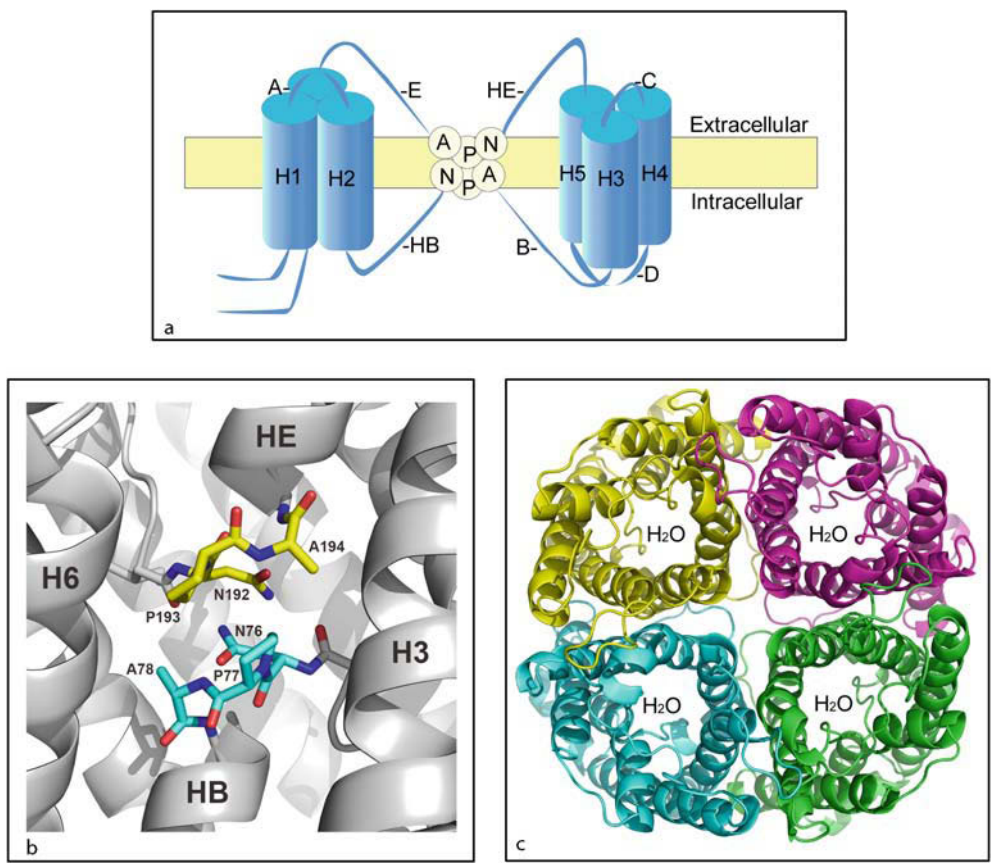
Background

Aquaporins are central players in mammalian physiology, but are also important in microorganisms and plants. The number of AQPs in plants is quite high; angiosperms species, for example, express approximately 35 different AQPs divided into four families on the basis of their sequence. Moreover, plant AQPs might be considered multifunctional channels for their different transport properties.

To date, 13 different AQPs have been identified in mammals (AQP0-AQP12). Most of these proteins allow efficient water transport through the cell membrane. However, several studies revealed that some AQPs also mediate the transport of various small molecules such as hydrogen peroxide, urea, glycerol or anions. On the basis of their different transport properties, AQPs can be divided in two major groups: classical AQPs and aquaglyceroporins. Functional studies revealed that all AQPs transport water to a particular extent. AQP0, 1, 2, 4 and 5 are classical aquaporins being only permeable to water, while AQP3, 7, 9, 10 and 12 can also facilitate the transport of other small molecules, such as glycerol and urea. AQP6 has been suggested to function as a pH-sensitive chloride channel, while AQP8 is permeable to urea and hydrogen peroxide. AQP7 and 9 also transport heavy metal salts, such as arsenite, while AQP9 is additionally permeable to other solutes, such as urea, linear polyols, purines, pyrimidines and nucleosides. The water transport properties of AQP11 are unknown, but it is impermeable to any of the small molecules mentioned above. Considering the importance of water transport in several biological processes and the expression and distribution of AQPs in all organisms, its likely that mutations in these genes contribute to various diseases.

Structure of AQPs

Hydrophobicity plots of AQPs indicated that these proteins consist of six transmembrane α-helices (H1–H6 in Fig. 1a) connected by five connecting loops (A–E), and flanked by cytosolic N- and C-termini. The second half of the molecule is an evolutionary duplicate and inverse orientation of the first half of the molecule. Loops B and E of the channel bend into the membrane with an α-helical conformation (HB, HE in Fig. 1b) and meet and each other at their so-called Asn-Pro-Ala (NPA) boxes. These NPA motifs are the hallmark of AQPs and form the actual selective pore of the channel, as at this location, the diameter is of that of a water molecule (3Å; Fig. 1a and b). Based on the narrowing of the channel from both membrane sides to this small



Aquaporins. Figure 1 (a) The hour-glass model. The scheme depicts the six transmembrane helices (H1–H6), the connecting loops A–E, including the helical parts of loops B ((H)B) and E (E(H)), and the conserved NPA (Asn-Pro-Ala) motif of canonical aquaporins. (b) Structure of the conserved NPA motif region, flanked by the indicated helices. (c) Crystallographic structure of AQP1 tetramer. The four water pores in a tetramer are indicated [1].

pore, an "hour-glass" structure model was proposed, which has later been proven by electron and X-ray crystal studies of AQP1 [1]. Movement of water through the channel occurs in single file and at a speed indifferent from water molecule movement within water. This latter item means that water does not interact with the channel at its narrowest point, i.e., the NPA box region. Indeed, six water molecules form a single file through the channel of AQP1 and hydrogen bonds only occur within the AQP pore between water and six residues (Gly74, Ala75, and His76 on the cytoplasmic side and Gly190, Cys191, and Gly192 on the extracellular side), but not at the NPA motif. At this latter point, water molecules reorient to form a transient hydrogen bond with the conserved asparagine in the NPA triplet, thereby impairing the conduction of protons. Interestingly, the most recently identified AQPs, AQP11 and AQP12, have their E-loop NPA motif, but the alanine in the loop B NPA motif is replaced by cysteine (AQP11) or threonine (AQP12). The functional consequences are at present unclear.

Although freeze-fracture experiments have demonstrated that monomers are assembled into stable tetramers in the membranes, radiation inactivation studies and, later, expression studies revealed that each monomer is a functional water channel (Fig. 1c).

Tissue Distribution of AQPs

The presently known mammalian AQP0-AQP12 have been localized in tissues involved in fluid transport as well as in nonfluid-transporting tissues (Table 1). Most AQPs are constitutively present in the plasma membrane, whereas some water channels can be triggered to shuttle between intracellular vesicles and the plasma membrane [2].

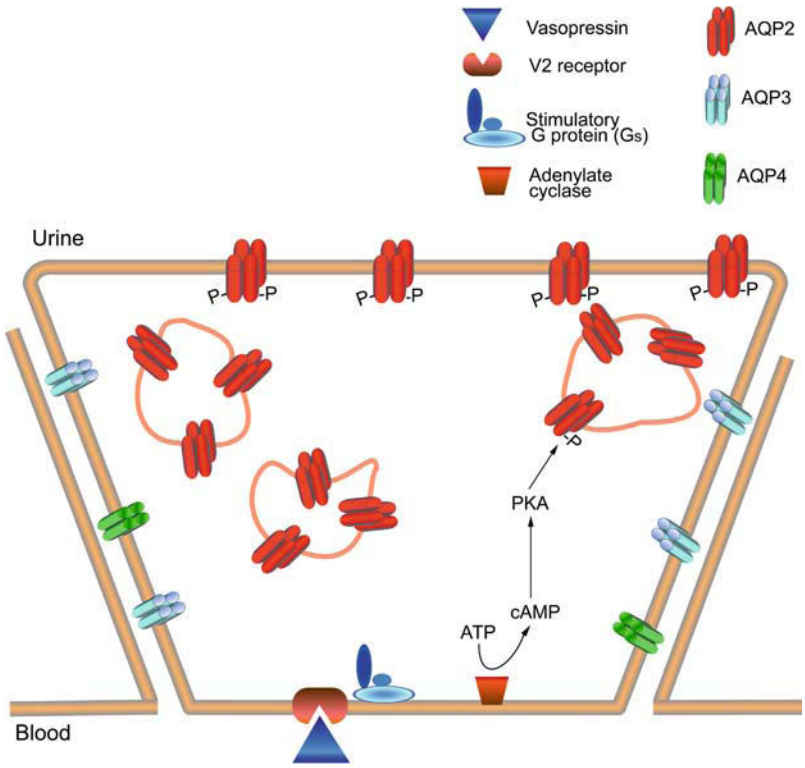
AQP0, formerly known as the Major Intrinsic Protein of 26 kDa (MIP26), is specifically expressed in the plasma membrane of eye lens fiber cells. It transports water to a low degree, but has also been implicated in cell adhesion and gap junction formation. Its main role is to maintain the transparency of the lens by maintaining a tight cellular connection to neighboring cells and/or by controlling the fluid circulation.

AQP1, till the renaming to AQPs referred to as CHIP28, is widely expressed throughout the body. AQP1 is located in the proximal tubule, thin descending limb of Henle and descending vasa recta in the kidney. Outside the kidney, AQP1 is present in endothelial cells of capillaries and small vessels throughout the whole digestive system, including salivary gland, esophagus, stomach, intestine, liver, gallbladder and pancreas. In addition, AQP1 is found in red blood cells, ear, eye, lung, male reproductive system and the choroid plexus. In contrast to its other locations, AQP1 shuttles to the apical membrane of cholangiocytes, which is under hormonal regulation.

The AQP2 water channel is highly expressed in the principal cells of renal collecting duct [3]. Whereas most AQPs are constitutively present on the plasma membrane, AQP2 shuttles between intracellular storage vesicles and the apical membrane. The localization of AQP2 within the principal cell is mainly controlled by the antidiuretic hormone vasopressin. In states of dehydration or hypovolemia, vasopressin is released into blood and binds its receptor on renal principal cells. Binding of vasopressin initiates a cAMP signaling cascade resulting in a fast translocation of AQP2 bearing vesicles to the apical membrane, rendering the membrane permeable to water (Fig. 2). In extrarenal tissues, AQP2 can be found in vas deferens and in the inner ear.

Aquaporins. Table 1 Localization of AQPs

AQP	Localization
0	Eye lens fiber cells
1	Kidney tubules, salivary gland, esophagus, stomach, intestine, liver, gallbladder, pancreas, red blood cells, ear, eye, lung, male reproductive system, the choroid plexus
2	Kidney collecting duct, vas deferens, inner ear
3	Kidney collecting duct, skin, bladder epithelium, digestive tract, respiratory tract, eye, brain
4	CNS, kidney collecting duct, glandular epithelia, airways, skeletal muscle, stomach, retina and ear
5	Glandular epithelia, lung epithelium, gastrointestinal tract, pancreas, ear
6	Kidney collecting duct
7	Adipose tissue, kidney proximal tubule, testis, gastrointestinal tract, immature dendritic cells and ear
8	Gallbladder, liver, pancreas, intestine, salivary gland, testis, heart, kidney, lung, placenta
9	Liver, intestinal wall, lung, leukocytes, testis, ear, brain
10	Small intestine
11	Kidney, liver, testis, brain
12	Pancreatic acinar cells



Aquaporins. Figure 2 Model showing the vasopressin dependent AQP2 trafficking in renal collecting duct cells. Vasopressin binding to its V2 receptor results in the activation and dissociation of a stimulatory G protein (Gs), of which the GTP-bound α-subunit activates adenylyl cyclase. The consequent increase in cAMP activates protein kinase A, which phosphorylate several proteins, including AQP2 at Ser256, which is located in its C-terminus. This event is essential for the steady-state redistribution of AQP2 to the apical membrane. Upon restored water homeostasis, blood AVP levels are decreased and AQP2 will be internalized from the apical membrane.

Similar to AQP1, AQP3 expression can be found in many different tissues, including kidney collecting duct, bladder epithelium, digestive tract, respiratory tract, eye and brain. In kidney, AQP3 is present in the basolateral membrane of collecting duct principal cells. In the digestive tract, AQP3 is expressed in epithelial cells ranging from the oral cavity to the stomach and from the distal colon to the anus. AQP3 is also expressed in skin, where it is involved in skin hydration and elasticity.

AQP4 is the predominant water channel in the central nervous system (CNS), where it is involved in maintaining brain water balance and neural signal transduction. It is mainly expressed in astroglial cells, which support the neurons. Outside the CNS, AQP4 has been found in the basolateral membrane of renal principal cells as well as in various glandular epithelia, airways, skeletal muscle, stomach, retina and ear.

AQP5 is expressed in lung epithelium, gastrointestinal tract, pancreas and ear. Several glandular epithelia also express AQP5, including airway submucosal glands, salivary glands, lachrymal glands and sweat glands suggesting a role for AQP5 in the release of airway fluids, saliva, tears and sweat.

AQP6 is expressed in the intercalated cells of the kidney collecting duct. This channel is hardly permeable to water, but capable of transporting anions, including chloride, and is therefore thought to play a role in maintenance of body acid-base balance or in intracellular vesicle acidification.

AQP7 is expressed in the proximal tubule of the kidney, testis, gastrointestinal tract, immature dendritic cells and ear. This glycerol channel is also highly expressed in adipocytes where it is thought to control the release of triglycerides.

Also AQP8 is widely expressed in organs including liver, pancreas, intestine, salivary gland, testis, heart, kidney, lung and placenta. AQP8 expression in gallbladder epithelium suggests a role in secretion of bile. Several groups also found AQP8 to be expressed in mitochondria, but a role for AQP8 in mitochondrial processes remains to be established.

AQP9, a channel highly permeable to water and solutes, is localized in liver, intestinal wall, lung, leukocytes, testis, ear and brain. Liver AQP9 is suggested to act in conjunction with AQP7 in fat metabolism. While AQP7 is involved in the release of glycerol from adipocytes, AQP9 facilitates its transport

into the hepatocytes. Based on its putative function in hepatocytes, AQP9 expression in the brain is suggested to play a role in brain energy metabolism.

AQP10 has only been identified in the small intestine so far and is thought to play a role in hormonal secretion. AQP11 is expressed in kidney, liver, testis and brain, but no function has been found so far. AQP12 has been identified in pancreatic acinar cells, where it is thought to facilitate the release of digestive enzymes into the pancreatic duct.

AQP-associated Pathologies

Knocking out genes and identification of mutations in the human genes provide information on the role of AQPs in normal physiology. The lack of some AQPs directly results in a disease phenotype, while the physiological role of many becomes clear when the putative function is challenged.

Mutations in two genes directly lead to a disease. Mutations in the AQP0 gene lead to dominantly inherited cataract. Single amino acid substitution in the AQP0 gene in both mice and humans result in proteins with impaired trafficking to the plasma membrane and cataract formation, due to loss of the integrity of the lens.

Mutations in the AQP2 gene can cause severe problems, as they result in nephrogenic diabetes insipidus (NDI), a disorder in which patients are unable to concentrate their urine. Congenital NDI can be caused by mutations in the V2R gene (X-linked NDI) or the AQP2 gene (autosomal recessive or dominant NDI) [4, 5]. Missense mutations in the AQP2 gene in recessive NDI affect amino acids located in between the first and last transmembrane domain. Although they might be functional water channels, the mutation leads to a misfolded protein, which is retained by the cellular quality control of the endoplasmic reticulum. In dominant NDI, the mutations are found in the AQP2 C-terminus, resulting in AQP2 mutants that are, in complex with wild-type AQP2, sorted to other subcellular destinations than the apical membrane. Consequently, inadequate amounts of AQP2 are expressed in the apical membrane, resulting in reduced water absorption and increased urine volumes.

Humans lacking AQP1 do not suffer from any severe symptoms. The only phenotype that can be observed in these individuals is a mild renal concentration defect.

Still, the list of diseases in which AQPs may play a role is expanding. AQP3 knockout mice developed NDI and showed polyuria. In addition, these mice showed dry skin and delayed wound healing. Humans lacking a functional AQP3, however, are symptom less. AQP4 knockout mice revealed an important role for AQP4 in recovery after brain injury. AQP5 expression is decreased in patients with Sjögren's syndrome, a disorder characterized by dry eyes, dry mouth and pulmonary problems, but a causal relationship has not been established yet. AQP7 expression is reduced in obese people compared to lean people, which may indicate that a lack of AQP7 in adipocytes may underlie congenital forms of obesity. The lack of AQP11 results in polycystic kidneys in mice, but whether such a relationship exists in humans is unknown.

Pharmacological Interventions

Although aquaporins play a fundamental role in the regulation of water homeostasis, specific pharmacological therapies are still not available. Several substances like mercurial derivatives, silver, and gold have been demonstrated to inhibit water permeability mediated by aquaporins in oocytes, but most of them are nonspecific and too toxic to be used *in vivo*. Recent studies have shown that tetra-ethyl ammonium (TEA) selectively inhibits AQP1, AQP2 and AQP4 but not AQP3 and AQP5, indicating that quaternary ammonium compounds and in particular TEA may be a good lead compound for the development of specific aquaporin inhibitors.

►Vasopressin/Oxytocin

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Aquaretic Agents

Substances which promote the elimination of water by the kidney without major losses of salts (e.g. conivaptan, tolvaptan, SR121463A/B). They are particularly useful in situations where excess water needs to be eliminated without affecting the salt metabolism, like eu- or hypervolemic hyponatraemia, congestive heart failure, some stages of hypertension and some metabolic states.

►Vasopressin/Oxytocin
►Aquaporins

Arachidonic Acid

The 20 carbon fatty acid, 5,8,11,14 – eicosatetranoic acid, an essential fatty acid that serves as a precursor for protaglandins.

►Prostanoids

Area Postrema

The area postrema is a circumventricular brain region positioned on the dorsal surface of the medulla on the floor of the fourth ventricle. The blood–brain barrier and the cerebrospinal fluid–brain barrier are absent in this region and consequently many substances that do not pass across capillaries in other regions of the brain can do so in the area postrema. The chemoreceptor trigger zone (CTZ), located in the lateral area postrema is sensitive to blood-borne emetogens. Nerves from the CTZ connect with the vomiting centre.

►Emesis

Area under the Curve

Area under the Curve (AUC) refers to the area under the curve in a plasma concentration-time curve. It is directly proportional to the amount of drug which has appeared in the blood ("central compartment"), irrespective of the route of administration and the rate at which the drug enters. The ►bioavailability of an orally administered drug can be determined by comparing the AUCs following oral and intravenous administration.

►Pharmacokinetics

L-Arginine

A substrate for the synthesis of NO that has a potential for improving endothelial dysfunction.

►Nitric Oxide (NO)
►NO Synthases

Arginine Vasopressin

►Vasopressin/Oxytocin

Aromatase

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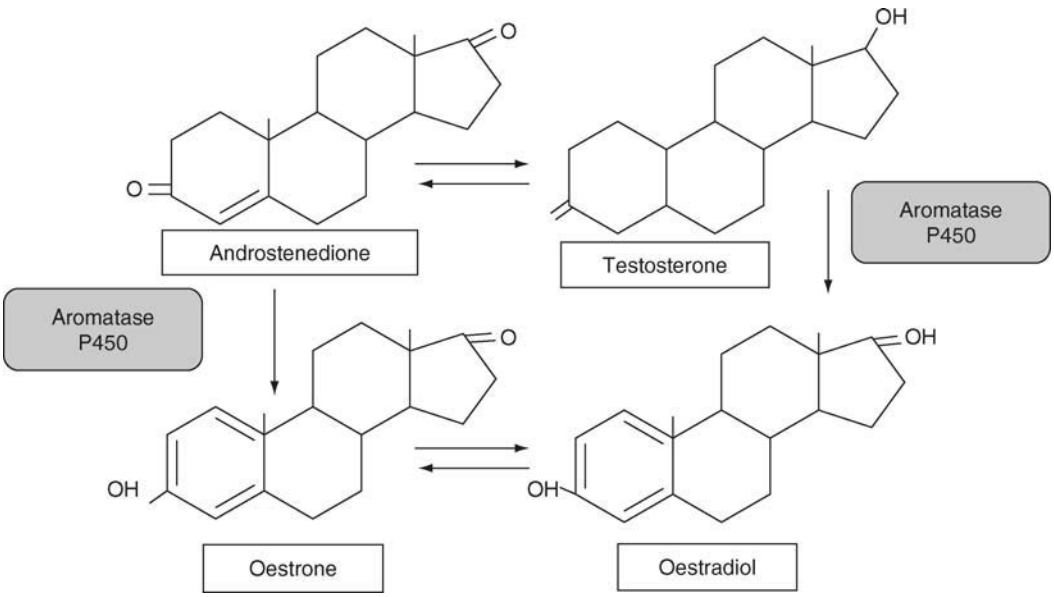
Definition

Aromatase is a cytochrome P450, hemoprotein-containing enzyme, located in the endoplasmic reticulum, which catalyzes the rate-limiting step in the conversion of ►androgens (androstenedione and testosterone), to ►estrogens (estrone and estradiol). Agents that inhibit aromatase (aromatase inhibitors) are widely used to prevent the development and progression of ►estrogen dependent breast cancers.

Basic Characteristics

Estrogen biosynthesis is mediated by the aromatase enzyme, which is a product of the CYP19 gene. The aromatase enzyme is a complex that consists of cyctochrome P450 hemoprotein and a flavoprotein, NADPH-cytochrome P450 reductase. This complex is responsible for catalyzing the conversion of steroidal C-19 androgens (androstenedione and testosterone) to C-18 estrogens (estrone and estradiol), which is the rate-limiting final step in the synthesis of estrogens (Fig. 1). This enzymatic reaction is comprised of three steps each of which requires 1M equiv. of NADPH and oxygen. The first step involves hydroxylation of the androgen substrate at C-19 to produce a 19-hydroxy intermediate. In the second step the 19-hydroxy intermediate is oxidized to produce a 19-oxo compound. The last step in the aromatization reaction is less well defined but is thought to involve the oxidative cleavage of the C10-19 bond to produce ►estrogens (estrone and estradiol) and formic acid.

Aromatase activity, and hence the capacity to synthesize estrogens, is found in a variety of tissues in the body. Gonadal sites include the ovaries in premenopausal women and the testes in men. Important extragonadal sites of aromatase activity include the placenta, chondrocytes and osteoblasts of bone, adipose tissue, muscle and brain. Aromatase plays an important



Aromatase. Figure 1 Androgens converted to estrogens by aromatase.

role in a number of important biological processes including breast development during puberty and uterine growth and bone maturation during adolescence. In adults aromatase influences bone mineralization, lipid metabolism and cardiovascular risk. In pregnant women it protects against the virlizing effects of fetal androgens.

Drugs

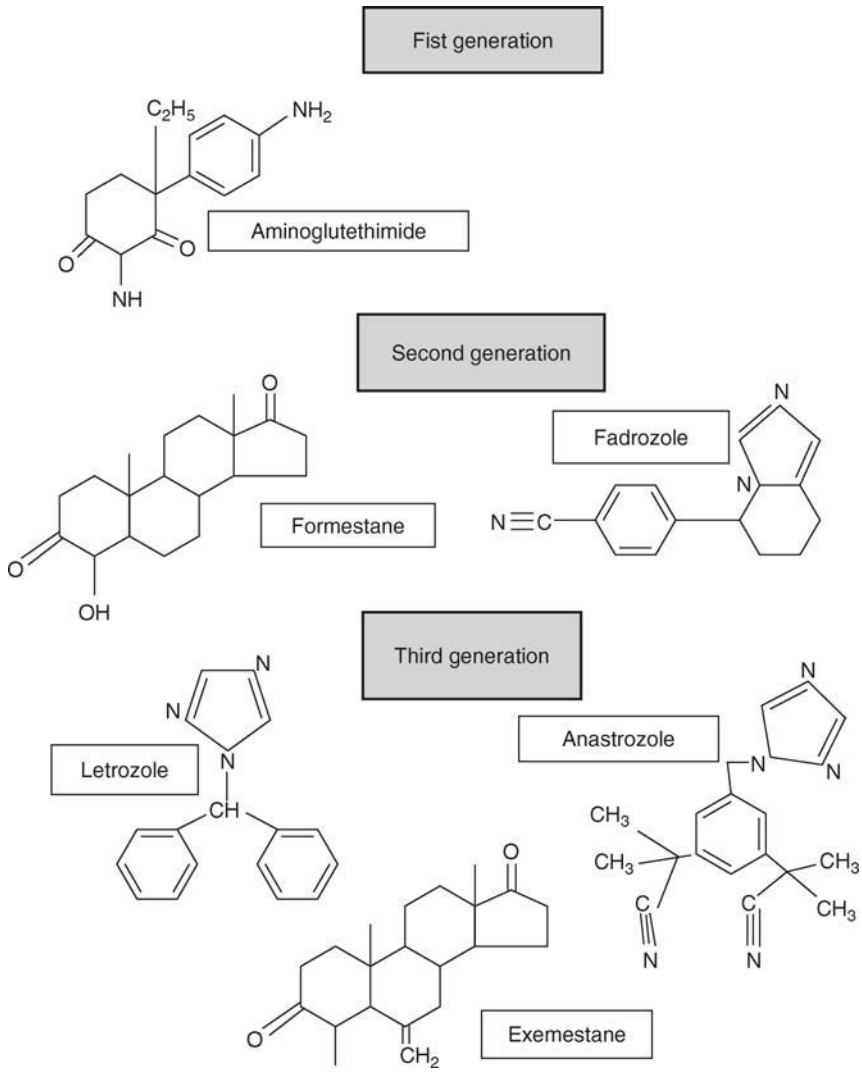
Estrogen is known to be an important stimulus in the development and progression of some breast tumors. Thus targeting the disruption of either the synthesis (i.e., inhibiting aromatase enzyme) or the activity (i.e., blocking ►estrogen receptors) of estrogens are potential mechanisms for the prevention and treatment of hormone sensitive breast cancer. Since aromatization is a unique reaction and is the terminal step of the estrogen biosynthetic pathway, agents that block this reaction would not potentially affect the production of other steroids.

In premenopausal women the ovary is the richest source of aromatase and hence estrogen. Aromatase is confined to the granulosa cells and is produced under the influence of ►gonadotropins (FSH and LH). Despite being a rich source of aromatase, three separate studies have shown that aromatase inhibitors are unable to sufficiently suppress ovarian estrogen production to postmenopausal levels. One explanation for this phenomenon may be a compensatory rise in gonadotrophins which maintains adequate estrogen production, despite the presence of the inhibitor. As such aromatase inhibitors cannot be used in premenopausal breast cancer patients. After menopause, ovarian production of estrogen ceases. However estrogen production continues from peripheral sources of aromatase activity that convert adrenal androgens to estrogens. Aromatase inhibitors have been shown to adequately suppress estrogen production in postmenopausal women, and in this setting are used in the treatment of both early and advanced stage ►estrogen receptor positive breast cancer.

Over the last 30 years a number of aromatase inhibitors (Fig. 2) have been developed. The first (aminoglutethimide) and second (Fadrozole and Fromestane) generation aromatase inhibitors are not commonly used due to their lack of specificity in inhibiting the aromatase enzyme and associated significant side-effects. Two types of third generation aromatase inhibitors are commercially available and have been shown to be either equal to or superior to tamoxifen in the treatment of metastatic estrogen receptor positive breast cancer. Type I (suicidal, noncompetitive) inhibitors bind irreversible with the aromatase enzyme thereby permanently blocking its activity. Exemestane is an example of a type I inhibitor. Type II inhibitors bind reversible with the aromatase enzyme, examples of which include letrozole and anastrozole.

Aminoglutethimide

Aminooglutethimide was the first aromatase inhibitor to be used in patients with metastatic breast cancer, where response rates of up to 30% have been reported. Unfortunately, due to its lack of selectivity for aromatase, it induced a medical adrenelectomy that resulted in suppression of aldosterone and cortisol. With the development of more selective aromatase



Aromatase. Figure 2 Aromatase Inhibitors.

inhibitors, aminoglutethimide is now rarely used for the treatment of breast cancer. It is occasionally used for the treatment of medical conditions involving excess hormone production such as ►Cushing's syndrome.

Anastrazole

Anastrazole is a nonsteroidal, type II, aromatase inhibitor that is 200 times more potent than aminoglutethimide. It is eliminated primarily via hepatic metabolism, has a terminal half life of 50 h with steady state concentrations achieved approximately 10 days with once daily dosing regimens. It is administered orally at a dose of 1 mg/day that achieves near maximal aromatase inhibition and hence estrogen suppression in breast cancer patients. No effect on adrenal steroidogenesis has been observed at up to ten times the daily recommended dose. When used in the metastatic setting, anastrozole has been shown to increase time to progression when compared to tamoxifen. In the ATAC (Arimidex, ►Tamoxifen, Alone or in Combination) trial over 9,000 patients with early stage breast cancer were randomized to 5 years of anastrozole 1 mg/day or 5 years of tamoxifen 20 mg/day or a combination of both. At a median follow up of 33 months, 47 months, and 68 months, compared to tamoxifen, anastrozole upfront significantly increased disease free survival and time to recurrence and reduced the risk of contra lateral breast cancer. The combination arm of the trial was closed as it was no more effacious than tamoxifen alone. Currently trials are ongoing evaluating anastrozole as chemopreventive agent for breast cancer.

Letrozole

Like anastrozole, letrozole is a third generation, type II nonsteroidal aromatase inhibitor. Renal excretion of its

inactive glucuronide metabolite represents its main pathway of clearance. It has a half-life of 2 days and at the recommended daily dose of 2.5 mg steady-state plasma levels is reached in 2–6 weeks. Letrozole has proved effective when used either sequentially after tamoxifen or upfront in the treatment of patients with early stage breast cancer. The MA-17 trial randomized approximately 5,000 postmenopausal breast cancer patients who had received 5 years of tamoxifen to either placebo or 5 years of letrozole. The Breast International Group (BIG) 1–98 trial randomized postmenopausal breast cancer patients to 5 years of tamoxifen, 5 years of letrozole, or 2 years of either agent (i.e., tamoxifen or letrozole) followed by three years of the other agent (i.e., tamoxifen or letrozole). In terms of disease free survival the MA-17 trial showed an advantage to switching to letrozole and the BIG 1–98 trial showed an advantage to up front letrozole with results awaited for the switching group.

Exemestane

Examestane is a type II, steroidal aromatase inhibitor with an androgen structure. It is metabolized by CYP3A4 enzyme and has a half-life of 27 h. At the recommended once daily dose of 25 mg no effect is seen on adrenal steroid production and maximal estrogen suppression is achieved in 7 days. In early breast cancer treatment it has been studied as a sequential agent after several years of tamoxifen. The Intergroup Exemestane Study (IES) randomized over 4000 patients to either 5 years of tamoxifen 20 mg/day or 2–3 years of tamoxifen followed by exemastane 25 mg/day. A significant reduction in disease free survival (hazard ratio, 0.76, $p = 0.0001$) and risk of contralateral breast cancer favoring the group switching to exemestane was observed.

Side Effects

Aromatase inhibitors are relatively well tolerated; however have a number of distinct side effects are observed that stem from the state of estrogen deprivation induced by aromatase inhibitors. Side effects include hot flashes, joint and muscle aches, vasomotor symptoms and vaginal dryness. Variable effects of aromatase inhibitors on lipid levels have been observed. Trials comparing third generation aromatase inhibitors to tamoxifen have also reported an increased risk of cardiovascular events in the group receiving aromatase inhibitors.

Other Uses

Aromatase inhibitors have also been used in premenopausal women for the treatment of ►endometriosis and to induce ovarian folliculogenesis as part of the treatment for infertility.

►Sex Steroid Receptors

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Aromatase Inhibitor

An aromatase inhibitor is a class of antiestrogens that inhibits the enzyme aromatase and by that means lowers the level of the estrogen estradiol. Aromatase catalyzes the conversion of testosterone to estradiol in many tissues including the adrenal glands, ovaries, placenta, testicles, adipose tissue, and brain. Estrogen is produced directly by the ovaries and is also made by the body using aromatase. Aromatase inhibitors cannot do anything about estrogen produced by the ovaries, but they do interfere with the body's use of aromatase.

►Sex Steroid Receptors: Androgen Receptor, Estrogen Receptors, Progesterone Receptor
►Aromatase
►Targeted Cancer Therapy
►P450 Mono-Oxygenase System

Arousal

Arousal is a state of vigilance regulated by subcortical parts of the nervous system, especially connections between the nuclei of the amygdala, the hypothalamus and the brain stem. These unconscious responses prepare the body for action.

In terms of sleep/wake regulation, the arousal systems are those that have highest activity during wake, for example the aminergic (noradrenaline, 5-HT, histamine) systems. The arousal systems inhibit, and are themselves inhibited by the GABAergic system emanating from the ventrolateral preoptic nucleus (VLPO), in a so-called "flip flop" arrangement that is stabilised via orexinergic activity.

►Psychostimulants
►Sleep

Array

Refers to the physical substrate to which biological samples are attached to create features (spots). In gene expression profiling arrays are hybridized with labeled sample and then scanned and analyzed to generate data.

►Microarray Technology

Arrestins

Arrestins act as adaptor proteins that bind to phosphorylated G protein-coupled receptors (GPCR) and link the receptors to clathrin-coated pits. β-Arrestins are essential in the internalization of many GPCRs.

►β-Adrenergic System
►G-protein-coupled receptors
►Tolerance and Desensitization

Arrhythmias

►Antiarrhythmic Drugs

Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

Synonyms
ARVD/C

Definition
Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited heart disorder with progressive replacement of right ventricular muscle by adipose and fibrous tissue. ARVD/C is associated with arrhythmia of right ventricular origin that may result in sudden death. Nine different loci are currently associated with ARVD/C, and causative mutations have so far been identified in five genes, RyR2, TGFβ-3, plakoglobin, desmoplakin and plakophilin.

►Ryanodine Receptor

Arteriogenesis

Arteriogenesis is the growth of collateral vessels from a pre-existing arteriolar network to bypass an ischemic area (e.g., following cardiac ischemia).

►Angiogenesis and Vascular Morphogenesis

Arteriosclerosis

►Atherosclerosis

Arylhydrocarbon Receptor

Synonyms
AhR

Definition
Members of the CYP1 family and some other drug metabolizing enzymes including UGT1A6 are collectively induced by polycyclic aromatic hydrocarbons (PAH) and other ligands (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) of this basic helix-loop-helix (bHLH) transcription factor. In the absence of ligand AhR is inactive while bound to HSP90 in the cytoplasm. Upon binding of ligand, it moves to the nucleus and dimerizes with Arnt (AhR nuclear translocator protein) to form the active transcription factor that binds to xenobiotic response elements (XRE) in the promotor regions of target genes. AhR furthermore plays a role in regulating hepatic cell regeneration.

►P450 Mono-Oxygenase System
►P450 Enzymes
►Nuclear Receptor Regulation of Drug-Metabolizing
►Dioxins
►PAS Domain

L-Ascorbic Acid

►Vitamin C

ASF Family of Transporters

The **A**mphiphilic **S**olute **F**acilitator family of transporters are simple in the sense that no specific source of energy is used for operation (such as hydrolysis of ATP or gradients of inorganic solutes).

►Organic Cation Transporters

Asn-linked Glycosylation

Asn-linked glycosylation is the addition of carbohydrate groups to peptides or proteins through specific glycosyltransferases. Glycosyltransferases within the lumen of the endoplasmic reticulum recognize an Asn-X-Ser/Thr motif (X can be any amino acid but not proline) and link carbohydrates via N-acetylglucosamine to the amino group of the asparagine residue.

►Protein Trafficking and Quality Control
►Intracellular Transport
►Palmitoylation
►Endothelins

ASON

ASON stands for antisense oligonucleotides.

►Antisense Oligonucleotides

Aspartyl Proteinases

Aspartyl proteinases are proteinases that utilize the terminal carboxyl moiety of the side chain of aspartic acid to effect peptide bond hydrolysis.

►Non-viral Peptidases

Aspirin

Aspirin is the brand name of acetylsalicylic acid. It is the most widely used analgesic, antipyretic and anti-inflammatory drug. Its main mode of action is irreversible acetylation of cyclooxygenases.

►Cyclooxygenases
►Non-steroidal Anti-inflammatory Drugs

Aspirin-like Drugs, Inflammation

►Non-steroidal Anti-inflammatory Drugs

Asthma

►Bronchial Asthma

Astrocytes

Category of glial cells in the vertebrate central nervous system with long radial processes. Astrocytes provide structural support to nerve cells and help to control their chemical and ionic extracellular environment.

►Interferons

Atherogenesis

Definition

Atherogenesis is the process that leads to changes in the arterial blood vessels, including deposition of cholesterol (atherosclerosis). It is the pathophysiological process behind the vast majority of heart attacks.

►Atherosclerosis
►HMG-CoA-Reductase Inhibitors (Statins)

Atherosclerosis

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Synonyms

Arteriosclerosis (see definition)

Definition

Atherosclerosis, from the Greek words *athera* – porridge, and *sclereni* – hardening, literally is a hardening of medium- and large-sized arteries specifically due to an atheromatous plaque, whereas arteriosclerosis is a more general term describing any hardening of medium and large arteries. A typical atherosclerotic ►plaque consists of a fibrous cap (A structure composed of a dense collagen-rich extracellular matrix with occasional smooth muscle cells, macrophages and T-cells that typically overlies the characteristic central lipid core of plaques.) overlying a lipid-rich core. In addition to the long recognized lipid accumulation, these lesions, known as atheromata, also harbor inflammation and cell recruitment and turnover (proliferation and death). Collectively, the process of atheroma development within an individual is called atherogenesis, and the overall result of the disease process is termed atherosclerosis.

Basic Mechanisms

General

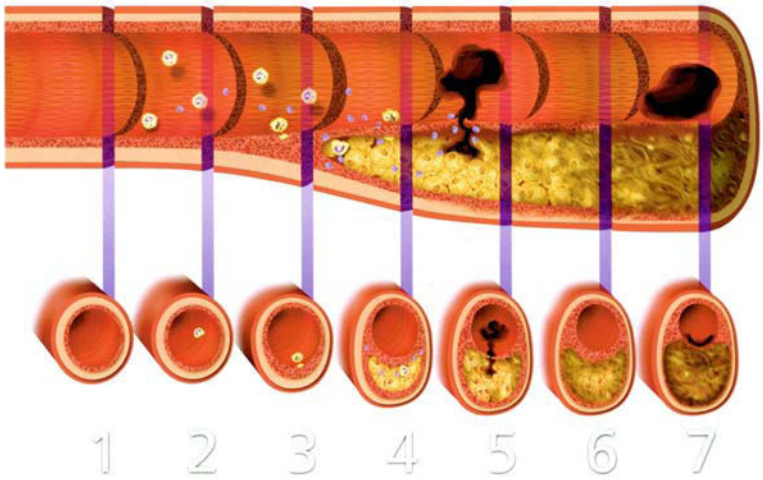
The traditional view of atherosclerosis as a bland cholesterol storage disease falters in the wake of extensive evidence that inflammation plays a central role in all stages of this pathology, from the initial lesion to the final devastating thrombotic complications [1, 2]. Atherosclerosis is a chronic systemic disease preferentially affecting particular circulatory beds; in the coronary arteries, it commonly causes ►angina pectoris and ►myocardial infarction, in the central nervous system it often leads to transient ischemic attack and ►ischemic stroke, and in the periphery it results in ►intermittent claudication and critical limb ischemia. The renal and splanchnic beds can also develop atherosclerosis. This disease may manifest clinically with chronic symptoms, such as stable angina or intermittent claudication, acutely as in myocardial infarction or cerebrovascular accident, or may remain clinically silent. Despite a broad array of pharmacologic and procedural interventions to combat this scourge, atherosclerosis remains the leading cause of death and disability in the developed world.

Lesion Initiation and Development of the Fatty Streak

Inflammation participates in atherosclerosis from its inception and onwards (Fig. 1). Even children can develop the "fatty streak," the initial lesion of atherosclerosis. Fatty streaks do not cause symptoms, and may progress to more complex lesions, or eventually disappear. Fatty streaks have focal increases in the content of lipoproteins within regions of the ►intima where they associate with constituents of the extracellular matrix such as proteoglycans, slowing their egress. This retention sequesters lipoproteins within the intima, isolating them from plasma antioxidants, thus favoring their oxidative modification. Oxidatively modified low-density lipoprotein particles (LDL) comprise an incompletely defined mixture, as both the lipid and protein moieties can undergo oxidative modification. Constituents of such modified lipoprotein particles can induce a local inflammatory response.

Endothelial cells (ECs) normally resist leukocyte adhesion. Proinflammatory stimuli that include high-saturated-fat diet, hypercholesterolemia, obesity, hyperglycemia, insulin resistance, hypertension, and smoking trigger the endothelial expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and P-selectin that mediate the attachment of circulating monocytes and lymphocytes. Interestingly, atherosclerotic lesions often form at bifurcations of arteries, regions characterized by a disturbed blood flow which reduces the activity of endothelial atheroprotective molecules. Laminar flow induces the expression of atheroprotective genes such as the antioxidant enzyme superoxide dismutase as well as the synthase that produces nitric oxide, an endogenous vasodilator that can also limit inflammation, for example by limiting VCAM-1 expression.

Chemoattractant factors that include monocyte chemoattractant protein-1 (MCP-1) produced by vascular wall cells in response to modified lipoproteins, direct the migration and diapedesis of adherent monocytes. MCP-1 binds to CCR2 (a chemokine receptor containing two adjacent cysteine residues) on the surface of the migrating monocyte to exert this effect. Experimental evidence and human observations support the involvement of several other chemokines in leukocyte recruitment into the nascent atherosclerotic lesion, including IL-8 and fractalkine. Within the intima, monocytes mature into macrophages under the influence of macrophage colony-stimulating factor (M-CSF), overexpressed in the inflamed intima. M-CSF stimulation also leads to increased macrophage expression of ►scavenger receptors, members of the pattern-recognition receptor superfamily, which engulf modified lipoproteins through receptor-mediated endocytosis. Accumulation of cholesteryl esters in the cytoplasm changes macrophages into ►foam cells, i.e. lipid-laden macrophages characteristic



Atherosclerosis. Figure 1 Initiation, progression, and complication of human coronary atherosclerotic plaque. Top, Longitudinal section of artery depicting "timeline" of human atherogenesis from normal artery (1) to atheroma that caused clinical manifestations by thrombosis or stenosis (5–7). Bottom, Cross sections of artery during various stages of atheroma evolution. 1, Normal artery. Note that in human arteries, the intimal layer is much better developed than in most other species. The intima of human arteries contains resident smooth muscle cells (SMCs) often as early as first year of life. 2, Lesion initiation occurs when endothelial cells, activated by risk factors such as hyperlipoproteinemia, express adhesion and chemoattractant molecules that recruit inflammatory leukocytes such as monocytes and T lymphocytes. Extracellular lipid begins to accumulate in intima at this stage. 3, Evolution to fibrofatty stage. Monocytes recruited to artery wall become macrophages and express scavenger receptors that bind modified lipoproteins. Macrophages become lipid-laden foam cells by engulfing modified lipoproteins. Leukocytes and resident vascular wall cells can secrete inflammatory cytokines and growth factors that amplify leukocyte recruitment and cause smooth muscle cell migration and proliferation. 4, As lesion progresses, inflammatory mediators cause expression of tissue factor, a potent procoagulant, and of matrix-degrading proteinases that weaken fibrous cap of plaque. 5, If fibrous cap ruptures at point of weakening, coagulation factors in blood can gain access to thrombogenic, tissue factor-containing lipid core, causing thrombosis on nonocclusive atherosclerotic plaque. If balance between prothrombotic and fibrinolytic mechanisms prevailing at that particular region and at that particular time is unfavorable, occlusive thrombus causing acute coronary syndromes may result. 6, When thrombus resorbs, products associated with thrombosis such as thrombin and mediators released from degranulating platelets, including platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), can cause healing response, leading to increased collagen accumulation and smooth muscle cell growth. In this manner, the fibrofatty lesion can evolve into advanced fibrous and often calcified plaque, one that may cause significant stenosis, and produce symptoms of stable angina pectoris. 7, In some cases, occlusive thrombi arise not from fracture of fibrous cap but from superficial erosion of endothelial layer. Resulting mural thrombus, again dependent on local prothrombotic and fibrinolytic balance, can cause acute myocardial infarction. Superficial erosions often complicate advanced and stenotic lesions, as shown here. However, superficial erosions do not necessarily occur after fibrous cap rupture, as depicted in this idealized diagram. (Libby P (2001) Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 104:365–372.)

of the early stages of atherosclerosis. In parallel, macrophages proliferate and amplify the inflammatory response through the secretion of numerous growth factors and cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β).

T-cells, representing the adaptive arm of the immune response, also play a critical role in atherogenesis, and enter lesions in response to the chemokines inducible protein-10 (IP-10), monokine induced by IFN-γ (MIG), and IFN-inducible T-cell α-chemoattractant (I-TAC), which bind CXCR3 (a chemokine receptor containing two cysteine residues separated by one amino acid), highly expressed by T lymphocytes in the plaque. The CD4+ subtype, which recognizes antigens presented as fragments bound to major histocompatibility complex (MHC) class II molecules, predominates in the lesion. Interestingly, CD4+ T-cells reactive to the disease-related antigens associated with oxidized LDL have been cloned from human lesions. The atherosclerotic lesion contains cytokines that promote a Th1 response, inducing activated T-cells to differentiate into Th1 effector cells. These cells amplify the local inflammatory activity by releasing proinflammatory cytokines such as IFN-γ and CD40 ligand (CD40L, CD154).

The atherosclerotic plaque overexpresses another chemoattractant, eotaxin, that may mediate mast cell

migration to the lesion through CXCR3. Multiple studies suggest that mast cells promote atherogenesis by releasing cytokines such as TNF-α, IFN-γ, and IL-6 as well as chemokines that attract other leukocytes and proteases that activate matrix metalloproteinases (MMPs) and participate in arterial remodeling. Although the subject of much debate for several decades, recent results in mice support a role for this cell type in lesion progression and complication.

Progression to Complex Plaque

Macrophages, T-cells, and mast cells infiltrate the lesion and localize particularly in the shoulder region where the atheroma grows. While accumulation of foam cells characterizes fatty streaks, deposition of fibrous tissue defines the more advanced atherosclerotic lesion. Smooth muscle cells (SMCs) synthesize the bulk of the extracellular matrix that characterizes this phase of plaque evolution. In response to platelet-derived growth factor (PDGF) released by activated macrophages and endothelial cells, SMCs migrate from the tunica media into the intima, where they proliferate under the influence of various growth factors and secrete extracellular matrix proteins, including interstitial collagen, especially in response to transforming growth factor-β (TGF-β) and PDGF. The ►fibrous cap covering the atherosclerotic plaque, formed during this phase, owes its biomechanical strength to interstitial collagen (types I and III).

Neovascularization arising from the artery's ►vasa vasorum contributes to lesion progression in many ways. First, it provides another entry route for leukocytes into established atherosclerotic lesions. Second, as these neovessels are friable, they can favor focal intraplaque hemorrhage that furnishes one mechanism for the discontinuous increments seen in plaque growth. Hemorrhage in turn generates thrombin, stimulating the release of PDGF from ECs as well as directly stimulating SMC proliferation and cytokine production.

CD40L plays an important role in this phase of atherogenesis. All the main cell types involved in atherosclerosis, including macrophages, T-cells, ECs, SMCs, and platelets, express this proinflammatory cytokine as well as its receptor, CD40. Ligation of CD40 triggers the expression of adhesion molecules and the secretion of numerous cytokines and MMPs involved in extracellular matrix degradation. Importantly, CD40L has a prothrombotic effect by inducing macrophage expression of ►tissue factor (also called thromboplastin, factor III, or CD142), which once exposed to factor VII initiates the coagulation cascade.

Plaque Rupture

Plaque rupture and the ensuing thrombosis commonly cause the most dreaded acute complications of atherosclerosis. In many cases, the culprit lesion of acute coronary artery thrombosis does not produce a critical arterial narrowing, rendering their identification a priori problematic by use of standard angiographic methods. Decades usually separate the initiation of atherosclerosis with the development of the fatty streak from the final thrombotic stages of this disease. This time lag results in part from the initial compensatory centrifugal remodeling ("compensatory enlargement") of the diseased vessel wall, allowing preservation of blood flow until the stenosis encroaches on >70% of the arterial lumen.

Indeed, it now appears that inflammatory activation rather than the degree of stenosis renders the plaque rupture-prone and precipitates thrombosis and resultant tissue ischemia. Advanced complex atheromata exhibit a paucity of SMCs at sites of rupture, and an abundant macrophage accumulation, key characteristics of plaques that have ruptured and caused fatal coronary thrombosis. Simply put, the fibrous cap is all that stands between coagulation factors in the circulation and the plaque core; the thinner the cap, the greater its propensity to rupture and cause thrombosis. Inflammation can interfere with the integrity of the cap's interstitial collagen by stimulating the destruction of existing collagen fibers while in parallel blocking the creation of new collagen. IFN-γ, secreted by activated T-cells, inhibits basal collagen production by SMCs. T-lymphocytes can also contribute to the control of collagenolysis. CD40L as well as IL-1 produced by T-cells induce macrophages to release interstitial collagenases, including MMPs 1, 8, and 13. Members of the cysteine protease family, such as Cathepsin S, can also participate in plaque evolution and destabilization.

Acute coronary syndromes most often result from a physical disruption of the fibrous cap, either frank cap fracture or superficial endothelial erosion, allowing the blood to make contact with the thrombogenic material in the lipid core or the subendothelial region of the intima. This contact initiates the formation of a thrombus, which can lead to a sudden and dramatic blockade of blood flow through the affected artery. If the thrombus is nonocclusive or transient, it may either be clinically silent or manifest as symptoms characteristic of ►unstable angina. Importantly, if collateral vessels have previously formed, for example, due to chronic ischemia produced by multivessel disease, even total occlusion of one coronary artery may not lead to an acute myocardial infarction.

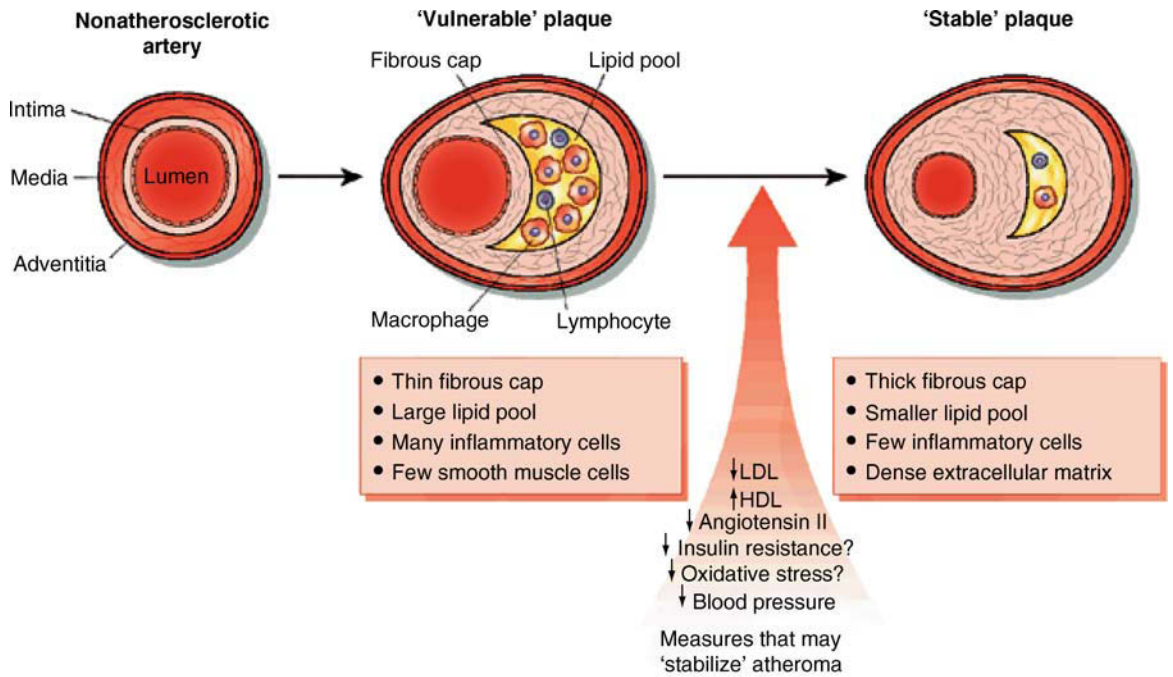
Pharmacological Intervention

General

Classic risk factors of atherosclerosis must first and foremost be fought with lifestyle interventions such as diet, physical activity, and smoking cessation. Indeed, and although it effectively relieves angina, simply

treating stenotic blood vessels by invasive procedures such as ►angioplasty or coronary artery bypass grafting has not been shown to prolong life in broad groups of patients.

Pharmacological management of risk factors through antihypertensive, antihyperlipidemic, and antidiabetic therapy coupled with antiplatelet drugs comprise the next step in the primary and secondary prevention of atherosclerosis, usually instituted together with lifestyle measures [3]. Moreover, medication can in some cases modify or dampen inflammatory processes (Fig. 2). The following review will emphasize the sometimes unforeseen antiinflammatory effects of certain classes of antiatherosclerotic drugs.



Atherosclerosis. Figure 2 Evolution and stabilization of rupture-prone, or "vulnerable," atherosclerotic plaques. The nonatherosclerotic artery (left) has a trilaminar structure; the intima is lined by endothelial cells (ECs) in contact with blood. The underlying media is composed largely of smooth musle cells (SMCs) and embedded in a dense extracellular matrix. The adventitia, the outermost layer, contains loose connective tissue and nerves. During the early stage in the development of atherosclerosis, the atheroma often grows outward and preserves the caliber of the lumen (middle). Such "compensatory enlargement" or "outward remodeling" explains in part why angiography underestimates the degree of atherosclerosis. Pathological studies have demonstrated that the majority of atheroma that have ruptured and triggered an acute myocardial infarction contain a prominent lipid pool and numerous inflammatory cells, in particular, macrophages. The activated inflammatory cells secrete mediators that thin and weaken the fibrous cap that overlies the lipid-rich core of the lesion by reducing synthesis and increasing degradation of collagen. SMC apoptosis may also contribute to depletion of collagen in the fibrous cap. Activated macrophages express tissue factor, a potent activator of coagulation cascade. Disruption of the thin fibrous cap of such vulnerable plaques causes the direct contact of blood coagulation factors to tissue factor and can trigger occlusive thrombus formation. A new therapeutic goal, stabilization of lesions, aims to reduce the incidence of acute coronary events by influencing the nature of the vulnerable plaque qualitatively or functionally rather than by shrinking the lesion (right). Lowering of LDL (low-density lipoprotein) can reduce cholesterol delivery, and increased HDL (high-density lipoprotein) may enhance cholesterol efflux from the atheroma. LDL reduction and inhibition of angiotensin II signaling may limit oxidative stress (for example, reactive oxygen species production, lipid peroxidation and oxidized LDL accumulation) in atheroma. Future research should evaluate the effects of other therapeutic measures on inflammatory processes mentioned in the text, extracellular matrix metabolism, the thrombotic/fibrinolytic balance, and other functional features of plaque, as well as the effects on lipids and the size of lesions. Conversion of unstable to stable plaques by altering their biological properties should prevent cardiovascular events such as myocardial infarction and stroke by a noninvasive strategy rather than helping in the traditional mechanical approach (bypass surgery, endarterectomy, or angioplasty). (Libby P, Ailkawa M (2002) Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med 8:1257–1262.)

Pharmacotherapy

Statins (3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors)

This lipid-lowering class of drugs efficiently lowers LDL cholesterol levels and reduces cardiovascular events significantly, even in patients with average LDL concentrations. Importantly, statins produce modest effects on the actual degree of arterial stenoses, reemphasizing that the functional state of the atherosclerotic plaque, and not merely its size, determines the propensity of a plaque to precipitate an acute coronary syndrome. This observation suggests that lipid lowering in and of itself may have actions beyond regression of stenoses. Indeed, several inflammatory markers that associate with cardiovascular disease risk, most notably ►C-reactive protein (CRP), decrease in response to statin treatment.

The antiinflammatory effects of statins likely result from their ability to inhibit the formation of mevalonic acid. Downstream products of this molecule include not only the end product, cholesterol, but also several isoprenoid intermediates that covalently modify ("prenylate") certain key intracellular signaling molecules. Statin treatment reduces leukocyte adhesion, accumulation of macrophages, MMPs, tissue factor, and other proinflammatory mediators. By acting on the MHC class II transactivator (CIITA), statins also interfere with antigen presentation and subsequent T-cell activation. Statin treatment can also limit platelet activation in some assays as well. All these results support the concept that in addition to their favorable effect on the lipid profile, statins can also exert an array of antiinflammatory and immunomodulatory actions.

Peroxisome Proliferator-Activated Receptor Agonists

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor family, and include three forms (α, γ, and δ) [4]. PPAR-α activators (fibrates such as gemfibrozil or fenofibrate) act as triglyceride-lowering/HDL-raising drugs, and PPAR-γ activators (thiazolidinediones such as pioglitazone or rosiglitazone) as insulin-sensitizing agents. The identity of endogenous PPAR ligands remains elusive, with recent work pointing towards a role for lipases such as lipoprotein lipase and endothelial lipase as well as lipoprotein substrates such as very low density lipoprotein (VLDL) and HDL in generating such ligands.

Current evidence suggests that PPAR activation may limit inflammation and hence atherosclerosis. Both PPAR-α and PPAR-γ can reduce T-cell activation, as shown by decreased production of IFN-γ. PPAR-α agonists also repress endothelial VCAM-1 expression and inhibit the inflammatory activation of vascular SMCs, while PPAR-γ agonists repress endothelial chemokine expression and decrease macrophage MMP production.

In humans, biomarker responses to PPAR agonists by and large support possible antiatherosclerotic benefits, although recent clinical trial results have not proven conclusive. Further large-scale trials should help define the role of PPAR agonist therapy on cardiovascular events in at-risk populations.

Angiotensin-Converting Enzyme Inhibitors/Angiotensin-Receptor Blockers

Agents that interfere with signaling of angiotensin II, originally designed to treat hypertension, may also have antiinflammatory effects relevant to atherosclerosis. In addition to its vasoconstrictor and sodium-retaining effects, angiotensin II may act as a proinflammatory cytokine that can elicit VCAM-1 and MCP-1 expression by endothelial cells. Treatment with inhibitors of angiotensin-converting enzyme can reduce signs of inflammation, thereby providing a potential link between antihypertensive and antiatherosclerotic therapy.

Aspirin (Acetylsalicylic Acid)

Randomized trials have clearly established that low-dose aspirin prevents arterial thrombosis, including first myocardial infarction in men, stroke in women, and recurrent vascular events among patients with known atherosclerotic disease. Due to the irreversible inactivation by acetylation of ►cyclooxygenases (COX)-1 and -2, key enzymes in platelet biology, only the generation of new platelets can reverse the antiaggregatory effect of aspirin. The products of COX-1 include thromboxane A2 (TXA2), which causes irreversible platelet aggregation and amplifies the platelet response secondary to stimuli such as thrombin, collagen, and adenosine diphosphate (ADP). Importantly, TXA2 is proatherogenic, and enhances endothelial adhesion molecule expression and subsequent leukocyte–endothelial interaction as well as vascular SMC proliferation. In addition to these specific effects of TXA2 directly abrogated by aspirin, platelet activation in general leads to the release of proinflammatory cytokines such as CD40L and PDGF, further heightening the inflammatory burden of atherosclerosis.

Future Directions

Vaccination Against Atherosclerosis. Parenteral immunization with oxidatively modified LDL can inhibit experimental atherosclerosis. This protection occurs in parallel with increased titers of antibody specific for the immunogen, and seems to depend mostly on humoral immunity. Although this approach remains unsubstantiated in humans, a vaccination strategy might protect against atherosclerosis and its complications, a proposition that would require rigorous testing in the clinic.

Mast Cell Regulation. Recent experiments have elucidated the deleterious role of mast cell activation in atherosclerotic mice. Atheromata from mast cell

deficient mice in compound mutation with atherosclerosis susceptibility demonstrate decreased lesion size, lipid deposition, T-cell and macrophage numbers, and apoptosis, but increased collagen content and fibrous cap development. Treatment of atherosclerotic mice with the mast cell stabilizer disodium cromoglycate yields similar results. This agent blocks the release of mast cell granular contents such as IL-6 and IFN-γ, which can activate other inflammatory cells present in the atheroma. In addition, treatment with disodium cromoglycate decreases plaque levels of cathepsins and MMPs, yielding plaques with features considered more stable in human lesions.

Given the routine use of mast cell stabilizers in the clinic, for example in the setting of asthma treatment, these preclinical results may stimulate clinical evaluation in humans.

►*Cannabinoid Receptors*. Of the two known cannabinoid receptors, CB2 is expressed predominantly on immune cells. In animals, activation of CB2 can ameliorate chronic inflammation in arthritis as well as experimental allergic encephalopathy. Recent findings suggest that cannabinoids can also benefit atherosclerosis. In atherosclerotic lesions, Δ9-tetrahydrocannabinol (THC) blocks IFN-γ secretion by T-cells and reduces macrophage infiltration by inhibiting the expression of the chemokine receptor CCR2. Interestingly, recent findings point toward PPAR-γ as an additional potential target for cannabinoid binding.

Determining whether cannabinoids may enter the list of antiatherosclerotic therapies will require further experimental evidence as well as clinical validation.

Conclusion

The immune response is central to atherosclerosis, from its initiation and development through its thrombotic complications. Indeed, our current understanding of the biology of this scourge differs greatly from the former perspective of atherosclerosis as a lipid storage problem. We now appreciate that the inflammatory activation state of the atheromatous plaque, which ultimately leads to a thin and rupture-prone fibrous cap, rather than the actual degree of luminal encroachment, influences the clinical manifestations of this disease. This enhanced understanding of plaque biology provides new insights into the diverse ways in which atherosclerosis can present clinically and why the disease may remain silent for prolonged periods of time, interrupted by acute complications.

Our new appreciation of the role of inflammation in atherosclerosis shows the way for translation of these novel biological insights to clinical practice, for example by aiding the identification of individuals at risk of adverse cardiovascular events [5]. In this context, inflammatory biomarkers such as CRP merit rigorous consideration for inclusion in risk assessment strategies. In addition, these scientific advances provide a framework to understand the mechanisms by which lifestyle modifications and certain medical therapies can reduce events by antiinflammatory actions that lead to stabilization of plaques. We can now conceive altering the biology of the atheroma rather than taking a mechanical approach to relieve stenosis by surgical or percutaneous revascularization. Finally, our expanded and deeper appreciation of the biology of atherogenesis should lead to new therapies as well as improved strategies for risk prediction and detection of silent disease, advances that should ultimately improve patient outcomes.

►HMG-CoA-Reductase-Inhibitors
►Peroxisome Proliferator-Activated Receptors (PPARs)
►ACE Inhibitors
►Antiplatelet Drugs

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Atherosclerotic Plaques

Atherosclerotic plaques are lesions in the arterial vessels which arise during the process of atherogenesis. Most cases of acute heart attacks are caused by rupture of an atherosclerotic plaque.

►Atherosclerosis
►HMG-CoA-Reductase-Inhibitors
►Antiplatelet Drugs
►ACE Inhibitors

Atopy

Atopy is the propensity to develop allergic reactions mediated by immunoglobulin E.

►Allergy
►Leukotrienes

ATP

Adenosine triphosphate (ATP) is a purine nucleotide involved in extracellular signalling, as well as acting as an intracellular energy source.

►Purinergic System

ATP-binding Cassette Transporter Superfamily

ATP-binding cassette (ABC) transporters (proteins) are characterized by having so-called ATP-binding cassette domains. ABC proteins function as pumps, channels, and channel regulators (receptors). They have multiple membrane-spanning segments and nucleotide-binding folds (domains) (NBFs or NBDs) in the cytoplasmic side, which contain highly conserved Walker motifs and an ABC signature sequence. Cystic fibrosis transmembrane conductance regulator, *P*-glycoprotein, canalicular multispecific organic anion transporter, and sulfonylurea receptor are typical ABC proteins.

►ABC Transporters
►MDR-ABC Transporters
►Multidrug Transporter
►Lysophospholipids
►Sterol Transporters
►ATP-dependent K^+ Channel

ATP-dependent K^+ Channels

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Synonyms

ATP-sensitive K^+ channel; ATP-regulated K^+ channel; K_{ATP} channel; SURx/Kir6.x channel

Definition

Key feature of K_{ATP} channels is their responsiveness to changes in intracellular ATP and ADP providing a means to couple electrical activity to cellular metabolism in many excitable cells (e.g., pancreatic beta-cells, neurons, and cardiac myocytes). K_{ATP} channels are assembled from alpha (►inward rectifier K^+ channel, Kir6.x) and beta (sulfonylurea receptor, SUR) subunits with obligate hetero-octameric stoichiometry (SUR/Kir6.x)$_4$. Four alpha subunits form the ion-conducting pore, which is surrounded by four beta subunits with receptor sites for drugs and ADP (Figs. 1a and b).

Basic Characteristics

Assembly

Trafficking from the endoplasmic reticulum to the cell surface is regulated by arginine-rich RKR motifs on both subunits in combination with a C-terminal signal on SUR. These motifs insure correct assembly of the complex and channel surface expression. ►14-3-3-proteins seem to be involved in this process. Shortly after synthesis, SUR1 and Kir6.2 presumably dimerize, protecting Kir6.2 from degradation. The SUR1/Kir6.2 dimers then assemble the octameric complex. SUR and Kir6.x subunits are the products of two pairs of genes on human chromosomes 11 and 12. *ABCC8* encodes for SUR1 (OMIM 600509) and lies in position 11p15.1, 5 kb upstream of the gene for Kir6.2 (*KCNJ11*, OMIM 600937). Encoding for SUR2A and SUR2B, *ABCC9* lies in position 12p12.1, 26.2 kb upstream of the gene for Kir6.1 (*KCNJ8*; OMIM 600935). SUR2A and SUR2B result from differential splicing of the terminal exon of *ABCC9*.

Tissue Distribution

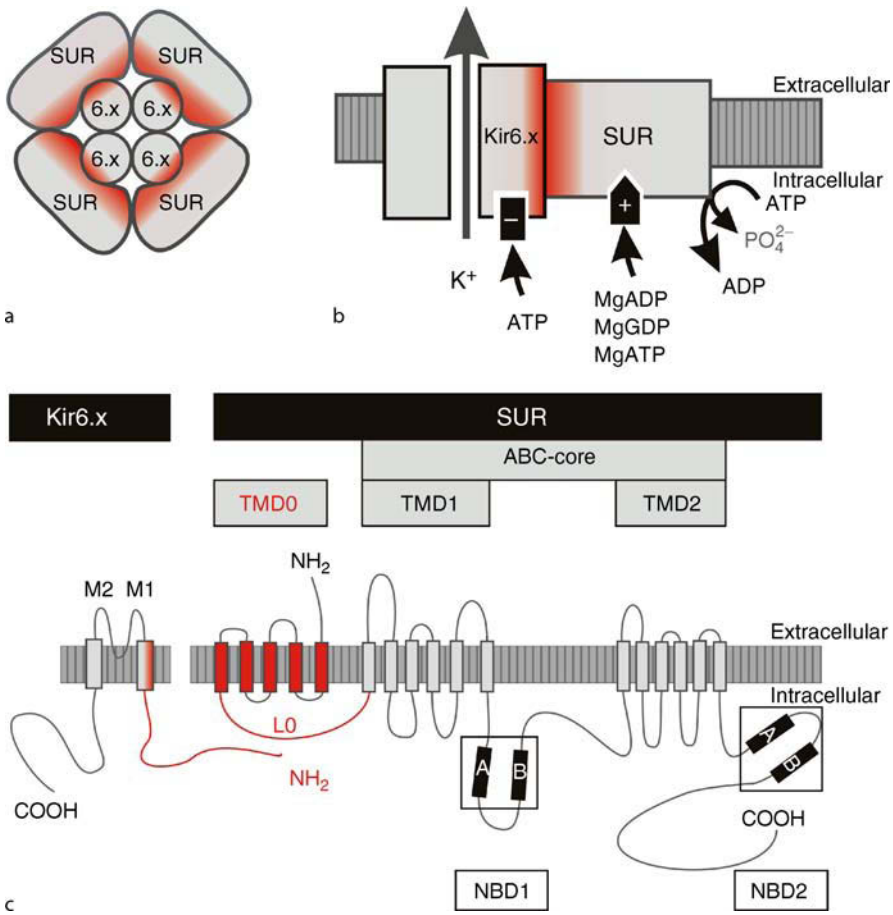
SUR1 assembles with Kir6.2, and (SUR1/Kir6.2)$_4$ channels are broadly distributed in the neuroendocrine system. (SUR2A/Kir6.2)$_4$ constitutes the K_{ATP} channels found in cardiac and skeletal muscle cells, and (SUR2B/Kir6.1)$_4$ those in vascular and nonvascular smooth muscle.

Kir6.x

Transmembrane helices M1 and M2 of the four Kir6.x subunits interact to form the ion-conducting pore of the channel. In analogy with a proposed mechanical mechanism for regulation of Kv channels (►voltage-gated K^+ channels), gating is presumably mediated by repositioning M1 and M2 through movement of a submembrane helix termed "the slide helix" (Fig. 2). The receptor site for inhibitory ATP has been localized at the interface between adjacent Kir6.x subunits, approximately 2 nm below the outer surface of the membrane.

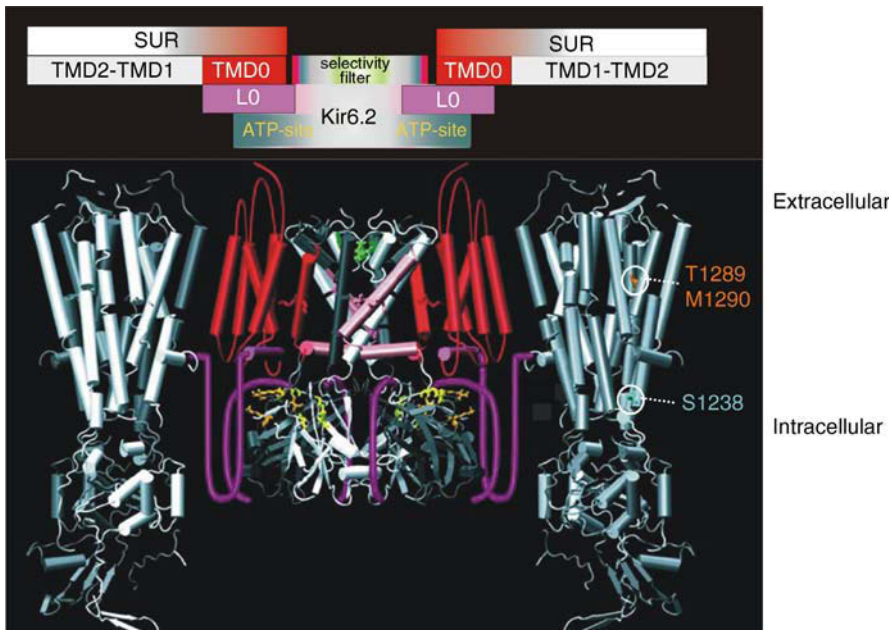
SURs

SURs are members of the ATP-binding cassette (ABC) family of proteins (►ABC transporters) with a typical ABC "core" consisting of two bundles of six



ATP-dependent K^+ Channels. Figure 1 (a) Tetradimeric architecture. K_{ATP} channels are assembled from alpha (Kir6.x) and beta (SUR) subunits with obligate hetero-octameric stoichiometry $(SUR/Kir6.x)_4$. Four alpha subunits form the ion-conducting pore, which is surrounded by four beta subunits. (b) Regulation by nucleotides. The receptor site for inhibitory ATP resides on the pore-forming Kir subunit, while activatory nucleotides bind to a separate nucleotide site on SUR. The NBDs of SUR bind and hydrolyse ATP. (c) Two-dimensional topology model of K_{ATP} channels. A and B symbolize the Walker A and B consensus motifs in the two NBDs, respectively; TMD: TransMembrane Domain, NBD: Nucleotide-Binding Domain, L0: intracellular linker between TMD0 and TMD1. Putative contact regions between Kir6.x (parts of M1 plus NH_2-terminus) and SUR (TMD0 plus L0) depicted in red.

transmembrane helices (TMD1 and 2) and two nucleotide-binding domains (NBD1 and 2) (Fig. 1c). Similar to several other ABCC proteins (i.e., ABCC1, 2, 3, 6, and 10), SURs have an additional amino terminal module that consists of a bundle of five transmembrane helices (TMD0). These additional helices are connected to the core via an intracellular linker termed "L0" (Fig. 2). In *ABCC8* and *9*, TMD0-L0 is the principal domain interacting with the Kir subunit. The SUR NBDs contain the canonical phosphate-binding Walker A and B motifs, the Q-loop, the signature sequence, and the H-loop, hallmarks of the ABC family. The SURs were among the first ABC proteins recognized to have degenerate, nonsymmetric NBDs with a noncanonical signature sequence, FSQGQ versus LSGGQ, in NBD2 and an aspartate (D) in place of the usual glutamate (E) adjacent to the highly conserved D in the Walker B motif. In analogy with other ABC proteins, ATP binding and hydrolysis are expected to drive dimerization of the SUR NBDs and produce concomitant rearrangements of TMD1 and TMD2. Typically coupled to substrate transport, SURs appear unique by transducing these conformational changes in modulation of channel gating. TMD0 plus subsequent intracellular loop L0 appear to be critical in this process. Analogous to the voltage sensor in Kv2.1 channels, L0 presumably affects gating through direct interaction with the Kir "slide helix" (Fig. 2). Localization of the site of action for activatory Mg-nucleotides (e.g., MgADP, MgATP, MgGDP) on SURs is still controversial. Cytosolic [MgADP] might enhance channel activity by lowering the off-rate of MgADP from NBD2 through product



ATP-dependent K+ Channels. Figure 2 Three-dimensional homology model of K_{ATP} channels. *Green*: pore selectivity filter, horizontal *pink* cylinder: the "slide helix", bundle of five *red* cylinders: TMD0, *purple*: L0 + distal parts of Kir amino termini, *gold* and *yellow*: residues involved in ATP-binding, S1238: critical for SU-binding, T1286 plus M1290 critical for K_{ATP}CO-binding. (Panel modified from [3]).

inhibition. Alternatively, evidence has been presented that Mg-nucleotides exert their activatory effect not by interaction with the catalytic NBD sites but by binding to a separate nucleotide site mainly formed by TMD2.

Role in Pancreatic Beta-Cells

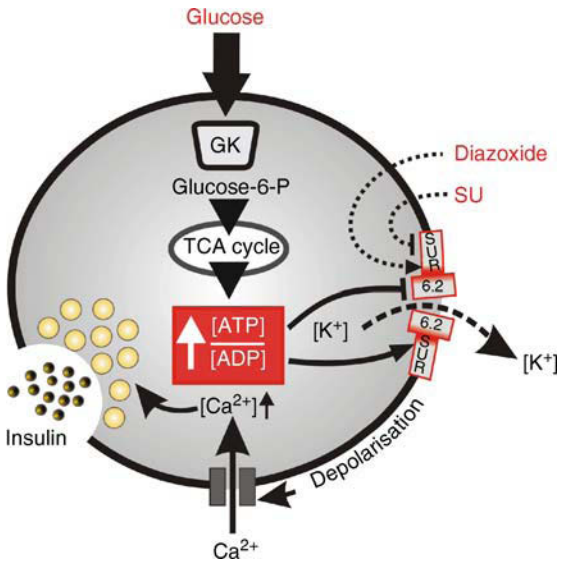
The role of K_{ATP} channels in cellular function is best understood in pancreatic beta-cells (Fig. 3). Insulin release is triggered by a rise of intracellular [Ca^{2+}] that results from Ca^{2+} influx through voltage-gated Ca^{2+} channels in the beta-cell plasma membrane. Under low serum [glucose], the Ca^{2+} channels are shut because the membrane is held hyperpolarized by potassium outward currents through K_{ATP} channels. When blood glucose levels are elevated, glucose is rapidly equilibrated across the beta-cell membrane via the GLUT2 transporter (►glucose transporters). In the next rate-limiting step, it is phosphorylated by glucokinase. Subsequent glycolysis and mitochondrial metabolism leads to changes in intracellular concentrations of adenine nucleotides and closure of K_{ATP} channels. This produces a membrane depolarization that opens ►voltage-dependent Ca^{2+} channels, initiating beta-cell electrical activity and Ca^{2+} influx. The subsequent rise in $[Ca^{2+}]_i$ triggers insulin release. In resting beta-cells, 98% of plasmalemmal K_{ATP} channels are closed and hence, insulin release is induced by reducing flux through residual 2%. Cytosolic free [ATP] not below 1mM, however, predicts that even in the resting state channel activity should be completely suppressed and a definite answer to this "ATP-paradox" is still pending. One possibility is that the concentration–inhibition curve is shifted to higher ATP levels through stimulatory effects of Mg-nucleotides. Alternatively, [ATP] in the immediate vicinity of the Kir6.2 binding pocket might be much lower than estimated by measurement of total cytosolic ATP.

Modulators

Phosphoinositides, long-chain acyl coenzyme As (CoAs), G proteins, and phosphorylation have been shown to modulate K_{ATP} function. The physiologic role of these modulators, however, has still to be established. Lower pH activates K_{ATP} channels by reducing their sensitivity to inhibitory ATP and may play a role in the regulation of vascular tone during hypercapnic acidosis. Zn^{2+} activates SUR1/Kir6.2 K_{ATP} channels via binding to two histidines on the extracellular face of SUR1. Zn^{2+} is present in high concentations in various regions of the CNS. In pancreatic islets it is coreleased with insulin and may play an autocrine role or serve to attenuate glucagon release from alpha cells.

Neuroprotection

Substantia nigra pars reticulata is the area with highest neuronal activity and metabolic rate in the brain. This region also shows highest expression rates of SUR1/Kir6.2 channels. These K_{ATP} channels are present



ATP-dependent K^+ Channels. Figure 3 Stimulation of insulin secretion in the beta-cell. A rise in extracellular [glucose] increases phosphorylation of glucose by glucokinase (GK), the rate-limiting enzyme for the metabolism of glucose in the beta-cell. Glycolysis results in conversion to pyruvate, which preferentially enters the mitochondria and fuels the tricarboxylic acid (TCA) cycle. The resultant increase in the [ATP/ADP] ratio leads to closure of K_{ATP} channels, depolarization of the plasma membrane, opening of voltage-gated Ca^{2+} channels, and an increase of the cytosolic Ca^{2+} concentration, which triggers the exocytosis of insulin. Sulfonylureas (SUs) inhibit the K_{ATP} channel and initiate the same chain of events, whereas diazoxide activates the channel, hyperpolarizes the beta-cell, lowers cytosolic Ca^{2+}, and inhibits insulin secretion. Glucose-6-P, glucose-6-phosphate.

throughout all regions of the CNS and confer neuroprotection by rapid hyperpolarization and thus minimization of energy consumption during metabolic stresses, like hypoxia and ischemia. In substantia nigra, SUR1/Kir6.2 channels confer a specific sensor function for ATP depletion thus enabling an early generalized protective response. Fast electrical silencing of GABAergic neurons within substantia nigra results in prevention of generalized seizure by disinhibition of distant projection areas (e.g., ventral thalamic nuclei, nuclei superior colliculi, pedunculopontine nucleus).

Central Glucose Homeostasis

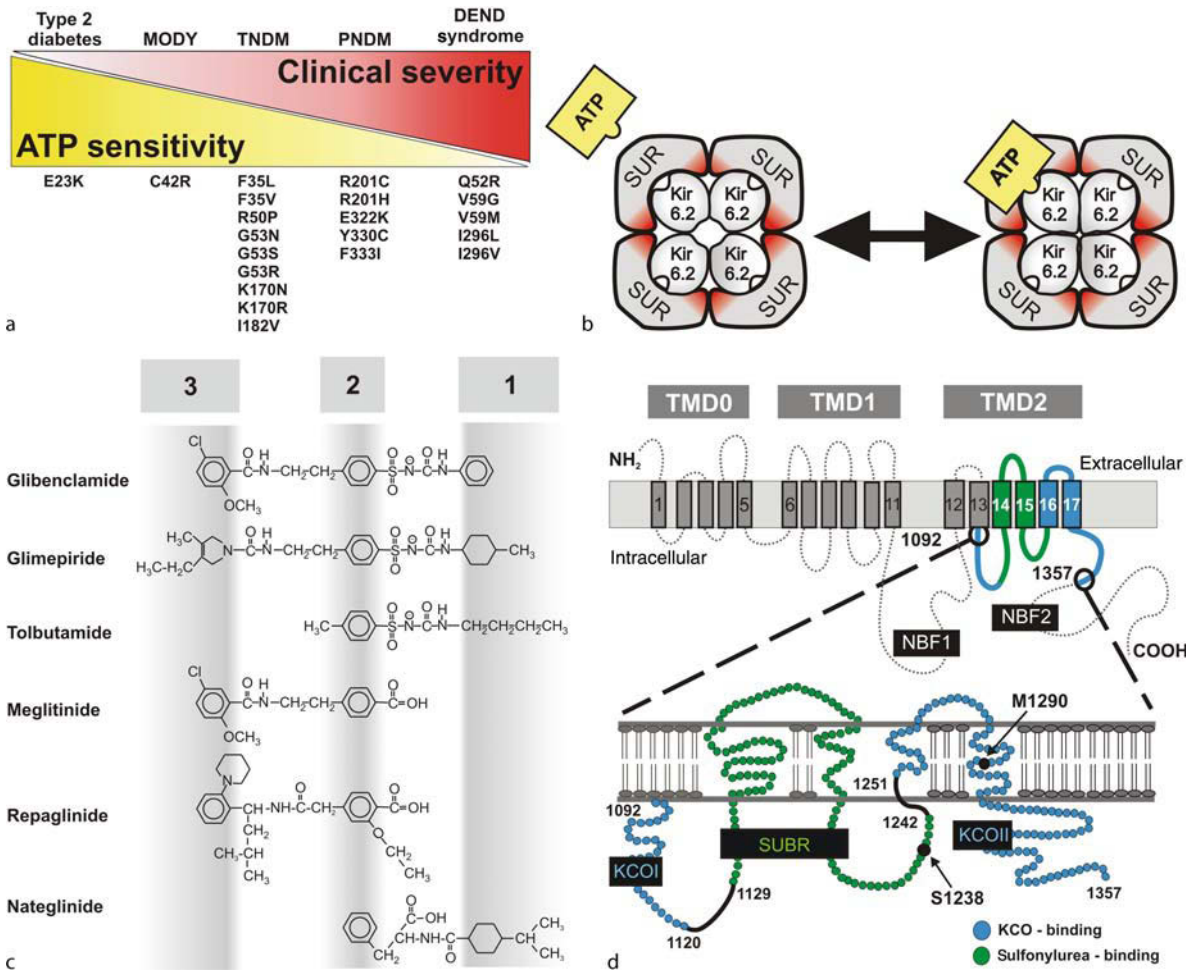
Central control of glucose homeostasis critically depends on the brain's ability to sense extracellular [glucose]. Within hypothalamus at least two types of neurons were identified which are presumably involved in this process. They are either glucose excited or glucose inhibited. Both types of neurons appear to be involved in the control of feeding, hepatic gluconeogenesis, and glucagon and epinephrine secretion. In glucose excited neurons, the sensing mechanism appears to be based on SUR1/Kir6.2 channels, analogous to that in pancreatic beta-cells. Consistently, glucose sensing was lost in Kir6.$2^{-/-}$ mice or intracerebral application of glibenclamide.

Hyperinsulinemic Hypoglycemia

Mutations in SUR1 and Kir6.2 are an established cause of ►hyperinsulinemic hypoglycemia of infancy (HHI), characterized by excessive insulin release independent from blood [glucose]. Most cases of HI are sporadic and the disease may result from homozygous or heterozygous mutations. Mutations in SUR1 (*ABCC8*) are the most common cause of HI, accounting for almost 50% of cases. More than 100 mutations have been described, distributed throughout the gene. Class I mutations are characterized by loss of K_{ATP} channels in the plasma membrane. This may result from impaired SUR1 synthesis, abnormal SUR1 maturation, defective channel assembly, or faulty surface membrane trafficking. Class II mutations impair the ability of MgADP to stimulate channel activity. In general, class II mutations cause a milder phenotype because residual channel activity remains. They can also be causative for leucine-sensitive HI (e.g., R1353H). Most class II mutations reside in the NBDs of SUR1. To date in Kir6.2 (*KCNJ11*) five mutations have been identified that cause HI. They also act by reducing, or abolishing K_{ATP} channel activity in the surface membrane, thus inducing permanent beta-cell depolarization, uncontrolled insulin release, and hypoglycemia.

Neonatal Diabetes

Neonatal ►diabetes mellitus (NDM) is characterized by hyperglycemia within the first 6 months of life. It is a rare disorder affecting 1 in 400,000 live births, and it may be either transient (TNDM) or permanent (PNDM). Approximately 50% of PNDM cases result from heterozygous gain-of-function mutations in Kir6.2 [2]. To date, more than 20 mutations in *KCNJ11* have been reported to cause NDM. They form striking clusters in the putative ATP-binding site (Fig. 2) and the cytosolic pore of the channel. Mutations within residues, V59 and R201, show highest frequency. A series of gain-of-function mutations cause a range of phenotypes with increasing severity (Fig. 4a). In most cases, PNDM is observed without additional symptoms. These patients show weak insulin release in response to i.v. glucose but may respond to sulfonylureas. Other mutations cause more severe phenotypes with delayed speech, walking, and muscle weakness in addition to neonatal diabetes. A third class of mutations (e.g., Q52R, V59G) produce the severe DEND syndrome (developmental delay, epilepsy, and neonatal diabetes), characterized by marked developmental retardation, muscle weakness, epilepsy, and dysmorphic



ATP-dependent K+ Channels. Figure 4 (a) Genotype–phenotype correlation for gain-of-function mutations in Kir6.2. (b) Stoichiometry of ATP-induced channel closure. (c) Structure of sulfonylurea and nonsulfonylurea hypoglycemic agents. *Grey*: lipophilic centers 1–3. (d) Regions within SUR critical for drug binding.

features besides hyperglycemia. Mutations that cause TNDM or maturity onset diabetes of the young (MODY) are also found. All PNDM mutations studied to date act by reducing the potency of ATP to close the channel and this effect correlates quite well with clinical phenotype. Channels are closed if just one of the four sites for inhibitory ATP is occupied and thus, strongly reduced ATP sensitivity is only observed in channels with four mutated subunits (Fig. 4b). Therefore, a marked increase in open probability is found in no more than 6% of the channels of heterozygotes. This effect, however, is sufficient to completely abolish insulin release (see earlier). In extrapancreatic tissues expressing Kir6.2 (skeletal muscle, cardiac muscle, and neurons throughout the brain) dependence on open probability appears less critical, explaining lack of additional symptoms in heterozygote patients with PNDM mutations (e.g., Q52R, V59G, and I296L). Mutations causing the severe DEND phenotype affect ATP sensitivity by increasing spontaneous open probability of the channel. Here, one mutated subunit is sufficient to strongly reduce ATP sensitivity thereby inducing additional symptoms in extrapancreatic tissues. To date two mutants within SUR1 are known (I1424V and H1023Y), resulting in hyperactive channels and PNDM.

Type 2 Diabetes Mellitus

Large-scale association studies indicate that a common variant (E23K) in Kir6.2 is strongly associated with an enhanced susceptibility to type 2 diabetes [1]. Although the effect is small, the high prevalence of the K allele (34%) makes this a significant population risk. The E23K polymorphism confers an increase in intrinsic open probability, with a consequent reduction in ATP sensitivity, and enhanced activation by Mg-nucleotides and long-chain acyl-CoAs. In glucose-tolerant subjects it was demonstrated to be associated with reduced insulin and increased glucagon secretion. Polymorphisms in HNF1alpha, HNF4alpha, and glucokinase (MODY), and in genes involved in mitochondrial metabolism, have also

been associated with an increased risk of type 2 diabetes. They might influence disease susceptibility by impairing metabolic regulation of K_{ATP} channel activity.

Arrhythmias and Heart Failure

Data from Kir6.2 knockouts suggest that SUR2A/Kir6.2 channels in the heart are required for the adaptive response to acute stress and chronic hemodynamic load. Thus, deficiency is presumed to be associated with stress-induced arrhythmias, defective structural remodelling, calcium-dependent maladaptation, and predisposition to heart failure. Consistently, two mutations were identified in exon 38 of the human *ABCC9* gene that resulted in dilated cardiomyopathy. A male who was diagnosed at age 55 and died from heart failure at age 60, had a 3-bp deletion followed by a 4-bp insertion (4570–4572delTTAinsAAAT) causing a frameshift at leu1524 and introducing four anomalous terminal residues followed by a premature stop codon. The patient died at age 60 and had no family history of dilated cardiomyopathy. A female who was diagnosed at age 40 had at 1513 an alanine to threonine substitution. Her father was diagnosed at age 54 and died at age 55 of heart failure. All three individuals had ventricular tachycardia with normal coronary angiography. Both mutations were not identified in unrelated healthy controls. The C-terminus of SUR proteins contributes to K_{ATP} channel trafficking, and the frameshift and missense SUR2A mutants, reconstituted with Kir6.2, had reduced expression in the plasma membrane. Yet, mutant K_{ATP} channel complexes formed functional channels with intact pore properties. Residues ala1513 and leu1524 flank the C-terminal beta-strand in close proximity to the signature Walker A motif, required for coordination of nucleotides in the catalytic pocket of ATP-binding cassette proteins. Replacement of ala1513 with a sterically larger and more hydrophilic threonine residue or truncation of the C-terminus caused by the frameshift would disrupt folding of the C-terminal beta-strand. Nucleotide-induced K_{ATP} channel gating was aberrant in both channel mutants, suggesting that structural alterations induced by the mutations distorted ADP-dependent pore regulation through SUR2A.

Prinzmetal Angina

Mice lacking the Kir6.1 gene had a phenotype resembling Prinzmetal angina. Spontaneous vasospasms of hypercontractive coronary arteries were associated with ST elevation, atrioventricular block, and a high rate of sudden death. Results presume *KCNJ8* and *ABCC9* as candidate genes for human disease.

Drugs

K_{ATP} channels are the targets for two classes of therapeutic agents, hypoglycaemic drugs like glibenclamide or nateglinide and potassium channel openers like diazoxide or P1075. In pancreatic beta-cells glibenclamide and analogous substances stimulate insulin secretion by closing K_{ATP} channels while diazoxide shifts the plasma membrane potential toward the potassium equilibrium potential, thus reducing electrical activity and inhibiting insulin release.

Sulfonylureas and Analogs

Hypoglycemic drugs comprise sulfonylureas (e.g., tolbutamide, glibenclamide, glimepiride) and nonsulfonylureas (e.g., meglitinide, repaglinide, nateglinide) (Fig. 4c). Except for repaglinide these compounds are weak organic acids (pKa values 3.1–6.8). Their protonated species diffuse rapidly across the plasma membrane, dissociate and gain access to their binding site via the cytoplasmic face of SURs. Structure–activity data suggest that high affinity SUR1 selective ligands require three lipophilic centers within their molecule. Center 1 (e.g., a cyclohexyl group in glibenclamide or a cyclohexane substituted with an isopropyl group in nateglinide) is the major determinant of selectivity toward SUR1. Drugs lacking this part of the molecule (e.g., meglitinide) show same affinity for SUR1 and SUR2 isoforms.

Localization of the Binding Site

Affinity of glibenclamide for SUR1 (K_D 0.72 nM) is 350 higher than that for SUR2A/B. Based on this difference a 114 amino acid segment within TMD2 (SUBR; C1129 – T1242) was identified as putative part of the receptor binding site (Fig. 4d). Substitution of S1238 within this region by Y strongly reduced glibenclamide affinity without affecting binding of meglitinide. Thus this residue was presumed to contribute to interaction with the center 1 region of the ligands. Centers 2 and 3 seem necessary for interaction with either of the SUR isoforms. Both L0 and the proximal amino terminus of Kir6.2 are likely to participate in formation of this part of the binding pocket.

Mechanism of Action

Every SUR subunit carries one site for inhibitory drugs, and hence there are four of these sites per channel complex. Analogous to ATP-induced inhibition (see earlier) occupation of just one of these sites is sufficient to close the channel. This effect is mediated by egalizing Mg-nucleotide-induced channel activation.

Therapeutic Use

Sulfonylureas and glinides are largely used to control hyperglycemia in the treatment of T2DM. In addition many patients with NDM show sulfonylurea sensitivity. Thus, in these patients the drugs appear as an alternative to insulin injections. To date, good glycemic control has been reported for several patients. In DEND patients, therapy with sulfonylureas and analogs might prove

helpful in the control of symptoms resulting from enhanced channel activity in extrapancreatic tissues (see earlier, paragraph "Neonatal Diabetes").

K_{ATP} Channel Openers

K_{ATP} channel openers (K_{ATP}COs) comprise a structurally diverse group of compounds (e.g., pinacidil, P1075, levcromakalim, rilmakalim, minoxidil sulphate, nicorandil, diazoxide) which exert their activatory effect on the channels by interaction with SURs. Some K_{ATP}COs are SUR1-selective (e.g., NN414), most, however, show clear selectivity for SUR2 isoforms (e.g., K_D of P1075 for SUR2B = 11 nM and for SUR1 = 1.02 mM).

Localization of the Binding Site

The cytosolic loop between TMs 13 and 14 (KCO I) and TMs 16–17 (KCO II) were identified as critical for K_{ATP}CO binding to SURs (Fig. 4d). T1286 and M1290 appeared to be particularly important. Close local association of sulfonylurea and KCO binding regions might represent the structural basis for negative allosteric coupling of the sites.

Mechanism of Action

K_{ATP}COs do not bind to SURs with defect NBDs indicating that affinity of the receptor site depends on the catalytic state. Similarly, defective NBDs egalize Mg-nucleotide-induced channel activation and are thus causative for HI (see earlier). In analogy to sulfonylureas K_{ATP}CO-induced channel activation is mediated by interaction with one of the four sites per tetradimeric complex. Occupation of additional sites did not induce stabilization of the open state.

Therapeutic Use

Diazoxide, pinacidil, cromakalim, minoxidil sulfate, and nicorandil have been extensively studied. Although there is a broad spectrum of potential therapeutic applications (e.g., hypoglycemia, hypertension, arrhythmias, angina pectoris, cardiac ischemia, asthma), at present none of these drugs is in widespread clinical use. Nicorandil is approved for the treatment of coronary artery disease. Minoxidil sulfate serves for the therapy of severe hypertension that responds poorly to other hypertensive medications. In addition, it is applied topically to stimulate hair growth. Diazoxide is used to suppress excessive insulin secretion in forms of hyperinsulinism (HI) with responsive K_{ATP} channels. HI caused by mutations in glucokinase (*GCK*), glutamate dehydrogenase (*GLUD1*), or short-chain l-3-hydroxyacyl-CoA dehydrogenase (*SCHAD*) responds well to diazoxide. Severe forms of HI caused by mutations in SUR1 or Kir6.2, however, are refractory to diazoxide and require subtotal pancreatectomy (see earlier, paragraph Hyperinsulinemic Hypoglycemia). In some HI SUR1 mutants (e.g., R1349H) diazoxide was shown to act as chemical ►chaperone and correct defective surface trafficking. This effect might prove valuable in future HI therapy.

►K^+ Channels
►Voltage-gated K^+ Channels
►Inward Rectifier K^+ Channels
►Diabetes Mellitus
►Antidiabetic Drugs other than Insulin

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ATP-powered Pump

►Table appendix: Membrane Transport Proteins

ATP-regulated K^+ Channel

►ATP-dependent K^+ Channel

ATP-sensitive K^+ Channel

►ATP-dependent K^+ Channel

Atrial Fibrillation

Cardiac arrhythmia with a rapid and irregular activity in different areas within the upper chambers (atria) of the heart is also known as atrial fibrillation.

►Cardiac Glycosides
►Antiarrhythmic Drugs

Atrial Natriuretic Peptide

The atrial natriuretic peptide (ANP) belongs to a family of hormones that have structural similarity and some biological actions in common, such as natriuresis and haemoconcentration. It is synthesized and secreted by the cardiac atrium in response to increased atrial pressure. ANP is believed to act physiologically in an opposing manner to AVP.

►Guanylyl Cyclases
►Vasopressin/Oxytocin

Attention Deficit Hyperactivity Disorder

Synonyms

ADHD

Definition

ADHD is a disorder of childhood. Key features of Attention Deficit Hyperactivity Disorder (ADHD) – with a highly differing prevalence of 0.1–10% – are distractibility and difficulties in sustaining attention and focusing on a task. These symptoms are associated with impulsiveness, regardless of consequences. Comorbidity is high; boy to girl ratio is 4:1. The diagnosis is made using the diagnostic and statistical manual of the American Psychiatric Society (DSM-IV). There is currently no biological test to confirm the diagnosis. The DSM-IV assessment distinguishes between three subtypes of ADHD where (i) inattentiveness, (ii) hyperactivity-impulsivity, or (iii) a combination is prevalent. The period of formal schooling is usually the most difficult life phase for persons with ADHD. At later stages, these individuals often find occupational or educational niches that accommodate their behavioral and cognitive idiosyncrasies. About 50% of children with ADHD continue to manifest dysfunctional symptoms into adulthood. There is relatively poorer occupational and educational outcome, greater psychiatric comorbidity than in control subjects, and significantly higher rates of socialization disorders and substance use disorders in adults in whom ADHD symptoms persist.

The most common treatment of ADHD is pharmacological. Psychostimulant drugs such as methylphenidate and amphetamine or atomoxetin, an inhibitor of the noradrenaline transporter can be prescribed. These agents elicit the non-exocytotic release of noradrenaline, serotonin, and dopamine via their cognate cell surface transporters. They have considerable abuse liability.

►Neurotransmitter Transporters
►Psychostimulants

AUC

AUC is the area under the (drug) concentration time curve.

►Area under the Curve
►Pharmacogenetics

Autacoid

Autacoids are literally ‘self-medicating agents’ that are liberated from or produced by cells in response to a stimulus. They differ from hormones in that they usually act locally after release, rather than reaching their target organ via the bloodstream.

►Emesis
►Prostonoids
►Histaminergic System

Autoantigen

Structures expressed in the organs of an individual against his own immune system can mount an immune response. Autoantigens can be organ specific (e.g. insulin) or present in all cells (e.g. DNA).

►Autoimmune Disease
►Immune Defense

Autocrine

A mode of action of a molecular messenger such as a cytokine or hormone in which the molecule binds to receptors on, and affects the function of, the cell type that produced it.

Autoimmune Disease

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Definition

The immune system provides powerful defense mechanisms against infective or toxic agents. Its hallwork is the high specificity with which harmful noxes can be recognized and discriminated. Although usually prevented, the immune system sometimes can react against components of the own body resulting in a great variety of disorders, the autoimmune diseases.

Basic Mechanisms

To survive in an environment which contains a plethora of life threatening infectious agents and other harmful noxes, higher organisms have developed special defense mechanisms contained in the immune system (→ immune defense). In vertebrates, it is based on two arms. The phylogenetically older part (which can be traced back to unicellular organsims) in human beings is represented by the phagocytic leukocytes such as the monocytes/macrophages and the various granulocytes. These cells possess a restricted number of receptors, including the Toll like receptors, which recognize structural patterns common in different strains of virusses, bacteria, fungi or parasites. The major defense mechanisms consist in phagocytosis and subsequent intracellular enzymatic degradation. This is supported by the secretion of many mediators which together form an inflammatory response (→ inflammation). This "innate immunity" is present from birth, and its reaction remains identical throughout life.

Later in evolution, the second arm evolved, built up by lymphocytes. These cells exist in two main classes: B-lymphocytes which differentiate in the bone marrow, and T-lymphocytes which differentiate in the thymus. B-lymphocytes secrete antibodies into the body fluids. T-lymphocytes exert cellular defense mechanisms, such as killing of e.g. virus infected cells. They also play a central role in regulating immune reactions (→ immune defense). The novel acquisition of this "adaptive immunity" consists of two connected properties: specificity and memory. Lymphocytes can recognize and distinguish an extremely large number of molecular structures (termed in immunology antigens); in human beings this ranges up to about 10^{18} of which approximately 10^8 are realized in an individual. Each lymphocyte carries only receptors of a single specificity. When mounting an immune response – e.g. against an invaded bacterium – cells recognizing their respective antigen proliferate and acquire their specific functions implicated in the defense mechanisms. Part of these antigen specific cells develop into memory cells, which possess the capacity to react faster and even more efficient against the same antigen (or the infective bearing this antigen). It is this property that allows an individual to adapt to an environment containing specific infectious agents.

The adaptive immune system contains effector mechanisms of its own such as cytotoxic T-lymphocytes. In most instances, however, it relies on the mechanisms of the innate immune system. Not only directs it the cells of the innate system specifically to the antigen, but also strongly enhances their capacity to deal with harmful noxes. Thus B-lymphocytes release antibodies – secreted forms of their antigen receptor – which bind to their antigens and thereby recruite and activate the phagocytic cells, and also soluble effector systems such as complement. T-lymphocytes in contact with their cognate antigens secrete (→) cytokines which boost the activity of the cells of the innate immune system.

Self Tolerance

The antigen receptors are proteins. A simple calculation shows that the antigen receptor cannot be inherited. More than 10^8 specificities, i.e. different proteins by far exceed the number of the 25–30,000 genes existing in human beings. The antigen receptors thus must be generated during the development of individual T- or B-lymphocytes. Both antigen receptors are composed of two chains. Although B- and T-cell receptors are different proteins, their generation follows closely related rules. For each of the respective receptor chains there exist inherited in the germ line two to three clusters containing up to less than 100 gene segments. These are assembled in the developing lymphocyte by somatic recombination to form the antigen binding part of the receptor chain resulting in a large combinatorial diversity. This is much increased by the insertion of random nucleotides at the junction of two gene segments during the somatic recombination.

It is clear that generated by such a random process the initial repertoire of antigen specifity in all individuals of human beings should be quite similar. This implies that initially in each individal there develop lymphocytes which recognize antigens of this very individual, i.e. ►autoantigens.

As most humans are healthy, at least do not destroy their own organs, there must exist mechanisms which eliminate the ►autoreactive lymphocytes. For T-lymphocytes this happens in the thymus. When cells with functional antigen receptors develop, those which react with self (= auto) antigens present in the thymus with high affinity undergo apoptosis (= programmed cell death). An elegant mechanism governed by the

transcriptional regulator AIRE (autoimmune regulator) provides that in special epithelial cells of the thymus tissue antigens of all organs of the body are ectopically (i.e. in an unusual place!) expressed. Those cells which leave the thymus to become the functional T-lymphocytes of the body thus exhibit the "central ►self tolerance" by not reacting with ►autoantigens in the healthy individual. Similar mechanisms are also operative for B-lymphocytes in the bone marrow. The self tolerance predominantly is secured by T-lymphocytes.

The mechanisms of the central self tolerance implies that T- and B-lymphocytes bearing receptors which recognize autoantigens with low affinity are present in the body. These cells are held under control by the mechanisms of peripheral tolerance. Several mechanisms contribute to this (Fig. 1). That T-lymphocytes are activated requires the binding of the antigen to its antigen receptor ("signal 1"), but also accessory signals (termed "signal 2") are necessary. The antigen (peptide) has to be presented to the T-lymphocytes bound to molecules of the major histocompatibility complex (MHC); to the central T-helper cells bound to MHC class II molecules present predominantly on dendritic cells, macrophages, or B-lymphocytes (→ immune defense).

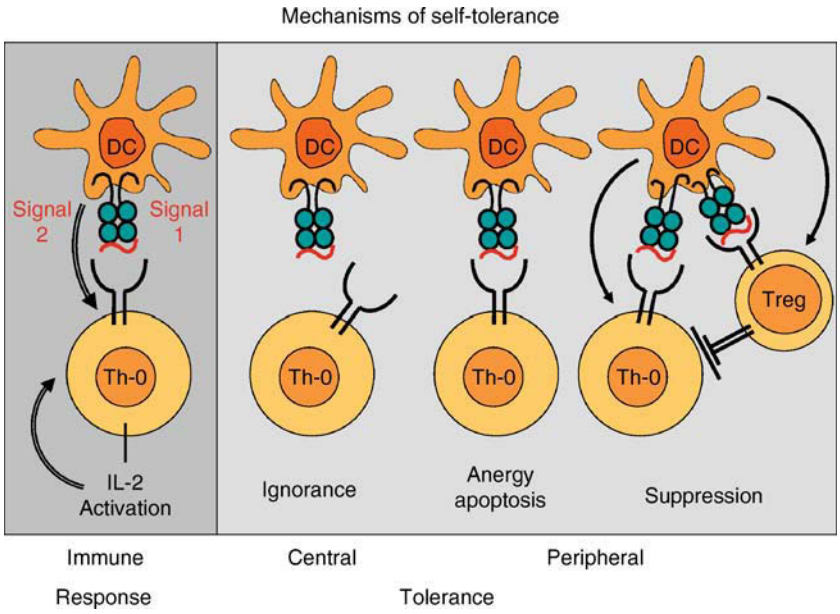
Peripheral self tolerance ensues when the strength of the antigen binding is below a certain threshold, which due to central tolerance holds generally for autoantigens. Also, self antigens elicit only weak signals 2, in sharp contrast to infectious agents. The generation of negative regulating cells (Treg), too, contributes to self tolerance

Breaking Self-Tolerance Leads to Autoimmune Disease

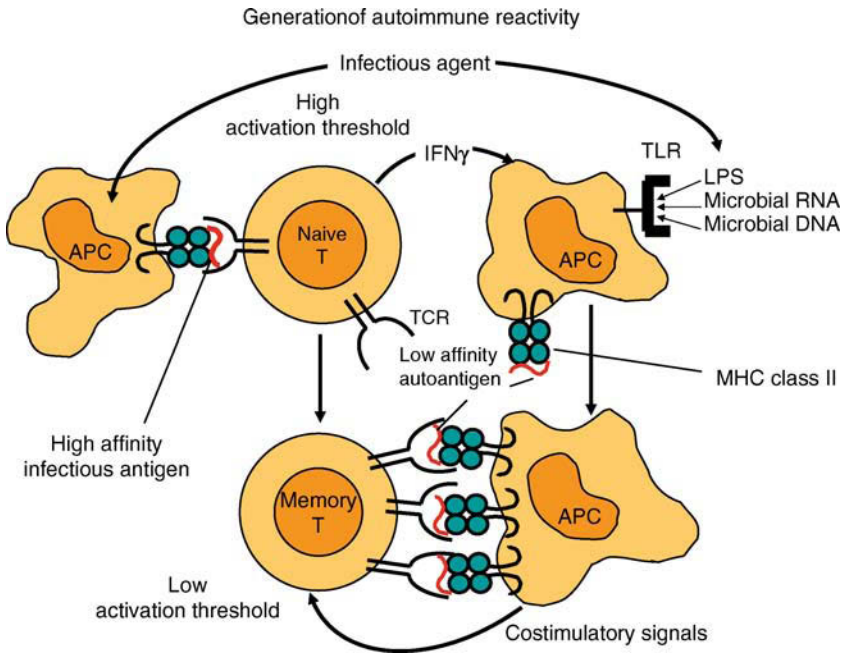
How self-tolerance is broken to result in autoimmune reactions leading to disease is one of the central issues that are still not completely understood. The crucial point is how the reaction barrier in T-lymphocytes is overcome. The trigger must come from the outside; accumulating evidence points to an infection. As T-lymphocytes recognize as their antigen short peptides (below 25 amino acids) one can imagine that proteins of the host as well as of microbes contain short stretches of a similar amino acid sequence. Indeed, numerous examples for this have been documented. Such a related microbial peptide can be bound by a T-cell receptor with an affinity above the threshold for activation. Moreover, other structures of the microbe can effectively generate signal 2 in the antigen-presenting cells. Both together then lead to activation of the T-lymphocytes. These proliferate and also provide sufficient help for the B-lymphocytes to also become activated.

Part of these T-lymphocytes transform into memory cells. These cells are different from their ancestors in that they are activated by a much lower antigen binding strength and also much less depend on signal 2. Now self-antigens can activate these T-lymphocytes. As during activation continuously new memory cells are formed, autoreactivity is sustained and autoimmune disease follows (Fig. 2).

Although this may suffice, additional factors contribute to this process. Activated T-lymphocytes secrete amongst other cytokines also interferon γ which



Autoimmune Disease. Figure 1 Mechanisms of self tolerance. DC, dendritic (antigen presenting) cell; T, T-lymphocyte; Th, T helper lymphocyte; Treg, T regulatory lymphocyte. For details see text.



Autoimmune Disease. Figure 2 Generation of autoreactivity. APC, antigen presenting cell; IFN, interferon; LPS, lipopolysaccharide; MHC, major histocompatibility complex; T, T-lymphocyte; TCR, T cell (antigen) receptor; TLR, toll like receptors. For details see text.

upregulates the expression of MHC molecules and thus improves antigen presentation. The activated antigen presenting cells also enhance the expression and secrete costimulatory molecules.

The activated T- and B-lymphocytes recruite and activate the cells of the innate immune system which mount an inflammatory reaction which impedes and eventually destructs the inflicted organ. On the other hand, by expressing and secreting costimulatory molecules the inflammatory cells also contribute to the activation of T-lymphocytes. Moreover, antigens not accessible to lymphocytes before and ("hidden antigens") may be exposed, such as cartilage. The local milieu of an inflammation thus not only executes an autoimmune reaction converting it into a disease, but also plays a central role in perpetuating autoimmunity.

Autoimmune Diseases

►Autoimmune diseases may inflict on each organ or cell. Manifestations range from affecting a single cell type and its specific function (such as the β-cell of the islands in the pancreas) to systemic diseases which have a detrimental effect on an entire organ system (e.g. the vasculature) of even many different organs. Table 1 summarizes some clinically important diseases.

Also, the outcome covers a large spectrum. Autoantibodies can specifically block an important protein (such as the gastric intrinsic factor required for the uptake of orally taken vitamin B12), or the receptor for → acetylcholine (as in myasthenia gravis), but also can lead to a continuous stimulation of a receptor (as in autoimmune thyreoiditis). Autoimmune reactions can lead to the destruction of a single cell type, the function of which is vital (as in type 1 diabetes (IDDM), where the loss of the β-cells results in the loss of insulin synthesis). In many cases autoimmune disease is associated with chronic inflammation. In fact, most chronic inflammatory diseases are today generally regarded as autoimmune with respect to their mechanisms of perpetuation. This includes rheumatoid arthritis (RA), chronic inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis, kidney diseases (glomerulonephritis, GN), mulitple sklerosis (MS), or arteriosklerosis. Accumulating evidence also points to an autoimmune component in a vast array of diseases up to psychiatric syndromes such as depressive disorders.

Type 1 Diabetes Mellitus (IDDM)

Blood sugar (blood glucose) in human beings is controlled by the secretion of (→) insulin by the beta (B- or β-) cells of the islands of Langerhans in the pancreas. Loss of insulin synthesis leads to (→) diabetes. Type 1 diabetes (insulin dependent diabetes mellitus, IDDM) begins in juveniles as an organ-specific autoimmune reaction, the destructive insulitis.

The cellular infiltrate contains macrophages, T- and B-lymphocytes. Lymphocytes regognize several autoantigens of the β-cells, including insulin and its precursor proinsulin; Accordingly, auto-antibodies against these antigens are also present. With time the insulitis leads to a

Autoimmune Disease. Table 1 Autoimmune diseases

Organ-specific autoimmune diseases
Hematologic diseases: autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, pernicous anemia
Kidney disease: Goodpasture syndrom, lipoid nephroses, minimal change glomerulonephritis
Diseases of the gastrointestinal tract: autoimmune chronic active hepatitis, autoimmune atrophic gastritis, Crohn's disease, ulcerative colitis
Neurological diseases: myasthenia gravis, multiple sklerosis, Guillain-Barré-syndrom
Endocrine diseases: autoimmune thyreoiditis, primary myxedema, Addison's disease, type 1 diabetes mellitus (IDDM)
Eye diseases: sympathetic ophthalmia, uveitis
Skin diseases: pemphigus, (sklerodermia), psoriasis
Systemic autoimmune diseases
Systemic lupus erythematosus
Rheumatoid arthritis
Mixed connective tissue disease (MCTD)
Sjögren syndrom
Polymyositis
Systemic sklerosis
Systemic vasculitis: Wegener's disease, giant cell arteritis, polyarteritis nodosa

complete and selective loss of the β-cells. What triggers the autoimmune reaction is not known. Epidermiological evidence points to virus infection as infections with coxackie virus B4.

Multiple Sklerosis

Multiple sklerosis is the most frequent neurological disease of jung adults in the western world. It results from a chronic inflammation of the central nervous system which leads to focal demyelinated lesons in brain and spinal cord. Although clinically different forms occur in the onset, it mostly proceeds intermittantly, where acute phases alternate with full or partial remissions. The disease unevitably ends in progression leading to increasing neurological defects, which lead do severe physical handicaps and eventually death.

For the pathogenesis of multiple sklerosis, autoimmune T-lymphocytes play a predominant role, which are directed against components of the neural myelin sheath. T-lymphocytes by secreting cytokines such as interferon γ maintain the chronic inflammation which destructs the myelin sheath. Also cytotoxic T-lymphocytes may participate directly. The cause of multiple sklerosis is unknown. Significantly increased antibody titers against several virusses, mostly the measles virus, point to a (latent) virus infection initiating the disease.

Rheumatoid Arthritis

Rheumatoid arthritis represents a chronic inflammatory disease of the joints. About 1% of the population in Germany suffers from this disease. Primarily the synovial membranes of the jounts are affected, however, the disease can also reach other organs such as the pleura, pericardium organ and skin blood vessels. The inflamed synovial membran becomes multicellular bearing infiltrates of predominantly lymphoid cells. The drastically increased synovial fluid (which causes swelling of the joints) contains many inflammatory cells, predominantly granulocytes, macrophages and lymphocytes. The inflammatory process leads to the destruction of the cartilage and eventually to the erosion of the bones. This is augmented by the mostly fibroblastic pannus which can invade the bone. A crucial pathophysiological role is played by the cytokines interleukin-1 and tumor necrosis factor. That rheumatoid arthritis represents an autoimmune disease is supported by many animal models. The putative autoantigens in the human disease are not known.

Systemic Lupus Erythematosis (SLE)

The disease affects predominantly young women. Nearly all of the patients suffer from symptoms such as fatigue, weight loss, and fever and have chronic arthritis. In addition, nearly all organs of the body can be affected to various degrees. Clinically, the severity of the disease can vary within wide ranges. In cases where organs are affected, the disease in former times was lethal without therapy within 10 years in 50% of the patients.

Several immunological abnormalities are found. Most important are pathogenic autoantibodies including antinuclear antibodies (amongst others against nucleoprotein particles or double stranded DNA). The blood plasma contains circulating immunocomplexes which result from an insufficient clearing due to exhaustion of complement. The regulation of the activation of T- and B-lymphocytes often exhibits abnormalities.

Chronic Inflammatory Bowel Disease

Two diseases make up for the majority of all inflammatory bowel diseases: Crohn's disease and ulcerative colitis. Crohn's disease is a chronic recurrent inflammation of all layers of the gut wall. Although it can occur anywhere, the major site of manifestation is the regional ileitis. In contrast, ulcerative colitis is restricted to the colon and the inflammation generally only affects the gut mucosa. In both diseases the inflammatory lesons contain lymphocytes which react with several gut-associated auto-antigens. In the blood also respective autoantibodies are found. To protect the host from infectious agents or toxic substances which are ingested, the gut relies on its barrier function in which its innate immune system plays a central role. At least in Crohn's disease a dysfunction of this system appears to be involved. The defective clearance of the commensal gut bacteria and the subsequent continuous flooding of the gut-associated lymphoid cells with them may lead to a perpetuating immune reaction which supports the chronic inflammation.

Pharmacological Intervention

The treatment of an autoimmune disease very much depends on the nature of the clinical outcome it causes. Although the formation of autoantibodies causes the inactivation of the gastric intrinsic factor, the subsequent shortage of vitamin B12 can be easily overcome by supplying it via an parenteral route. Lifelong immunosuppression (with all its side effects) thus is inappropriate. When, however, as in sympathetic ophtalmia, after damage of the first eye the second eye is endangered, an even drastic immunosuppression is mandatory.

A more differentiated discussion is required regarding type 1 diabetes mellitus. Although injected, now mostly human, insulin can substitute the lost function of the destroyed β-cells, it does it in an unphysiological way, requiring restrictions in the way of life. In addition, concomittant disease may cause severe problems and long-term damages cannot fully be avoided. When the disease begins, theoretically immunosuppression should be able to halt it leaving some β-cells alive to secrete insulin in a regulated manner. Clinical studies with ciclosporin (→ immunossuppressants) based on such considerations were diappointing. Thus, at least with the immunosuppressive drugs available at present, type 1 diabetes is not an indication for immunosuppressive therapy.

As discussed above, the vast majority of autoimmune diseases is associated with chronic inflammation. The therapy thus rests on two principles:

1. Antiinflammation
2. Immunosuppression

The antiinflammatory drugs include the nonsteroidal antiinflammatory drugs (NSAIDs → analgesics) → cyclooxigenases), the disease modifying antirheumatic drugs (DMARDs), such as → methotrexate or → glucocorticoids. More recently, specific cytokine inhibitos were included which block the action of central mediators of inflammation such as tumor necrosis factor (infliximab, adalimumab, etanercept) or interleukin-1 (anakinra) (→ cytokines).

Amongst the → immunosuppressive agents besides the → glucocorticoids the modern nontoxic drugs have become drugs of choice including ciclosporin, tacrolimus or ascomycin.

Paving the way for a new class of drugs effective in autoimmune diseases, immunomodulators have been introduced very recently (mostly to treat rheumatoid arthritis). Abatacept by blocking accessory signals (see Fig. 1) prevents the activation of (autoimmune) T-lymphocytes. Rituximanb (which has become a standard drug for treating chronic lymphatic leucemia) by decreasing the number of B-lymphocytes inhibits the formation of autoantibodies. As B-lymphocytes in chronic situation become the predominant antigen presenting cells, the activation of (autoimmune) T-lymphocytes is also impeded. Interferon β (→ interferons) has become a valuable drug to reduce the number of relapse rates multiple sklerosis. Its major effect appears to be based on tightening the (disease-associated loose) blood brain barrier, and thus preventing autoimmune lymphocytes from entering the central nervous system.

►Interferons
►Immunosuppressive Agents

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Autonomic Nervous System

The part of the vertebrate nervous system that regulates involuntary action, such as the intestines, heart and glands: it is divided into the sympathetic nervous system and the parasympathetic nervous system.

►α-Adrenergic System
►β-Adrenergic System
►Muscarinic Receptors
►Nicotinic Receptors
►Purinergic System

Autophagy

Autophagy derived from latin words "self eating" is a normal regulated cell process where cytoplasmic materials are degraded through the lysosomal machinery and the contents reused by the cell. During this process, organelles like mitochondria together with long-lived proteins are sequestred in a double-membrane vesicle delivered and degrade in lysosomes inside the cell. Autophagy is activated in case of nutrient deprivation and plays a crucial role in the destruction of bacteria, viruses, and unnecessary proteins aggregates in cell.

►Phospholipid Kinases

Autoreactive Lymphocytes

T- or B-lymphocytes which react with autoantigens. In healthy individuals kept under control by the mechanisms of self tolerance.

►Autoimmune Disease

Autoreceptor

A receptor on nerve endings within a synapse that responds to the released neurotransmitter from that neuron. This then feeds back to the same neuron and negatively regulates the synthesis and release of that neurotransmitter.

►Synaptic Transmission
►α-Adrenergic System
►Muscarinic Receptors
►Histaminergic System

Autosomal Dominant Hypocalcemia (ADH)

A form of hypoparathyroidism (hypofunction of the parathyroid glands) caused by the presence of activating mutations in the CaR, usually in the heterozygous state.

►Ca^{2+}-Sensing Receptor

Autotaxin

Autotaxin is a lysophospholipase D that occurs in plasma and serum and cleaves lysophosphatidylcholine, thereby forming lysophosphatidic acid (LPA). This enzyme occurs as a ~125 kDa protein, attached to intracellular vesicles with a single transmembrane domain, and as a soluble extracellular enzyme generated from the former by proteolytic processing and secretion. Autotaxin appears to be a major source of extracellular LPA. In mice expressing only one allele of autotaxin, plasma levels of LPA are half as high as in control mice. Mice with homozygous autotaxin deficiency died around embryonic day 10 with major vascular defects in yolk sac and embryo. They also had allantois malformation, neural tube defects and asymmetric headfolds. These symptoms strongly resemble the phenotype of $G\alpha_{13}$ knockout mice, suggesting that LPA-GPCR predominantly signal through $G\alpha_{13}$ in early development.

►Lysophospholipids

Axon

Long nerve-cell process transmitting the action potential and ending as the synapse.

Axon Reflex

This is an unconventional reflex mediated by capsaicin-sensitive primary afferent neurons. In fact, an adequate stimulus can directly excite a peripheral terminal

generating an action potential. The action potential orthodromically conveys the stimulus to the spinal cord (and eventually triggers a conventional reflex) and/or antidromically invades another peripheral branch of the neurons and induces the release of neuropeptides (and other mediators) at a peripheral site (axon reflex) distal to the site of stimulation.

►Tachykinins and their Receptors

Axonal Guidance

During the development of the nervous system growing axons find their ways to their final target sites due to the presence of a variety of attractive and repulsive cues in the extracellular environment. These so-called guidance factors act especially on the axonal growth cone which is localized at the tip of the growing axon. Several conserved families of axon guidance factors have been identified. Slits are secreted proteins which repel growth cones by activating Robo (roundabout) class receptors. Netrins can attract or repel axons via their receptors DCC and UNC5. Ephrins are transmembrane proteins that activate Ephs which are receptor tyrosine kinases. The ephrin/Eph system can mediate repulsive as well as attractive signals. Finally, the semaphorins which act mainly via plexins and neuropilins are primarily repulsive. There is growing evidence that the understanding of the molecular mechanisms of axonal guidance may also provide new approaches for the improvement of regenerative processes after neuronal injury.

►Plexins

Axonal Membrane

The axonal membrane is a lipid bilayer in the nerve fibre. Ionic channels and other proteins are located in the membrane to achieve electrical activity. Action potentials are generated and conducted along the membrane.

►Local Anaesthetics

Azole

Antifungal Drugs.