L-NAME

L-NAME (N-nitro-L-arginine methyl ester), like L-NMMA, is a structural analogue of L-arginine and competes with L-arginine for NO-synthase, which uses L-arginine as a substrate for the formation of NO. L-NMMA and L-NAME are very effective NO-synthesis inhibitors, both in vitro and in vivo.

►NO Synthase

β-Lactam Antibiotics

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Synonyms

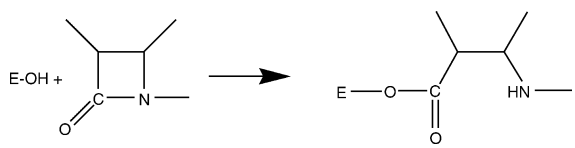
Wall peptidoglycan inhibitors

Definition

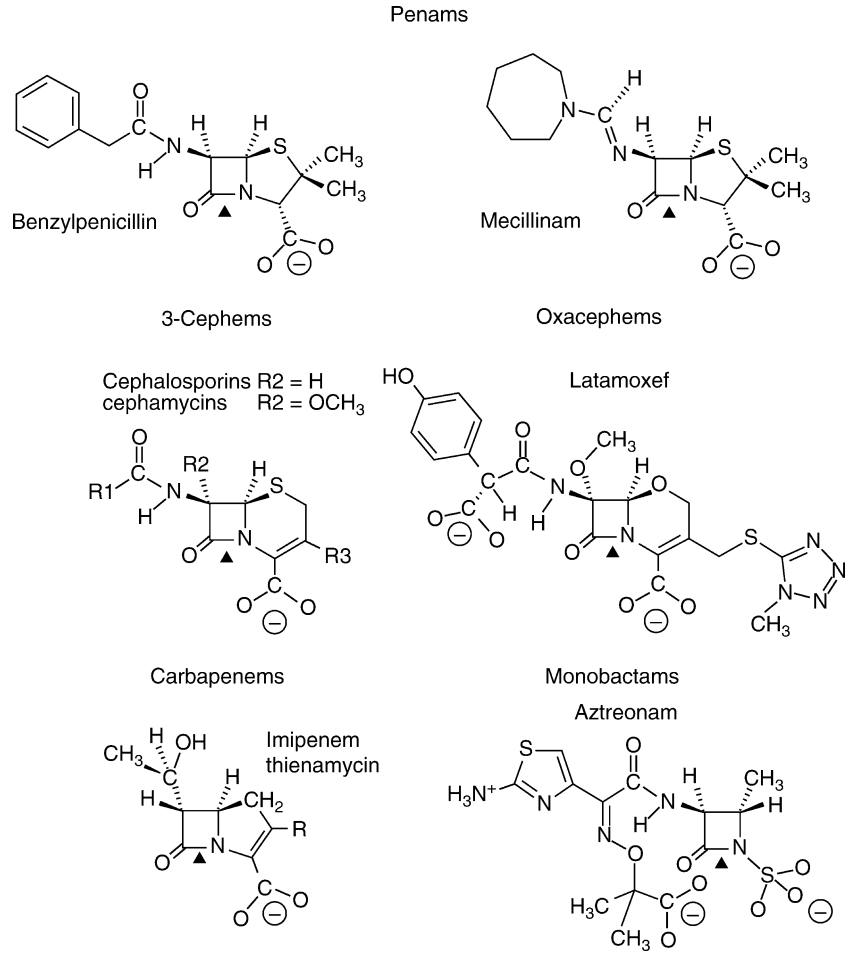
►β-Lactam antibiotics are bicyclic or monocyclic azetidinone ring-containing compounds (Fig. 1). They kill bacteria by preventing the assembly of (4–3) peptidoglycans. These covalently closed net-like polymers form the matrix of the cell wall by which the bacteria can divide and multiply, despite their high internal osmotic pressure.

Mechanism of Action

►Peptidoglycans of the (4–3) and (3–3) types (Fig. 2) are comprised of glycan chains made of alternating β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues [1]. The D-lactyl groups on carbon C_3 of the muramic acids are substituted by L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanine stem tetrapeptides. In the (4–3) peptidoglycans, peptides borne by adjacent glycan chains are cross-linked through direct linkages or cross bridges (comprising one or several intervening amino acid residues) that extend from the D-alanine residue at position 4 of a stem peptide to the ω-amino group at position 3 of another stem peptide. Lipid II (Fig. 3) is the immediate biosynthetic precursor. A disaccharide bearing an L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanyl-D-alanine stem pentapeptide (the diamino acid residue of which can be either free, i.e., unsubstituted, or substituted by one or several amino acid residues, i.e., branched) is exposed on the outer face of the plasma membrane, linked to a C_{55}-undecaprenyl via a pyrophosphate. From this precursor, the formation of polymeric (4–3) peptidoglycans relies on glycosyl transferases ensuring glycan chain elongation and acyl transferases ensuring peptide cross linking. Acyl transferases of the SXXK superfamily (►SXXK Acyl Transferases, also called DD-transpeptidases, with X denoting a variable amino acid residue) are implicated in cross linking and they exhibit a specific bar code in the form of three motifs, SXXK, SXN (or analogue) and KTG (or analogue), occurring at equivalent places and roughly with the same spacing along the polypeptide chains [2]. As a result of the polypeptide folding, the motifs are brought close to each other at the immediate boundary of the catalytic centre between an all-α domain and an α/β domain. SXXK acyl transferases identify *N*-acyl-D-alanyl-D-alanine sequences as carbonyl donors, produce a *N*-acyl-D-alanyl moiety linked as an ester to the serine residue of the invariant SXXK motif and transfer the peptidyl moiety onto an amino group (transpeptidation) or a water molecule (carboxypeptidation). SXXK acyl transferases identify penicillin (used as a generic term for β-lactam antibiotics) as a suicide carbonyl donor. Because the serine-ester linked penicilloyl enzyme that SXXK acyl transferases produce is rather stable, they are immobilized, at least for a long time, in the form of penicillin-binding proteins, in short PBPs. The inactivation reaction can be written



where E-OH is the enzyme, with –OH the hydroxyl group of its active-site serine residue. Kinetically, the interaction is described by a 3-step model



β-Lactam Antibiotics. Figure 1 Bicyclic (penams, 3-cephems, oxacephems and carbapenems) and monocyclic (aztreonam) β-lactam antibiotics. Rupture of the scissile amide bond of the azetidinone ring (arrow) by the SXXK acyl transferases implicated in (4–3) peptidoglycan synthesis results in the formation of long-lived, serine-linked acyl ester derivatives. The inactivated enzymes behave as penicillin-binding proteins or PBPs.

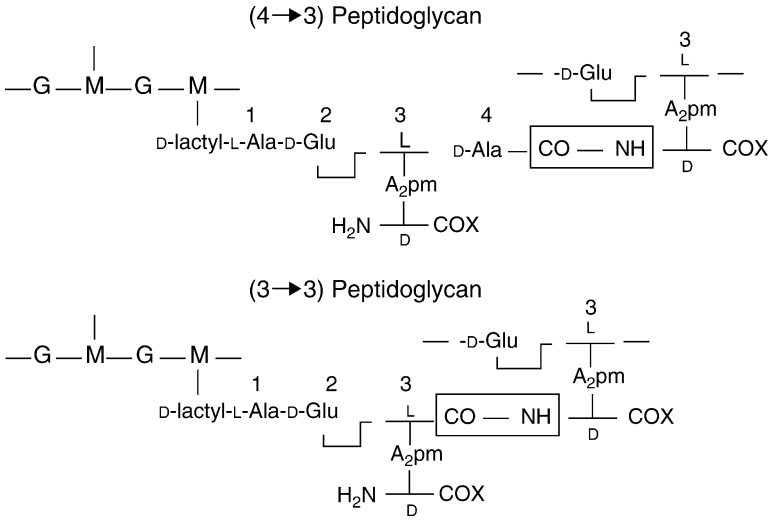
$$E + I \underset{}{\overset{K}{\rightleftharpoons}} EI \xrightarrow{k_2} EI^{*} \xrightarrow{k_3} E + P(s)$$

where I is the β-lactam, P(s) the product(s) resulting from the very slow decay of the EI* penicilloyl enzyme. K is the dissociation constant of the EI, a non-covalent complex and k_2 and k_3 first-order rate constants. In all cases, k_3 has been found to be very small and K is generally large so that the sensitivity of a PBP to a β-lactam is best characterized by the k_2/K ratio [3]. This ratio varies from 1,000,000 $M^{-1}s^{-1}$ for the most sensitive PBP to less than 1 for the most resistant.

Lethal Target Proteins

A constellation of genes code for PBPs of varying amino acid sequences and functionalities. PBPs occur as free-standing polypeptides and as protein fusions. This combinatorial system of structural modules results in a large increase in diversity.

►SXXK PBP fusions of classes A and B are the lethal targets of β-lactam antibiotics. The PBP fusions of class A are comprised of an SXXK acyl transferase module of class A, linked to the carboxy end of a glycosyl transferase module having its own five motif-bar code, itself linked to the carboxy end of a membrane anchor [2]. They convert the disaccharide–pentapeptide units borne by lipid II precursor molecules into nascent polymeric (4–3) peptidoglycans. Glycan chain elongation is catalysed by the transglycosylase module. Peptide cross linking between elongated glycan chains is then carried out by the associated SXXK acyl transferase module. The PBP fusions of class B are comprised of an SXXK acyl transferase module of class B, bound to the carboxy end of a linker module having its own three-motif bar code, itself linked to a membrane anchor. They are components of morphogenetic apparatus that controls wall expansion, ensures cell-shape maintenance and carries out septum formation. Likely, the linker modules ensure that the



β-Lactam Antibiotics. Figure 2 (4–3) Peptidoglycan (the synthesis of which is susceptible to β-lactam antibiotics) and (3–3) peptidoglycan (the synthesis of which is resistant to antibiotics) in *Escherichia coli* and *Mycobacterium tuberculosis*. G: *N*-acetylglucosamine. M: *N*-acetylmuramic acid (i.e., *N*-acetylglucosamine with a D-lactyl substituent on carbon C_3). A_2pm: *meso*-diaminopimelic acid. In *E. coli* and *M. tuberculosis*, the stem peptides are unbranched. COX: COOH (in *E. coli*) or $CONH_2$ (in *M. tuberculosis*).

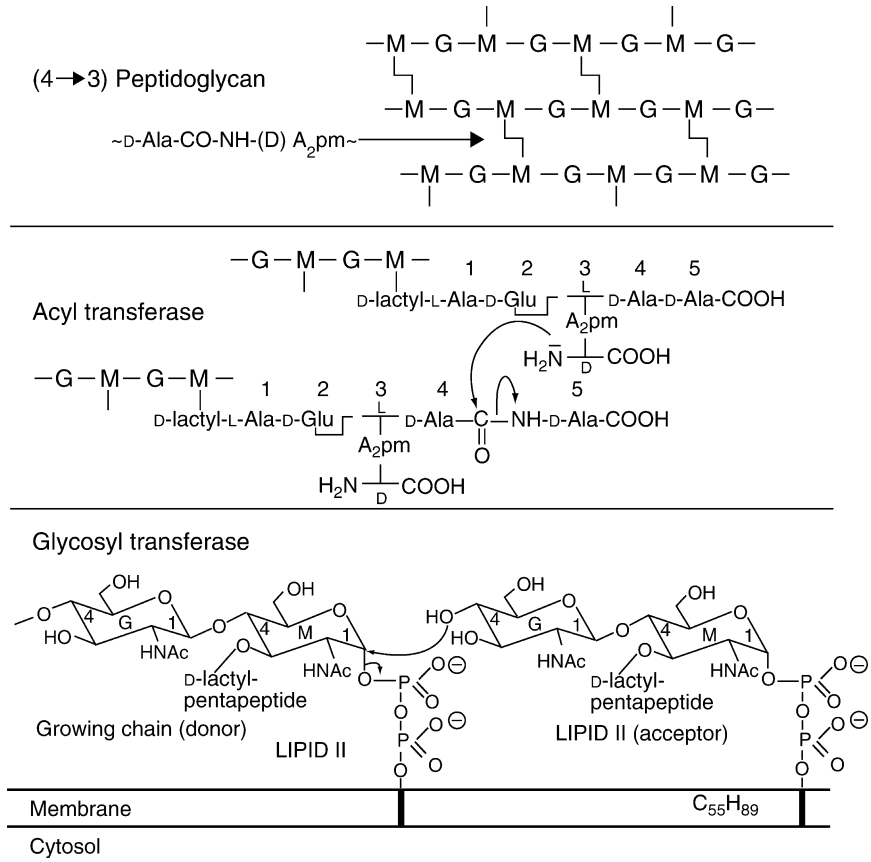
associated SXXK acyl transferase modules are positioned in an active conformation within the morphogenetic apparatus where they need to be. Binding of β-lactam antibiotics to the SXXK acyl transferase modules of the PBP fusions leads to the formation of Henri–Michaelis complexes that exhibit ligand- and enzyme-specific hydrogen bonding networks. *Escherichia coli* is killed in a number of ways; via cell lysis as a result of the selective inactivation of the class A PBP1a and PBP1b (which can substitute for each other) by cephaloridine and cefsulodin; via transformation of the cells into round bodies as a result of the selective inactivation of the cell-cycle subclass B2 PBP2 by mecillinam and thienamycin; via cell filamentation as a result of the selective inactivation of the cell-cycle subclass B3 PBP3 by mezlocillin, cefaperazone, cefotaxime, cefuroxime, cephalothin and aztreonam; or via different combinations of these morphological alterations by ampicillin, benzylpenicillin, carbenicillin and cefoxitin.

β-Lactamase-Mediated Resistance

►SXXK free-standing PBPs are peptidoglycan-hydrolases of one kind or another. Loss of these auxilliary cell-cycle proteins causes varying morphological aberrations, but is not fatal at least in the laboratory environment. Similarly, conversion of free-standing PBPs into β-lactam antibiotic-hydrolysing enzymes, with loss of peptidase activity and conservation of the polypeptide fold, gives rise to the *SXXK β-lactamases*. There are three classes of SXXK β-lactamases A, C and D, which are easily distinguished on the basis of their primary structures, although the tertiary structures are clearly similar and also related to those of the acyl transferase modules of PBPs. In particular, in addition to the characteristic SXXK, motifs reminiscent of the bar code SXN and KTG groups are found in nearly superimposable positions. The catalytic pathway of β-lactamases follows the 3-step model shown above but with good substrates, the k_3 value can be higher than 1000 s^{-1} [4].

The class A enzymes have M_r values around 30,000. Their substrate specificities are quite variable and a large number of enzymes have emerged in response to the selective pressure exerted by the sometimes abusive utilization of antibiotics. Some of these "new enzymes" are variants of previously known enzymes, with only a limited number of mutations (1–4) but a significantly broadened substrate spectrum while others exhibit significantly different sequences. The first category is exemplified by the numerous TEM variants whose activity can be extended to third and fourth generation cephalosporins and the second by the NMCA and SME enzymes which, in contrast to all other SXXK β-lactamases, hydrolyse carbapenems with high efficiency. Despite these specificity differences, the tertiary structures of all class A β-lactamases are nearly superimposable.

The class C enzymes have M_r values of 39,000 and exhibit more uniform properties. They hydrolyse benzyl- and phenoxymethyl penicillin relatively well (turn-over numbers of 20–70 s^{-1}), ampicillin and amoxicillin 10- to 20-fold less rapidly and extremely poorly the other penicillins (generally due to low k_3 values). The early cephalosporins (cephalothin,



β-Lactam Antibiotics. Figure 3 Lipid II precursor (bottom) and polymeric (4–3) peptidoglycan of *Escherichia coli* and *Mycobacterium tuberculosis*. Glycosyl transferase and acyl transferase-catalysed reactions. G: *N*-acetylglucosamine. M: *N*-acetylmuramic acid. A_2pm: *meso*-diaminopimelic acid. (3–3) Peptidoglycan cross linking (Fig. 2) may proceed via the formation, in a penicillin-resistant manner, of a *N*-acyl-L-diaminoacyl moiety linked as an ester to the serine residue and the transfer of the peptidyl moiety to the ω-amino group of the diamino acid residue of another peptide.

cephalexin, cefazolin) are well hydrolysed but the later generation compounds also exhibit low to very low k_3 values, as do imipenem and aztreonam. A few mutations are known to extend the spectrum of some enzymes but the most currently utilized bacterial strategy is overproduction by deregulation of the biosynthetic control mechanism, so that the MICs of poor or even very poor substrates can increase significantly [5].

Class D enzymes (M_r of about 27,000) exhibit a high activity versus isoxazolyl penicillins, such as oxacillin and are referred to as the OXA-family. Surprisingly, the amino group of the SXXK lysine residues is carboxylated in the most active forms of the enzymes. Penicillins are generally better substrates than cephalosporins but mutations have been found which confer extended activity spectra to these enzymes.

Inactivators of class A β-lactamases (clavulanate, sulbactam, tazobactam) are themselves β-lactams and act as suicide substrates. They can be used in combination with β-lactamase sensitive compounds (ampicillin, amoxicillin) but are not clinically useful against class C and class D producers. Some TEM mutants have also been selected that exhibit reduced sensitivity to these inactivators.

The class B metallo-β-lactamases have emerged more recently as a clinical problem but they are particularly dangerous since many of them hydrolyse all know β-lactams, with the exception of monobactams. In particular, they hydrolyse the suicide substrates mentioned above, as well as carbapenems that usually escape the activity of all the SXXK enzymes, with the exception of the NMCA group.

Three subclasses B1, B2 and B3 can be distinguished on the basis of the sequences. B1 and B3 enzymes are optimally active with 2 Zn^{2+}ions, while the B2 enzymes are inhibited by the second Zn^{2+}. These B2 enzymes also exhibit a very narrow activity spectrum and only hydrolyse carbapenems. The 3D structures of representative members of each subfamily have been

solved and they highlight a typical αββα fold, completely unrelated to that of the SXXK enzymes.
The production of β-lactamases can be inducible or constitutive and the genes carried by the chromosome or plasmids, some genes being parts of integrons. Several clinical strains produce up to three distinct enzymes.

PBP-Mediated Resistance

There are at least two modes of intrinsic resistance to β-lactam antibiotics. Determinants conferring a decreased susceptibility to β-lactam antibiotics evolve by the accumulation of point mutations in genes that code for essential PBP fusions of classes A and/or B. The shuffling and capture of DNA sequences from commensal *Streptococci* having a reduced susceptibility to the drug, give rise to *Streptococcus pneumoniae* pathogens in which mosaic genes code for mosaic PBP fusions of classes A and/or B of decreased affinity for the drug. Mosaic and wildtype PBP fusions of the same class (and the same subclass) differ by up to 15% amino acid residues. Mosaic PBPs occur also in *Neisseria meningitidis* and *Neisseria gonorrhoeae* strains.

Other strategies leading to an increased resistance are the transfer of a complete gene encoding a resistant PBP from a non-pathogenic related species to yield the Methicillin Resistant *Staphylococcus aureus* (MRSA) and the overproduction by *Enterococci* of a pre-existing but minor resistant PBP, which can further mutate to even more resistant forms. Some bacteria can also manufacture a (3–3) peptidoglycan (Fig. 2) with the help of a penicillin-resistant LD-transpeptidase, which is not an SXXK enzyme. Indeed, a low proportion of (3–3) cross links have been found in the walls of *Enterococcus faecium*, *Mycobacterium tuberculosis* and *Mycobacterium leprae*. The quantitative influence of these cross links on the resistance level remains to be determined but the synthesis of (3–3) peptidoglycan, combined with that of a set of relatively resistant SXXK PBPs and the limited permeability of the mycolic acid layer might explain the lack of efficiency of β-lactam antibiotics as therapeutic agents against tuberculosis and leprosy [6].

Outer Membrane Permeability and Active Efflux Systems

In Gram-negative bacteria, diffusion of β-lactam antibiotics into the periplasm (where the activity of PBPs takes place) occurs via the channels that porins create in the outer membrane. The number and properties of the porin molecules are such that diffusion is relatively rapid in *E. coli* but much slower in *Enterobacter* and *Pseudomonas*. Mutants can be selected after the permeability of porin channels or their number has been decreased. A slow diffusion into the periplasm becomes a particularly important factor when it is combined with the presence of a β-lactamase (even in low concentration or when poorly active) in the same cellular compartment. Note that in *Mycobacteria*, the mycolic acid layer plays a role similar to that of the outer membrane in Gram-negatives. Finally, β-lactams bearing a hydrophobic side-chain can be ejected from the periplasm by the active efflux systems AcrAB-TolC in *E. coli* and *Salmonella typhimurium* and MexAB-OprM in *Pseudomonas*. Overexpression of these proteins can significantly increase the level of resistance.

Clinical Use

Because the SXXK PBPs are specific to the prokaryotes, the β-lactam antibiotics have a high selective toxicity without marked side effects except for possible allergic reactions. Resistance is a problem of great concern. The use of antibiotics fuels the continuing emergence and spreading of novel β-lactamases and intrinsic resistance determinants among bacterial pathogens.

►Microbial Resistance to Drugs
►Quinolones
►Ribosomal Protein Synthesis Inhibitors

L

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β-Lactamases

►β-Lactam Antibiotics.

Lateral Hypothalamic Area

The lateral hypothalamic area has been identified as a feeding centre by studies involving electric stimulation and discrete lesions. Neurons in the lateral hypothalamic area and the neighbouring perifornical area express neuropeptides that stimulate feeding when injected into cerebral ventricles (orexins 1 and 2, melanin-concentrating hormone (MCH)).

►Appetite Control

Laxatives

Laxatives, also called purgatives or cathartics, are substances used to hasten the transit of food through the intestine. Laxatives function by different mechanisms. Bulk laxatives, like methylcellulose or bran, contain agents like polysaccharide polymers, which are not fermented by the normal processes of digestion. They retain water in the gut lumen and promote bowel movements. Osmotic laxatives consist of poorly absorbable solutes such as salines containing magnesium cations or phosphate anions or non-digestable sugars and alcohols (glycerin, lactulose, sorbitol or mannitol). Osmotic laxatives retain an increased volume of fluid in the lumen of the bowel by osmosis, which accelerates the transfer of the bowel contents. Faecal softeners alter the consistency of the faeces. They are also called emollients and contain surface-active compounds similar to detergents, like docusate salts. Stimulant laxatives/purgatives increase the motility of the gut (peristalsis) and stimulate water and electrolyte secretion by the mucosa. Stimulant laxatives, which can cause abdominal cramps and deterioration of intestinal function, include diphenylmethane derivatives (bisacodyl, phenolphthalein), anthraquinone laxatives (derivatives of plants such as aloe, cascara or senna) and ricinoleic acid.

LCAT

►Lecithin-Cholesterol Acyltransferase

LD_{50}

►Lethal Dose

LDL-Cholesterol

►Low-Density-Lipoprotein

LDL-Receptors

LDL-receptors are receptors on the cell surface that remove LDL (and some other forms of) lipoproteins from the plasma into the cell.

►HMG-CoA-Reductase Inhibitors (Statins)
►Lipoprotein Metabolism
►Low-Density Lipo-protein Receptor Gene Family

Lead

A lead is a hit compound that displays specificity and potency against a target in a library screen and continues to show the initial positive dose-dependent response in more complex models such as cells and animals.

Lead Discovery by NMR

►SAR-by-NMR

Lecithin-Cholesterol Acyltransferase

Enzyme that converts free cholesterol to cholesteryl ester on HDL.

►Lipoprotein Metabolism

Leonardo

►14-3-3 Proteins

Leptin

The cytokine leptin is secreted by adipocytes (fat cells) in proportion to the size of the adipose depot and circulates via the bloodstream to the brain, where it ultimately affects feeding behavior, endocrine systems including reproductive function and, at least in rodents, energy expenditure. The major effect of Leptin is on the hypothalamous, where it suppresses appetite and hence food intake. Leptin exerts its effects via binding to the leptin receptor in the brain (specifically in the hypothalamus), which activates the JAK-STAT Pathway.

►Appetite Control
►Adipokines

Lethal Dose

The lethal dose (LD_{50}) is a measure of the toxicity of a compound. In an LD_{50} toxicity test, various doses of a drug are administered to groups of animals, and the mortality in each group is determined. The lethal dose 50 is the dose, which is lethal for 50% of a group of animals.

Leucin Zipper

A leucine zipper is a structural motif present in a large class of transcription factors. These dimeric proteins contain two extended alpha helices that "grip" the DNA molecule much like a pair of scissors at adjacent major grooves. The coiled-coil dimerization domain contains precisely spaced leucine residues which are required for the interaction of the two monomers. Some DNA-binding proteins with this general motif contain other hydrophobic amino acids in these positions; hence, this structural motif is generally called a basic zipper.

Leukotrienes

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Synonyms

►Cysteinyl leukotrienes; B-leukotriene

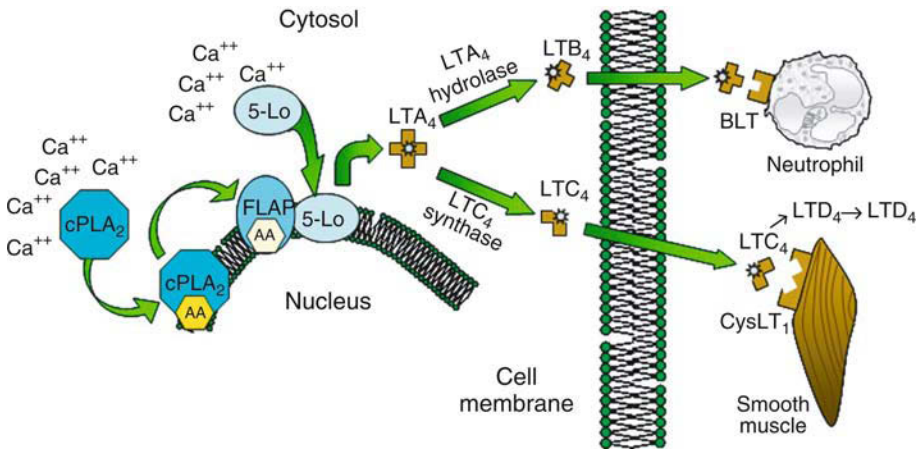
Definition

Leukotrienes belong to a large family of lipid mediators, termed eicosanoids, which are derived from arachidonic acid (AA) and released from the cell membrane by phospholipases. AA is subsequently converted by the enzyme, 5-lipoxygenase (5-LO) and a protein cofactor, ►5-lipoxygense activating protein (FLAP), into two bioactive classes of leukotriene (LT): (i) LTB_4 and (ii) ►cysteinyl leukotrienes (cysLTs), LTC_4, D_4, and E_4. Leukotrienes do not exist in the state preformed in cells. These compounds play a significant role in allergic responses and contribute variably to the bronchoconstrictor response in human ►bronchial asthma, and possibly, chronic obstructive pulmonary disease.

Basic Characteristics

Synthesis and Metabolism

The site of synthesis of leukotrienes is largely or completely in the nuclear membrane. LTs are produced from cell membrane phospholipid-associated arachidonic acid (AA), which is liberated by 85 kDa cytosolic group IVAPLA_2 (cPLA_2) (Fig. 1) or phospholipase C (not shown) in response to cell activation. The secretory phospholipase group V PLA_2 (g$VPLA_2$) also is capable of inducing leukotriene synthesis, probably from phosphatidylcholine-rich membrane hydrolysis at both plasma or perinuclear membrane [1]. AA is converted to 5-HETE and subsequently to an unstable epoxide LTA_4 ([5(*s*)-trans-5,6-oxido-7,9-trans11,14-cis-eicosatetranonic acid]), a central intermediary in LT biosynthesis. LTs are synthesized in the cells by 5-LO which acts in concert with FLAP, a protein essential for 5-LO to function enzymatically in intact cells. In neutrophils, LTA_4 is transformed by LTA hydrolase to dihydroxy LTB_4, whereas in ►eosinophils, macrophages, basophils, and mast cells that express LTC_4 synthase, the terminal enzyme involved in cysLT synthesis, LTA_4 is conjugated with the tripeptide glutathione to form LTC_4. Outside of the cell, LTC_4 is rapidly converted by γ-glutamyltranspeptidase into LTD_4. LTD_4 is converted more slowly into LTE_4 by a dipeptidase, which is secreted into the urine and is sometimes used (controversially) as a marker for cysLT synthesis. Leukotriene B_4, a noncysteine



Leukotrienes. Figure 1 Schema representing cellular biosynthesis of cysteinyl leukotrienes (cysLTs) and BLT at the nuclear membrane. In this schema, $cPLA_2$ has migrated from the cytosol to the nuclear envelope and 5-LO is secreted from the nucleus to bind with FLAP and produce the metabolite, LTA_4, which is synthesized by 5-LO from 5-HPETE (not shown) in two steps. LTA_4 is converted by LTC_4 synthase which after LTC_4, is converted rapidly to LTD_4 outside of the inflammatory cell. Note that a specific hydrolase (LTA_4 hydrolase) also catalyzes the formation of LTB_4, a non-cysLT, which has its own receptor. See text for details.

containing dihydroxy leukotriene, binds to its own BLT receptor. The cysLTs are characterized by a side chain containing 3, 2, or 1 amino acid(s) (cysteine is always present); there are two or more specific cysLT receptors in tissues at various sites including airway smooth muscle, vascular smooth muscle, and inflammatory cells themselves. Both cysLT receptors have been characterized and cloned. LTD_4 binds to high affinity $CysLT_1$ receptor while $CysLT_2$ receptor is most preferred target for LTC_4.

Recent investigations have suggested that cysLT may also be synthesized in the cytosol in lipid bodies that are formed during cellular activation in eosinophils [2]. It has been suggested that all enzyme and transport systems essential to cysLT synthesis exist in these lipid bodies, and hence synthesis could occur without translocation of $cPLA_2$ to the nuclear envelope. Likewise, it has been suggested that 5-LO is not stored within the nucleus, but rather is an enzyme dispersed throughout the cytosol, which migrates to the perinuclear membrane and cytosolic lipid bodies upon cellular activation. Recently, the role of 14 kDa secretory PLA_2 in leukotriene synthesis has been explored in human granulocytes [1]. Studies demonstrated that $gVPLA_2$ is capable of causing AA synthesis and subsequent LT secretion by both $cPLA_2$-dependent and -independent pathways. Among the secretory enzymes, $gVPLA_2$ is unique because it can bind cell membranes by two distinct mechanisms: (i) cell surface proteoglycan through a cluster of cationic residues at the carboxy terminus of the enzyme and (ii) direct interfacial binding to membrane phosphatidylcholine. In eosinophils, $gVPLA_2$ is internalized and binds to perinuclear membrane to generate AA and subsequent translocation of cytosolic 5-LO to perinuclear membrane. Accordingly, LTC_4 secretion caused by $gVPLA_2$ in eosinophils does not involve ERK-1/2 mediated $cPLA_2$ phosphorylation. By contrast, LTB_4 secretion caused by $gVPLA_2$ in neutrophils is mediated through activation of the $cPLA_2$ pathway. These novel pathways for $gVPLA_2$ are fully established in granulocytes; however, the role of other 14 kDa $sPLA_2$ isoforms in LT synthesis in vitro remains an area of considerable interest. $sPLA_2$s have been identified in the bronchoalveolar lavage fluid of asthmatic subjects.

Leukotriene Receptors

There are two distinct receptor types for LTB_4 (BLT_1 and BLT_2 receptors) and two for cysLTs ($CysLT_1$ and $CysLT_2$ receptors). Classification is based mainly on functional data as derived from recent cloning and biochemical characterization. For LTB_4, high affinity BLT_1-receptor shows high degree of specificity for LTB_4 (Kd 0.15–1 nM) and is only expressed in inflammatory cells. Recently identified low affinity BLT_2-receptor has a higher Kd value for LTB_4 (23 nM) and is expressed ubiquitously, in contrast to BLT_1, in leukocytes.

The cysLTs are a family of potent bioactive lipids that act through two structurally divergent G protein coupled $CysLT_1$ and $CysLT_2$ receptors. mRNA expression demonstrates the $CysLT_1$ gene, which is located on the X chromosome and is identified in macrophages, smooth muscle cells, leukocytes, lung, and spleen. The $CysLT_1$ receptor was originally identified on the basis of its contractile properties for bronchial smooth muscle. The agonist for the $CysLT_1$ receptor are

$LTD_4 > LTC_4 > LTE_4$. The second receptor, $CysLT_2$, binds equally to LTD_4 and LTC_4; mRNA is expressed in heart, leukocytes, spleen, brain, placenta, and lymph nodes. To date, the functional role of $CysLT_2$ receptor remains unclear. The current nomenclature is summarized in Table 2, together with order of potency of the LTs, names of some selective antagonist drugs and expression in human and mouse tissues/cells.

Role of Leukotrienes in Inflammatory Diseases

LTB_4, a B-leukotriene, is a potent chemotaxin for neutrophils and T-cells and a weak chemotaxin for human eosinophils. In human neutrophils, LTB_4 causes translocation of intracellular calcium that initiates an autocrine pattern of stimulated cellular activity. It promotes the adhesion of neutrophils to the vascular endothelium and enhances the migration across the endothelial wall into the surrounding tissues. LTB_4 also causes the release of toxic oxygen radicals, lysosomal enzymes, and chemokines/cytokines from proinflammatory cells. Some studies have shown that LTB_4 may cause contraction of both human bronchus and guinea pig parenchymal strips in vitro; however, blockade of LTB_4 receptor has little or no therapeutic action in human asthma.

The cysLTs were formerly known as SRS-A (slow reacting substance of anaphylaxis) because of the slow, sustained contraction of airway smooth muscle that resulted from the secretion of this substance(s) upon mast cell activation. Human bronchi may have a homogenous population of $CysLT_1$ receptors, whereas guinea pig trachea have both $CysLT_{1/2}$ receptors. Although originally identified in tissue mast cells, eosinophils, and mast cells, both have substantial cysLT synthetic capacity. Because the eosinophil is ubiquitous in asthma and allergic disease and because it is capable of cysLT synthesis, this cell has generally been regarded as primary mechanism for sustained cysLT secretion in allergic states (Table 1). However, recent publications have suggested the eosinophil secretion is relatively minimal compared to mast cell secretory capacity for cysLTs.

Asthmatic exacerbations are associated with chemotaxis of eosinophils from the peripheral blood across endothelial membranes into the parenchyma and lumen of the conducting airways of the lung. This process is facilitated by the upregulation of β_1- and β_2-integrins, which bind to counterligands of the immunoglobulin supergene family on the endothelial surface. Diapedesis is facilitated by the presence of these ligands on both sides of the endothelium (lumenal and parencymal). The migration of eosinophils from the peripheral blood to human airways provides a continuous reservoir for leukotriene synthesis in allergic and asthma reactions. The 85 kDa $cPLA_2$ that catalyzes the first steps of leukotriene synthesis also serves as a messenger protein for cellular adhesion, likely by the synthesis of lysophospholipid, which is the by-product of membrane hydrolysis in which AA is also synthesized.

Leukotrienes play a role in both allergy and asthma [5]. While cysLTs are highly efficacious bronchoconstricting agents when administered exogenously to human asthmatics (Fig. 2) (1000-fold the potency of histamine), a substantial role for cysLT in asthmatic bronchoconstriction has not been established. Amelioration of inflammatory response by corticosteroids in both asthma and allergic reactions is highly efficacious and does not appear to result from blockade of leukotriene synthesis. No other chronic inflammatory disease has been linked directly to cysLT secretion despite the capacity for these compounds to cause edema and inflammatory cell migration.

Drugs

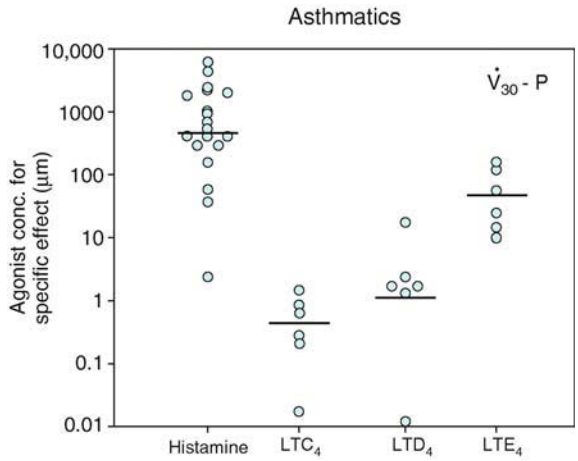
The effects of leukotrienes can be blocked at several levels. Inhibitors of FLAP or 5-LO inhibit LT synthesis at all levels. However, FLAP antagonists developed to date have been too hepatotoxic for human use. Zileuton, a 5-LO synthase inhibiting drug, also demonstrated some hepatotoxicity in a small percentage of patients, which was nonetheless entirely reversible. However, the short half-life of this compound requires four times daily

Leukotrienes. Table 1 Pharmacologic actions of the leukotrienes

LTB_4	Cysteinyl LTs (LTC_4, LTD_4, LTE_4)
PMN aggregration	Airway smooth muscle contraction
PMN chemotaxis	Constriction of conducting airways
Nuclear transcription	Contraction of guinea pig parenchyma
Exudation of plasma	Secretion of mucus
Translocation of calcium	Fluid leakage from venules
Stimulation of PLA_2 (guinea pig)	Edema formation
Contraction of human bronchus (?)	Chemoattraction of eosinophils
Contraction of guinea pig parenchyma (?)	Autocrine activation of PLA_2 (guinea pig)
No effect with LTRA	Blocked by LTRA

Reprinted with permission from [3].

administration, and accordingly, this compound is rarely prescribed. The most widely used antiLT drugs are the ►leukotriene receptor antagonists (LTRAs; see Table 2), which were synthesized before the cloning of the $CysLT_1$ receptor. These include pranlukast, which is widely used in Japan, and zafirlukast and montelukast, which are used in the USA, Europe, and Asia. In controlled studies in human asthmatics, these compounds cause 5–8% improvement in bronchoconstriction as measured by the forced expiratory volume in 1 s (FEV_1). LTRAs have been shown to reduce the need for inhaled corticosteroids (ICS) and β-adrenoceptor agonists in human asthma. Additional studies have suggested that addition of LTRA to ICS or β- adrenoceptor agonists augments the improvement in FEV_1. Nonetheless, LTRAs are fairly expensive and substantially less efficacious than either long-acting β-adrenergic agonists (LABA) or ICS, particularly when LABA and ICS are used in combination. Accordingly, the use of LTRAs is often relegated to either mild asthma or as supplemental therapy for patients failing to respond to other drugs.



Leukotrienes. Figure 2 Relative airway responses of asthmatic patients to inhaled cysteinyl leukotrines versus histamine. CysLTs have up to 1,000-fold greater potency in causing bronchoconstriction than histamine. Reprinted with permission from (2).

There are few definitive data to substantiate the efficacy of LTRA therapy in refractory asthma, except for patients with "aspirin-sensitive asthma." This is a fairly uncommon form of asthma that occurs generally in adults who often have no prior (i.e., childhood) history of asthma or ►atopy, may have nasal polyposis, and who often are dependent upon oral corticosteroids for control of their asthma. This syndrome is not specific to aspirin but is provoked by any inhibitors of the cycloxygenase-1 (COX-1) pathway. These patients have been shown to have a genetic defect that causes

Leukotrienes. Table 2 Leukotriene receptor type [4]

Nomenclature	BLT_1 receptor	$CysLT_1$ receptor (LTD_4 receptor)	$CysLT_2$ receptor (LTC_4 receptor)
Relative potency of agonists	LTB_4 > 12(R)-HETE (cysLTs are inactive)	$LTD_4 = LTC_4 > LTE_4$[a]	$LTC_4 > LTD_4 > LTE_4$[a]
Chromosome	Human: 14q11.2–q12	Human: Xq13.2–21.1	Human: 13q14.12–q21.1
	Mouse: 14	Mouse: X-D	Mouse: 14-D1
Selective antagonists (IC_{50})	LY-255883 (>10 nM)	Montelukast (MK476)	none
	CP-195543 (570 nM)	ICI198615	
	U-75302 (1 μM)	Pobilukast (SFK104353)	
	CP-105696 (58 nM)	Zafirlukast (ICI204219)	
	ZK-158252 (260 nM)	Pranlukast (ONO1078)	
Expression (Human)	*Tissues*: Leukocytes, thymus, spleen, liver, ovary *Cells*: PBLs, neutrophils,T-cells, dendritic cells, mast cells, eosinophils, macrophages, leukocytes	*Tissues*: spleen, small intestine, placenta, lung smooth muscle, *Cells*: bronchial smooth muscle, $CD34^+$ hemapoietic progenitor cells, monocytes, macrophages, mast cells, eosinophils, neutrophils, PBLs, human umbilical vein endothelial cells	*Tissues*: heart, skeletal muscle, spleen, brain, lymp node, adrenal medulla, lung, human pumonary/ saphenous vein *Cells*: monocytes, macrophages, mast cells, eosinophils, cardiac muscle, coronary artery, PBLs
Expression (Mouse)	*Tissues*: lungs, *Cells*: myeloid leukocytes, neutrophils, T-cells, macrophages, mast cells, eosinophils	*Tissues*: lung, skin, small intestine *Cells:* macrophages, fibroblasts, leukocytes	*Tissues:* lung, skin, brain, small intestine, spleen *Cells:* macrophages, fibroblasts, endothelial cells, leukocytes

[a]Partial agonist in some tissues.

overexpression of the enzyme LTC_4 synthase. However, the mechanism by which bronchoconstriction is provoked by COX-1 inhibition remains unexplained. In such patients, LTRAs are specifically indicated. There is limited evidence suggesting that patients with aspirin-sensitive asthma can use selective COX-2 inhibitors safely; however, COX-2-specific analgesics still are not recommended for use in aspirin-sensitive asthma by the FDA in the USA and may themselves be cardiotoxic.

Although some studies have shown no change in the excretion of LTE_4 in the urine of patients treated with corticosteroids, other investigations indicated that corticosteroids inhibit $cPLA_2$ translocation to the nuclear membrane in inflammatory cells, thus attenuating stimulated synthesis of cysLTs. This would suggest that oral and possibly inhaled corticosteroids may also inhibit production of cysLTs both directly (see above) and indirectly by causing necrosis and apoptosis of eosinophils.

In the USA, LTRAs have largely replaced theophylline as the incremental drug for the treatment of moderate and severe asthma, where LABA plus ICS alone do not provide adequate control. For patients with mild persistent asthma, LTRAs have been designated as a suitable substitute for low dose ICS by the National Asthma Education Panel Program (NAEPP) of the National Heart and Lung Institute (National Institutes of Health). However, inhaled ICS are more efficacious.

Therapeutic Response to Antileukotriene Drugs

When given acutely to patients who have no prior exposure to LTRA therapy, FEV_1 increases measurably within 30 min and results in a maximal improvement of about 8% in 1 h. For short acting drugs (e.g., zileuton), this response returns rapidly to baseline, and the drug must initially be administered in four doses daily. In this regard, the initial effects of 5-LO inhibition are more like bronchodilators than disease modifying agents. However, with prolonged use (>60 days), there is little difference between peak and trough response in FEV_1, even if zileuton administration is decreased to twice daily. This acquired, longer acting effect has been suggested to imply a disease modifying effect of antileukotriene therapy. In some studies, even acute administration of LTRAs has caused a modest decrease in eosinophil migration into asthmatic airways and the presence of the cysLT receptor, which has been recently identified on eosinophils, suggests a possible mechanism for this action. Other recent studies indicate that the LTRA, montelukast, if infused intravenously causes rapid incremental increase in FEV_1 in patients seeking treatment for acute asthma. The reason for the improved efficacy by intravenous infusion is unclear, but further investigations are examining the future role of LTRAs as potential rescue drugs in acute asthma.

LTRAs are extremely safe for patient use. However, the present generation of LTRAs is only modestly efficacious. Many patients show no clinically meaningful response, and current recommendations suggest a 1 month trial period to determine if patients will benefit from these drugs. With the exception of aspirin-sensitive asthmatics, there is currently no means for predicting which patients or under what circumstances antileukotrine therapies will be effective.

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Levodopa

►L-DOPA / Levodopa
►Dopamine System
►Anti-Parkinson Drugs

Lewy Bodies

Lewy bodies are typical in neuronal degeneration, which is accompanied by the presence of these eosinophilic intracellular inclusions of 5–25 μm diameter in a proportion of still surviving neurons. Lewy bodies contain neurofilament, tubulin, microtubule-associated proteins 1 and 2, and gelsolin, an actin-modulating protein.

►Anti-Parkinson Drugs

LH-RH Agonist

LH-RH (Luteinizing Hormone-Releasing Hormone) agonists are drugs that inhibit the secretion of sex hormones. In men, LH-RH agonists cause testosterone levels to fall. In women, LH-RH agonists cause the levels of estrogen and other sex hormones to fall.

►Contraceptives
►Targeted Cancer Therapy

Liddle's Syndrome

Liddle's syndrome is an autosomal dominant disorder that is caused by persistent hyperactivity of the epithelial Na channel. Its symptoms mimic aldosterone excess, but plasma aldosterone levels are actually reduced (pseudoaldosteronism). The disease is characterized by early onset arterial hypertension, hypokalemia, and metabolic alkalosis.

Disease-causing mutations are found in the cytoplasmic regulatory region of the β and γ subunits of the epithelial sodium channel (ENaC) genes. In general, patients with Liddle's syndrome can be treated successfully with the ENaC inhibitor amiloride.

►Diuretics
►Epithelial Na^+ Channels

Ligand

A ligand can be an antagonist or agonist that binds to a receptor.

Ligand-Gated Ion Channels

Ion channels that are opened by binding of a neurotransmitter (or drug) to a receptor domain on extracellular sites of the channel protein(s) are defined as ligand-gated. Nicotinic acetylcholine, glutamate, γ-aminobutyric $acid_A$ ($GABA_A$) and glycine receptors are examples of this type of receptor-linked ion channel.

►Benzodiazepines
►Ca^{2+} Channels
►Ionotropic Glutamate Receptors
►Nicotinic Receptors
►Non-selective Cation Channels
►K^+ Channels
►Serotoninergic System
►Purinergic System
►Table Appendix: Receptor Proteins
►Transmembrane Signaling
►Glyciu Receptor
►GABAergic System

Limbic System

Collection of interconnected subcortical and cortical brain structures (including hypothalamus, amygdala, and hippocampus) integrating multimodal intero- and exteroceptive information to produce coherent neuroendocrine and behavioral output, and to support memory functions.

►Orexins

Linkage Disequilibrium (LD)

Polymorphisms in the human genome are often not independently transmitted; i.e., a polymorphism is associated with particular variants present on the same chromosome. Recombination erodes this association, but for physically close polymorphisms (e.g., within a gene), the correlation, known as LD, persists over time.

►Pharmacogenomics

Lipid-lowering Drugs

Lipid-lowering drugs are drugs that affect the lipoprotein metabolism and that used in therapy to lower plasma lipids (cholesterol, triglycerides). The main classes of

drugs used clinically are statins (HMG-CoA reductase inhibitors), anion exchange resins (e.g. cholestyramine and cholestipol), fibrates (bezafibrate, gemfibrozil or clofibrate) and other drugs like nicotinic acid.

►HMG-CoA-Reductase-Inhibitors
►Fibrates
►Anion Exchange Resins

Lipid Metabolism

►Lipoprotein Metabolism

Lipid Modifications

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Synonyms

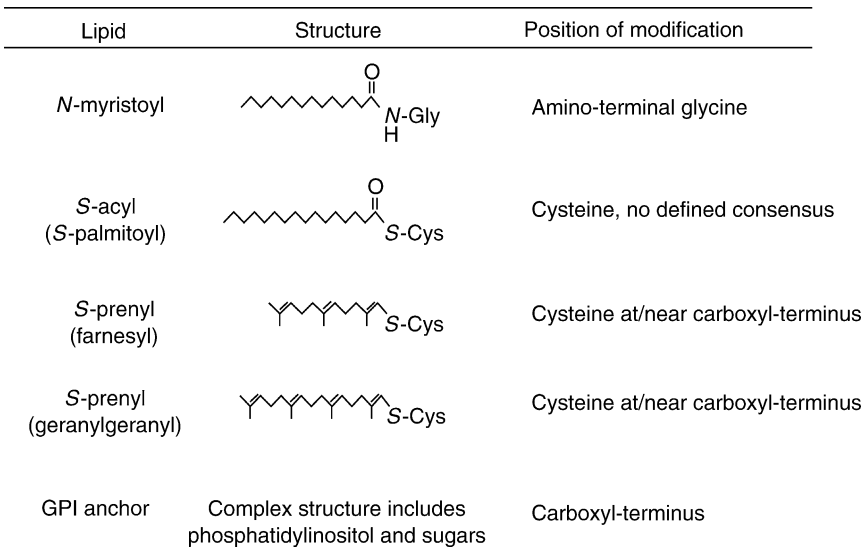
Lipidation; *S*-acylation; *N*-myristoylation; Myristoylation; *S*-prenylation; Prenylation; Palmitoylation; Isoprenylation; ►GPI anchors; Glypiation

Definition

Covalent attachment of lipid moieties to proteins plays important roles in the cellular localization and function of a broad spectrum of proteins in all eukaryotic cells. Such proteins, commonly referred to as lipidated proteins, are classified based on the identity of the attached lipid (Fig. 1). Each specific type of lipid has unique properties that confer distinct functional attributes to its protein host. *S*-acylated proteins, commonly referred to as palmitoylated proteins, generally contain the 16-carbon saturated acyl group palmitoyl attached via a labile thioester bond to cysteine residue(s), although other fatty acyl chains may substitute for palmitoyl group. *N*-myristoylated proteins contain the saturated 14-carbon myristoyl group attached via amide bond formation to amino-terminal glycine residues. *S*-prenylated proteins contain one of two ►isoprenoid lipids, either the 15-carbon farnesyl or 20-carbon geranylgeranyl. The fourth major class of lipidated proteins is those containing the glycosylphosphatidylinositol (GPI) moiety, a large and complex structure of which the lipid component is an entire phospholipid.

Basic Mechanisms

S-acylated proteins include many GTP-binding regulatory proteins (G proteins), including most α subunits of ►heterotrimeric G-proteins and also many members of the Ras superfamily of monomeric G proteins, a number of G protein-coupled receptors, several non-receptor ►tyrosine kinases, and a number of other signaling molecules. *S*-acylation is posttranslational and reversible, a property that allows the cell to control



Lipid Modifications. Figure 1 Major classes of lipid-modified proteins.

the modification state, and hence the localization and biological activity, of the protein [1]. The lipid substrates for *S*-acylation are ►acyl-CoA molecules. The molecular mechanism of *S*-acylation is only recently being elucidated; the first identified acyltransferase specifically involved in the process being the product of the *Skinny Hedgehog* (*Ski*) gene in Drosophila. *Ski* orthologs have also been identified in mammalian genomes. This acyltransferase appears to specifically process secreted proteins, in particular a molecule important in development termed Hedgehog.

The process by which cytoplasmic proteins are subject to *S*-acylation is also becoming clarified. The breakthrough in this arena came from genetic screens in yeast, in which two related *S*-palmitoyltransferases were uncovered that are termed Erf2 and Akr1. These two gene products share a common sequence referred to as a DHHC (aspartate-histidine-histidine-cysteine) motif, and a large number of genes encoding DHHC-motif proteins can be identified in all eukaryotes for which genome information is available. Although biochemical details of the enzymatic function of DHHC-motif proteins are still rather limited, the available evidence is consistent with the DHHC domain playing a direct role in the protein acyltransferase reaction.

In addition, nonenzymatic acylation of cysteine thiols on proteins incubated in the presence of acyl-CoA has been described, although the biological importance of this process is still unclear.

N-myristoylated proteins include select α subunits of heterotrimeric G proteins, a number of nonreceptor tyrosine kinases, a few monomeric G-proteins, and several other proteins important in biological regulation [2]. There is some overlap between *S*-acylated and *N*-myristoylated proteins such that many contain both lipid modifications. Such "dual lipidation" can have important consequences, most notably the localization of the dually modified species to distinct membrane subdomains termed ►lipid rafts or ►caveolae. *N*-myristoylation is a stable modification of proteins. The myristoyl moiety is attached to the protein cotranslationally by the enzyme myristoyl CoA: protein *N*-myristoyltransferase (NMT); the lipid substrate is myristoyl-CoA. NMT has been extensively studied in regard to substrate utilization and kinetic properties, and crystal structures of fungal enzymes are available. There are two distinct but closely related genes encoding NMTs in mammalian cells, *NMT1* and *NMT2*, although differences between the two isoforms have not yet been described.

S-prenylation is the most recent of the four major types of lipid modifications to be described. As with *S*-acylation, *S*-prenylation is posttranslational. The lipid substrates for these modifications are farnesyl diphosphate and geranylgeranyl diphosphate. The mechanism involves attachment of the isoprenoid lipid to cysteine residues at or near the carboxyl terminus through a stable thioether bond [3]. Two distinct classes of *S*-prenylated proteins exist in eukaryotic organisms. Proteins containing a cysteine residue fourth from the carboxyl terminus (the so-called CaaX-motif) can be modified by either the 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid by one of two closely related termed protein farnesyltransferase (FTase) and protein geranylgeranyltransferase type 1 (GGTase-1); these enzymes are collectively referred to as CaaX prenyltransferases [4]. FTase and GGTase-1 are αβ heterodimers; the α subunits of the enzymes are identical while the β subunits are the products of distinct, but related, genes. The identity of the "X" residue of the CaaX motif dictates which of the two enzymes recognize the substrate protein. Following prenylation, most CaaX-type proteins are further processed by proteolytic removal of the three carboxyl-terminal residues (i.e., the -aaX) by the Rce1 CaaX protease and methylation of the now-exposed carboxyl group of the prenylcysteine by the Icmt methyltransferase. A number of *S*-prenylated proteins are also subject to *S*-acylation at a nearby cysteine residue to produce a dually lipidated molecule, although this type of dual modification does not apparently target the protein to the same type of membrane subdomain as the dual acylation noted above.

The second class of *S*-prenylated proteins is a member of the Rab family of proteins, which is involved in membrane trafficking in cells. These proteins are geranylgeranylated at two cysteine residues at or very near to their carboxyl-terminus by protein geranylgeranyltransferase type 2 (GGTase-2), also known as Rab GGTase [5]. GGTase-2 is also a αβ heterodimer and its subunits show significant sequence similarity to the corresponding subunits of the CaaX prenyltransferases [6]. However, unlike the CaaX prenyltransferases, monomeric Rab proteins are not substrates for GGTase-2. In order to be processed by the enzyme, newly synthesized Rab proteins first bind and form a stable complex with a protein termed Rep, and it is the Rab–Rep complex that is recognized by GGTase-II. The enzyme acts in a processive fashion, attaching both geranylgeranyl groups to closely spaced cysteine residues at or near the carboxyl-terminus of the Rab proteins. All three of the protein prenyltransferases (FTase, GGTase-1, GGTase-2) have been extensively characterized in regard to substrate recognition, mechanism, and structure.

GPI-anchored proteins constitute a quite diverse family of cell-surface molecules that participate in such processes as nutrient uptake, cell adhesion, and membrane signaling events [3]. All GPI-linked proteins are destined for the cell surface via trafficking through the secretory pathway, where they acquire the

preassembled GPI moiety. The entire procedure, which involves assembly of the GPI moiety from ►phosphatidylinositol and sugars and proteolytic processing of the target protein to expose the GPI addition site at the carboxyl-terminus of the protein, involves a number of gene products. Many of the genes associated with GPI biosynthesis have been cloned by complementation of GPI-deficient mammalian cell lines and temperature-sensitive yeast GPI mutants. The precise mechanisms through which these enzymes work in concert to produce a GPI-anchored protein are just now beginning to be elucidated.

Pharmacological Intervention

Since the mechanisms of protein *S*-acylation are so poorly understood, there is little in the way of good pharmacological agents that target this process. One compound that has been used with modest success is cerulenin, which apparently mimics the acyl-CoA substrate in the reaction catalyzed by the putative *S*-acyltransferase that acts on intracellular proteins. In contrast, there has been substantial effort to identify and characterize specific inhibitors of *N*-myristoylation [2]. Genetic and biochemical studies have established NMT as a target for development of anti-fungal drugs. The enzyme is also a potential target for the development of antiviral and antineoplastic agents. Both peptidic and nonpeptidic inhibitors of NMTs, particularly fungal NMTs, have been described.

There has been enormous effort in the past 10 years to develop pharmacological agents targeting the *S*-prenylation by CaaX prenyltransferases, especially FTase [4]. This is primarily due to the interest in one subset of *S*-prenylated proteins, the Ras proteins, due to the important role of Ras in ►oncogenesis. Ras proteins are modified by the 15-carbon farnesyl isoprenoid, and farnesylation of these proteins is indispensable for both normal biological activity and oncogenic transformation. Selective inhibitors of FTase, termed FTIs, can reverse Ras-mediated oncogenic transformation of cells, and several are in clinical development as anticancer therapeutics. Literally hundreds of potent inhibitors of FTase, and many of GGTase-1, have been identified using several strategies, including design of analogs of the CaaX peptide and isoprenoid substrates and by high-throughput screening of natural product and compound libraries. These compounds can be placed into four distinct categories: mimics of CaaX tetrapeptides, mimics of FPP, ►bisubstrate analogs, and organic compounds selected from natural product and chemical libraries. There has been relatively little development of pharmacological agents targeting GGTase-2. It is likely, however, that some of the isoprenoid analogs that show activity against GGTase-1 will also have inhibitory activity on GGTase-2 given that both enzymes use the same isoprenoid substrate.

There is also increasing interest in developing pharmacological agents targeting the biosynthesis of GPI-anchored proteins [3]. Such proteins are particularly abundant on the surface of a number of protozoan organisms. Several devastating tropical diseases such as African sleeping sickness and Chagas disease are caused by protozoan parasites that rely heavily on cell-surface GPI-anchored proteins for both inhabiting their host and escaping immune detection. In studies primarily involving gene disruption approaches, several of the enzymes involved in GPI biosynthesis have been identified as attractive targets for development of antiparasitics. To date, there is very little publically available information on specific inhibitors of the enzymes involved in GPI biosynthesis and attachment, but it is likely that such information will be forthcoming.

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Lipid Phosphate Phosphohydrolases

Lipid phosphate phosphohydrolases (LPPs), formerly called type 2 phosphatidate phosphohydrolases (PAP-2), catalyse the dephosphorylation of bioactive phospholipids (phosphatidic acid, ceramide-1-phosphate) and lysophospholipids (lysophosphatidic acid, sphingosine-1-phosphate). The substrate selectivity of individual LPPs is broad in contrast to the related sphingosine-1-phosphate phosphatase. LPPs are characterized by a lack of requirement for Mg^{2+} and insensitivity to N-ethylmaleimide. Three subtypes (LPP-1, LPP-2, LPP-3) have been identified in mammals. These enzymes have six putative transmembrane domains and three highly conserved domains that are characteristic of a phosphatase superfamily. Whether LPPs cleave extracellular mediators or rather have an influence on intracellular lipid phosphate concentrations is still a matter of debate.

Lipid Rafts

Lipid rafts are specific subdomains of the plasma membrane that are enriched in cholesterol and sphingolipids; many signaling molecules are apparently concentrated in these subdomains.

►Lipid Modifications

Lipid Transfer Proteins

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Synonyms

Plasma lipid transfer proteins

Definition

Plasma lipid transfer proteins, which include the cholesteryl-ester-transfer-protein (CETP; previously known as lipid transfer protein I, LTP-I) and the phospholipid-transfer-protein (PLTP; previously known as lipid transfer protein II, LTP-II) mediate the transfer of lipids (cholesteryl esters, triglycerides and phospholipids) between lipoproteins present in human plasma. These proteins significantly affect plasma lipoprotein concentration and composition.

Basic Characteristics

Gene and Protein Characteristics

The genes coding for CETP and PLTP belong to one gene family, which also includes lipopolysaccharide-binding-protein (LBP) and bactericidal/permeability-increasing-protein (BPI). This common descent not only becomes clear from a considerable sequence similarity (45–65% homology at the cDNA level), but also from substantial conservation of exon/intron transitions.

The gene encoding CETP is located on the long arm of chromosome 16 (16q12-21), spans approximately 25 kbp and harbors 16 exons and 15 introns. The molecule mass of its mature protein product is 74 kDa. Major sites of CETP gene expression in humans are the liver, spleen and adipose tissue, with lower levels of expression in the small intestine, adrenals, kidney and heart. CETP present in human plasma seems to originate predominantly from the liver and adipose tissue, although data that have assessed this directly in man are lacking. The majority of human plasma CETP (~90%) is bound to ►high-density lipoprotein (HDL) particles. An important regulatory factor of CETP gene expression is hypercholesterolemia, which induces gene transcription. Sequences necessary for this cholesterol-responsiveness are located in the natural flanking regions of the human CETP gene, upstream of the transcription start site. This regulatory sequence concerns an element of two direct repeats separated by four nucleotides (DR4 element). This element enhances CETP gene expression following activation through a heterodimer, containing the transcription factors Liver X Receptor (LXR) and Retinoid X Receptor (RXR). In order to enhance gene transcription, these transcription factors require activation through ligands, which include cholesterol and its oxysterols.

The gene coding for PLTP has been mapped to chromosome 20 (20q12-13.1), where also the genes for LBP and BPI are situated. The PLTP gene contains 13.3 kbp, with similar organization as compared to CETP (16 exons-15 introns). Purified PLTP has a molecule mass of 81 kDa. PLTP is expressed in a variety of tissues including placenta, pancreas, lung, kidney, heart, liver, adipose tissue, skeletal muscle and brain. Macrophages also express PLTP and may contribute significantly to plasma PLTP activity levels. PLTP gene expression can be upregulated by cholesterol in a similar way as described for CETP. In addition, PLTP gene expression is subject to regulation via response elements for the peroxisome proliferator activated receptor (PPAR) alpha and the farnesoid X-activated receptor (FXR).

Detailed protein structures have been reported for BPI and CETP. Given the aforementioned similarities within this gene family, these protein structures serve as a likely model for the protein structure of PLTP. CETP and BPI are elongated molecules, shaped like a boomerang. There are two domains with similar folds, and a central beta-sheet domain between these two domains. The molecules contain two lipid-binding sites, one in each domain near the interface of the barrels and the central beta-sheet.

Function

In humans, CETP and PLTP are directly involved in the transfer of lipids between different ►lipoprotein classes. Through their action, these lipid transfer proteins have major effects on the concentration and composition of HDL. This section further describes the physiological function of CETP and PLTP in humans.

CETP mediates the exchange of cholesteryl esters and triglycerides between HDL and the proatherogenic,

apoB-containing lipoprotein fractions, predominantly ►very-low density lipoprotein (VLDL). Specifically, triglycerides are transported from VLDL to HDL, with cholesteryl esters transferred in the opposite direction. It is generally acknowledged that CETP itself has no preference for the type of lipoprotein in its substrate specificity. The VLDL–HDL axis is likely to result from the presence of plasma lipid transfer inhibitor protein (LTIP), which is associated almost exclusively with ►low-density lipoprotein (LDL) and inhibits the CETP-mediated transfer of cholesteryl esters and triglycerides. LTIP suppresses the involvement of LDL, thereby directing the CETP activity towards the remaining lipoprotein fractions, VLDL and HDL. The CETP-mediated lipid exchange is an equimolar and energy neutral process. The exchange seems to occur by a shuttle mechanism, rather then by the formation of a complex between CETP and donor and acceptor lipoproteins. In addition to LTIP, composition and concentration of lipoprotein substrates are important regulatory factors of the CETP-mediated exchange activity, and under physiological conditions, the net mass of VLDL-triglycerides may be rate limiting. The relevance of CETP for human lipid metabolism became evident after the identification of humans with partial or near complete loss of CETP activity due to the presence of a mutation in one or both alleles of the CETP gene. In these subjects, who predominantly live in Japan, (partial) loss of plasma CETP reduces the ability of cholesteryl esters to be transported out of HDL. This results in a net increase in plasma HDL cholesterol (HDL-C) levels, with the appearance of HDL particles that have increased in size. This change is paralleled by increased concentrations of ►apolipoprotein A-I (apoA-I), which results from delayed catabolism of these large sized HDLs. In some cases of severe CETP deficiency, the reduced transfer of cholesteryl esters from HDL to VLDL furthermore gives also rise to mildly decreased levels of LDL cholesterol (LDL-C), which originates from VLDL. Recently, the first Caucasian family was described with partial CETP deficiency due to a novel splice site mutation in the CETP gene.

PLTP is responsible for the majority of phospholipid transfer activity in human plasma. Specifically, it transfers surface phospholipids from VLDL to HDL upon lipolysis of triglycerides present in VLDL. The exact mechanism by which PLTP exerts its activity is yet unknown. The best indications for an important role in lipid metabolism have been gained from knockout experiments in mice, which show severe reduction of plasma levels of HDL-C and apoA-I. This is most likely the result of increased catabolism of HDL particles that are small in size as a result of phospholipid depletion. In addition to the maintenance of normal plasma HDL-C and apoA-I concentration, PLTP is also involved in a process called HDL conversion. Shortly summarized, this cascade of processes leads to fusion of HDL particles into larger particles, with a concomitant release of lipid-poor apoA-I. This newly formed lipid-poor apoA-I is a pivotal particle in the uptake of free cholesterol from peripheral cells via efflux n through ABCA1. This is a potentially antiatherogenic function of PLTP, but – surprisingly – overexpression of human PLTP in mice results in increased susceptibility for diet-induced atherosclerosis and a reduction of plasma HDL-C, again due to hypercatabolism of apoA-I. At present, no reports exist on genetic PLTP deficiency in humans. Therefore, any hypothesis on the relevance of PLTP activity for human lipid metabolism remains to be confirmed. When it comes to humans, plasma PLTP activity is increased in insulin-resistant individuals with high plasma triglycerides and low HDL-C, as well as in patients with diabetes mellitus. In patients with type 2 diabetes, PLTP activity is positively associated with intima-media thickness, which is a surrogate measure for the risk of atherosclerotic disease. In addition, elevated PLTP activity is decreased by statin treatment.

Drugs

Plasma HDL-C concentration is negatively associated with occurrence of cardiovascular disease, independent of age, gender, LDL-C and other established risk factors. Consequently, there is great interest for pharmacological interventions to raise HDL-C. Among the drugs that are currently available, statins, fibrates and nicotinic acid exert such an increasing effect on plasma HDL-C concentrations. These drugs, however, are limited in their HDL-C raising efficacy (statins: +3% to +9%, fibrates: +0% to +11%, nicotinic acid: +7% to +23%) or exhibit significant side effects (nicotinic acid: flushes). The high levels of HDL-C observed in case of CETP deficiency have led to the development of strategies to raise HDL-C by inhibition of CETP activity in humans. Two such strategies are currently in advanced stage of development, i.e., vaccine-induced inhibition and inhibition of CETP activity through small-molecule compounds. Among these, the latter has attracted most attention. The effects of CETP inhibition through small-molecule compounds parallel those observed in genetic CETP deficient subjects to a large extent: increase of plasma HDL-C and apoA-I concentration, with the occurrence of large sized HDL particles due to cholesteryl ester enrichment. In case of potent CETP inhibition, plasma levels of LDL-C and apoB are also decreased. With respect to the effects on cardiovascular disease exerted by these lipoprotein changes, data from epidemiological studies might justify optimism. However, clinical trials assessing the consequences of CETP inhibition on surrogate and clinical cardiovascular endpoints are required. One such trial has recently been terminated due to an increased number of lethal cardiovascular events occurring in the CETP-inhibition group. At the moment,

it remains to be established whether this relates to intrinsic characteristics of the study drug or to the mechanism of CETP-inhibition in general.

Due to the absence of human models for PLTP deficiency, our knowledge about the relevance of plasma PLTP activity for human lipid metabolism is still incomplete. No investigational drugs are available that specifically target the activity of this protein.

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Lipidation

►Lipid Modifications

Lipopolysaccharide

A lipopolysaccharide (LPS) is any compound consisting of covalently linked lipids and polysaccharides. The term is used more frequently to denote a cell wall component from Gram-negative bacteria. LPS has endotoxin activities and is a polyclonal stimulator of B-lymphocytes.

►Neutrophils
►Inflammation

Lipoprotein Lipase

Endothelial anchored enzyme in muscle and adipose tissue primarily responsible for hydrolysis of chylomicron and VLDL triglycerides.

►Lipoprotein Metabolism

Lipoprotein Metabolism

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Synonyms

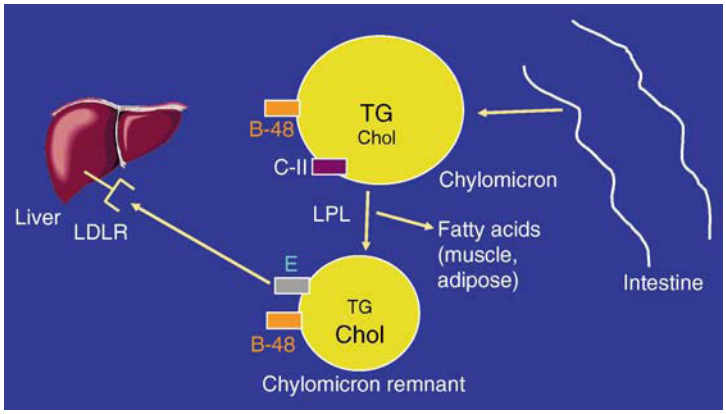
Lipid metabolism; Cholesterol metabolism

Definition

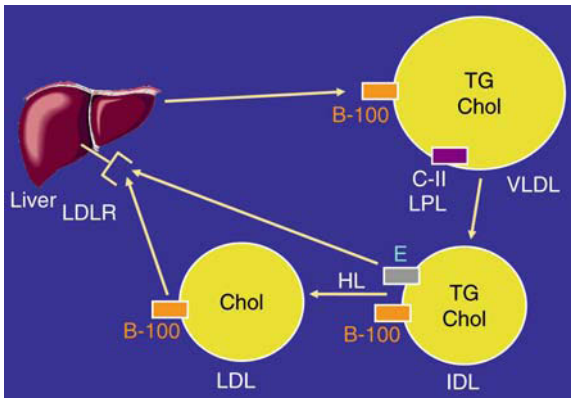
►Lipoprotein metabolism is the process by which hydrophobic lipids, namely triglycerides and cholesterol, are transported within the interstitial fluid and plasma. It includes the transport of energy in the form of triglycerides from intestine and liver to muscles and adipose, as well as the transport of cholesterol both from intestine and liver to peripheral tissues, as well as from peripheral tissues back to the liver.

Basic Mechanisms

Lipoproteins are large macromolecular complexes, which transport triglycerides and cholesterol within the blood. Triglycerides are a key component of energy transport and metabolism, and cholesterol is an essential component of all cells and required for steroidogenesis. The structure of lipoproteins is a hydrophobic neutral lipid core consisting of triglycerides and cholesteryl esters, surrounded by amphipathic phospholipids and specialized proteins known as ►apolipoproteins. Phospholipids serve to permit interaction with the aqueous environment, and apolipoproteins are required for the structural integrity of lipoproteins and direct their metabolic interactions with enzymes, lipid transport proteins, and cell surface receptors. The five major families of lipoproteins are ►chylomicrons, ►very-low-density lipoproteins (VLDL), ►intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and ►high-density lipoproteins (HDL). Chylomicrons are the largest and most lipid-rich lipoproteins, whereas HDL are the smallest lipoproteins and contain the least amount of lipid. In the exogenous pathway of lipid transport (Fig. 1), dietary fat and cholesterol are absorbed by intestinal enterocytes and incorporated into chylomicrons, which contain the major structural apolipoprotein B-48. Chylomicrons are initially secreted into lymph thus bypassing the hepatic first pass effect. Once they gain entry into the systemic circulation they bind to ►lipoprotein lipase (LPL) on the luminal surface of the capillary endothelium of tissues (►endothelial lipase), especially muscle and adipose tissue. The LPL hydrolyzes the triglycerides (apolipoprotein C-II on the chylomicron surface is a required cofactor for LPL). The free fatty



Lipoprotein Metabolism. Figure 1 Exogenous pathway of lipoprotein metabolism.

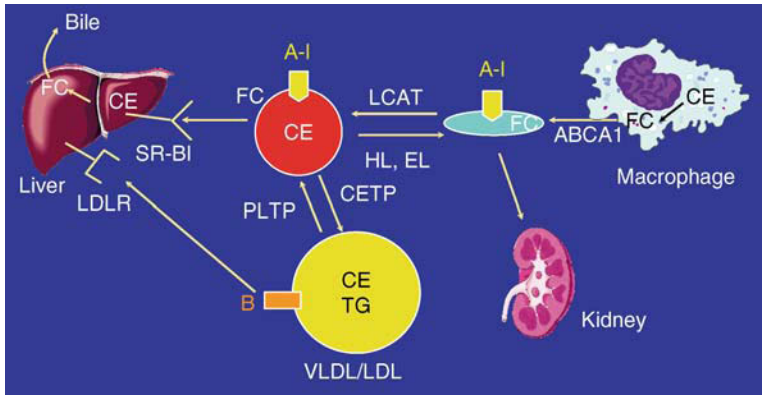


Lipoprotein Metabolism. Figure 2 Endogenous pathway of lipoprotein metabolism.

acids enter the tissue to be used for energy (muscle) or storage (adipose) and the triglyceride-depleted chylomicron remnant (CMR) is released. CMRs are taken up by the liver by binding of apolipoprotein E to the LDL receptor and the LDL receptor-related protein (LRP). In the endogenous pathway of lipid transport (Fig. 2), the liver synthesizes triglycerides and cholesteryl esters and packages them into VLDL, which contain the major structural apolipoprotein B-100. VLDLs are hydrolyzed by LPL to form intermediate density lipoproteins (IDL). IDL can be taken up by the liver via binding of apolipoprotein E to the LDL receptor or LRP. Alternatively, the triglyceride and phospholipid in IDL can be hydrolyzed by ►hepatic lipase (HL) within the hepatic sinusoids to form LDL. LDL can be taken up by peripheral cells or by the liver by the binding of apolipoprotein B-100 to the LDL receptor.

The pathways of HDL metabolism and reverse cholesterol transport are complex (Fig. 3, [1]). HDL and its major apolipoprotein apoA-I are synthesized by both the intestine and the liver. A second major HDL protein apoA-II is made only by the liver. Nascent HDL interacts with peripheral cells to facilitate the removal of excess free cholesterol through a process that is facilitated by the cellular protein ATP-binding cassette protein A1 (ABCA1). Some of the acquired free cholesterol is esterified to cholesteryl ester on the HDL particle by the action of the enzyme ►lecithin-cholesterol acyltransferase (LCAT) and the nascent HDL particle becomes the larger HDL_3. HDL acquires further cholesteryl ester by continued LCAT action and eventually becomes the even larger HDL_2. HDL_2 can selectively transfer both cholesteryl ester and free cholesterol to the liver via an HDL receptor in the liver called scavenger receptor BI (SR-BI). Cholesteryl esters can also be transferred from HDL_2 to apoB-containing lipoproteins such as VLDL and LDL via the action of the ►cholesteryl ester transfer protein (CETP) and then returned to the liver by hepatic uptake of LDL. HDL_2 triglycerides and phospholipids can be hydrolyzed by HL and endothelial lipase (EL) to remodel it to HDL_3. Cholesterol derived from HDL contributes to the hepatic cholesterol pool used for bile acid synthesis and the cholesterol is eventually excreted into the bile and feces as bile acid or free cholesterol.

Disorders of lipoprotein metabolism involve perturbations which cause elevation of triglycerides and/or cholesterol, reduction of HDL-C, or alteration of properties of lipoproteins, such as their size or composition. These perturbations can be genetic (primary) or occur as a result of other diseases, conditions, or drugs (secondary). Some of the most important secondary disorders include hypothyroidism, diabetes mellitus, renal disease, and alcohol use. Hypothyroidism causes elevated LDL-C levels due primarily to downregulation of the LDL receptor. Insulin-resistance and type 2 diabetes mellitus result in impaired capacity to catabolize chylomicrons and VLDL, as well as excess hepatic triglyceride and VLDL production. Chronic kidney disease, including but not limited to end-stage



Lipoprotein Metabolism. Figure 3 HDL metabolism and reverse cholesterol transport.

renal disease, is associated with moderate hypertriglyceridemia due to a defect in triglyceride lipolysis and remnant clearance. Nephrotic syndrome is associated with a more pronounced hyperlipidemia involving both elevated triglycerides and cholesterol due to hepatic overproduction of VLDL. Alcohol consumption inhibits oxidation of free fatty acids by the liver, which stimulates hepatic triglyceride synthesis and secretion of VLDL; the usual lipoprotein pattern associated with alcohol consumption is moderate hypertriglyceridemia.

Inherited disorders of lipoprotein metabolism have provided important insights into the molecular regulation of lipoprotein metabolism in humans. The familial hyperchylomicronemia syndrome (FCS) (also known as Type I hyperlipoproteinemia), characterized by severely elevated triglycerides, is caused by genetic deficiency of either ►LPL or apoC-II, demonstrating the essential nature of each of these protein for the hydrolysis of chylomicron triglycerides. Familial dysbetalipoproteinemia (FD) (also known as Type III hyperlipoproteinemia), characterized by elevation in apoB-containing lipoprotein remnant particles containing both triglycerides and cholesterol, is caused by specific mutations in the gene for apolipoprotein E (apoE), most commonly homozygosity for the relatively common apoE2 variant. This proved the essential nature of apoE for normal clearance of lipoprotein remnants by the liver. Familial hypercholesterolemia (FH) (also known as Type IIa hyperlipoproteinemia), characterized by elevation in LDL cholesterol, is caused by loss-of-function mutations in the LDL receptor. Homozygotes are more severely affected than heterozygotes, demonstrating the gene dose effect of the LDL receptor on LDL-C levels. This disorder established the key role of the LDL receptor in regulating plasma levels of LDL-C through mediating uptake of LDL by the liver. Mutations in other genes have been subsequently discovered to regulate the hepatic expression of the LDL receptor and thus influence plasma LDL-C levels. These include loss-of-function mutations in the *autosomal recessive hypercholesterolemia* (*ARH*) gene, a recessive trait, and gain-of-function mutations in the *proprotein convertase subtilisin/kexin type 9* (*PCSK9*) gene, an autosomal dominant trait. Sitosterolemia is caused by mutations in one of two members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family, ABCG5 and ABCG8, resulting in increased cholesterol in the liver and secondary downregulation of the LDL receptor. Finally, mutations in the receptor binding region of apoB-100, the ligand for the LDL receptor, can impair its binding to the LDL receptor and delay the clearance of LDL, a condition known as familial defective apoB-100 (FDB).

Genetic conditions causing low cholesterol have also provided important insights and novel pharmacologic targets. Abetalipoproteinemia is characterized by absence of plasma apoB-containing lipoproteins and is caused by mutations in the gene encoding microsomal triglyceride transfer protein (MTP), a protein that transfers lipids to nascent chylomicrons and VLDL in the intestine and liver, respectively. Familial hypobetalipoproteinemia is characterized by markedly reduced LDL-C and apoB levels and is caused by mutations in apoB that generally result in premature truncation that either reduce secretion and/or accelerate catabolism. Finally, loss-of-function mutations in *PCSK9* have been shown to cause low LDL-C levels and substantial lifetime protection from CHD. All three of these gene products are targets for the development of new therapies for reducing LDL-C levels.

Genetic disorders of HDL metabolism have also resulted in greater understanding of the molecular regulation of HDL metabolism. Nonsense or missense mutations in apoA-I can result in substantially reduced HDL-C levels due to rapid catabolism of structurally abnormal or truncated apoA-I proteins. Tangier disease is a rare autosomal codominant disorder characterized by markedly low HDL-C and apoA-I levels and caused

by mutations in the gene *ABCA1*. ABCA1 facilitates efflux of cholesterol from cells to lipid-poor apoA-I, and in its absence apoA-I is not appropriately lipidated and is rapidly cleared from the circulation. LCAT deficiency causes markedly low levels of HDL-C, establishing the importance of cholesterol esterification by LCAT in the maintenance of normal HDL metabolism. Finally, CETP deficiency causes markedly elevated levels of HDL-C, establishing the importance of cholesteryl ester by CETP in the maintenance of normal HDL metabolism. Again, all of these gene products are targets of new HDL-based therapies.

Pharmacologic Relevance

LDL Cholesterol Reduction

Disorders of lipoprotein metabolism are important risk factors for atherosclerotic vascular disease (ASCVD). Thus the field of lipoprotein metabolism has been a fertile area for the development of drugs that modulate the levels of plasma lipoproteins. Intervention with drugs to reduce ►LDL cholesterol has been proven to decrease the risk of cardiovascular events [2]. Intervention with drugs to reduce triglycerides or raise HDL-C has not yet been definitively proven to reduce cardiovascular events. The early clinical trials for LDL-C reduction utilized niacin, bile acid sequestrants, and even the surgical approach of partial ileal bypass to reduce serum cholesterol levels. However, the bulk of the data supporting LDL-C reduction comes from the clinical outcome trials with ►HMG CoA-reductase inhibitors (statins). Early studies focused on patients with preexisting CHD, including the Scandinavian Simvastatin Survival Study (4S), the Cholesterol and Recurrent Events (CARE) study, and the Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) trial and established the efficacy of statin therapy in reducing cardiovascular events in patients with CHD. Early primary prevention trials included the West of Scotland Coronary Prevention Study (WOSCOPS) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) and confirmed that the benefits of LDL cholesterol reduction extend to the primary prevention setting. Subsequent studies have expanded downward the range of baseline LDL-C levels for which statin therapy is beneficial as well as the target LDL that is effective [2], and include the Heart Protection Study (HPS), the Anglo-Scandinavian Cardiac Outcomes Trial Lipid-lowering Arm (ASCOT-LLA), the Collaborative Atorvastatin Diabetes Study (CARDS), the Treat to New Targets (TNT) trial, and the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) study. These studies strongly support the "lower is better" approach to reducing LDL-C as applied to high-risk patients.

Thus drug therapy for LDL-C reduction is widely used. Statins are the cornerstone of LDL-C reducing drug therapy. By inhibiting cholesterol biosynthesis in the liver, statins lead to increased hepatic LDL receptor expression. There are six statins currently available: lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, and rosuvastatin. There is wide interindividual variation in the initial response to a statin, but once a patient is on a statin, the doubling of the statin dose produces a very predictable 6% further reduction in LDL-C. Some patients treated with statins develop muscle fatigue or pain, and severe myopathy and rhabdomyolysis have been reported. The risk of statin-associated myopathy is increased by the administration of drugs that interfere with the cytochrome P450 metabolism of statins [3]. Due to both inadequate reduction of LDL-C in some patients as well as statin intolerance in others, there is a clinical need for LDL-reducing drug therapy beyond statins. The most commonly used class is that of cholesterol absorption inhibitors; currently ezetimibe is the only drug in this class clinically available. Ezetimibe binds to and inhibits the function of NPC1L1, a major cholesterol transporter in the intestinal enterocyte responsible for absorption of luminal cholesterol derived both from diet as a well as from bile. The inhibition of intestinal cholesterol absorption reduces hepatic cholesterol content and results in upregulation of hepatic LDL receptor expression. Ezetimibe at the 10 mg dose reduces cholesterol absorption by about 60% and reduces LDL-C levels by about 18% on average as monotherapy, and to a similar extent when used in combination with a statin. Ezetimibe is used in combination with statins to further reduce LDL-C levels and in patients who are statin-intolerant. In some cases, a third class of LDL-lowering drugs, bile acid sequestrants, is needed. Bile acid sequestrants bind bile acids in the intestine, prevent their reabsorption, and accelerate their loss in the feces. In order to maintain an adequate bile acid pool, the liver diverts cholesterol to bile acid synthesis, resulting in decreased hepatic cholesterol and upregulation of hepatic LDL receptor expression. Cholestyramine and colestipol are insoluble resins that must be suspended in liquid and colesevalam is available as large tablets, and relatively large amounts of bile acid sequestrants must be used to achieve effective LDL reduction.

Inability to achieve LDL-C goals with existing drug therapy remains an important unmet medical need. Advances in our understanding of the molecular regulation of LDL metabolism have generated new pharmacologic targets. These include the following: (i) inhibition of squalene synthase, another important enzyme in the cholesterol biosynthetic pathway; (ii) inhibition of MTP, which reduces VLDL production

and has been shown to reduce LDL-C levels; (iii) inhibition of apoB production using an antisense oligonucleotide approach; (iv) inhibition of PCSK9 based on the persuasive genetic data reviewed above.

The data supporting reduction in triglycerides or raising of HDL-C are much less abundant [4]. Fibrates and nicotinic acid are the most effective drugs in lowering TGs and raising HDL-C. Fibrates are agonists of PPARα and are most effective as triglyceride lowering drugs, with modest effects in raising HDL-C. Fibrates lower triglycerides by activating PPARα to stimulate ►LPL (enhancing triglyceride hydrolysis) and reduce hepatic apoC-III synthesis (enhancing clearance of TG-rich lipoproteins). A limited number of clinical trials with fibrates support the concept that they may reduce cardiovascular risk, particularly in patients with the phenotype of insulin-resistance. Increasingly, fibrates are being used in combination with statins. Nicotinic acid, or niacin, is a B-complex vitamin that in high doses lowers triglycerides and raises HDL-C levels. Niacin acutely reduces free fatty acid levels through inhibition of adipocyte triglyceride lipolysis, an effect now known to be mediated by the niacin receptor GPR109A, a G protein coupled receptor primarily expressed on adipocytes. Whether this mechanism accounts for the triglyceride lowering and HDL-C raising associated with niacin therapy is uncertain. The clinical use of niacin is limited by prostaglandin-mediated cutaneous flushing which is also mediated by GPR109A expressed on dermal macrophages. Data that niacin reduces cardiovascular risk is limited, but are consistent with a cardioprotective effect. Patients with combined hyperlipidemia may achieve their LDL-C goal with a statin alone, but frequently have persistently elevated triglycerides and often do not achieve their non-HDL-C goal. In this situation, the addition of either niacin or a fibrate to the statin can be highly effective in reducing the triglycerides and non-HDL-C levels. The therapy of low HDL-C is a major unmet medical need and new therapies designed to raise HDL-C levels or promote HDL function are under active development [1].

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Lipoproteins

The main transport form of lipids in the circulation. They are spherical macromolecules of 10–1200 nm diameter composed of a core of neutral lipids (mostly cholesterol ester and triglycerides) surrounded by an amphipathic shell of polar phospholipids and cholesterol. Embedded in the shell of lipoproteins are apolipoproteins that are essential for assembly of the particles in tissues that secrete lipoproteins, and for their recognition by target cells.

►Lipoprotein Metabolism
►Lipid Transfer Proteins
►HMG-CoA-Reductase Inhibitors
►Low-Density Lipoprotein Receptor Gene Family

Liposomes

Phospholipid vesicles, uncoated or polyethylenglycol-coated. They can be used to vehicle drugs, antibodies or nucleic acids to target cells.

5-Lipoxygenase

5-lipoxygenase is the enzyme causing catalysis of arachidonic acid into leukotriene A4.

►Leukotrienes

Lipoxygenases

►Leukotrienes

Lithocholic Acid

3α-Hydroxy-5β-cholanoic acid, a hepatotoxic and cholestatic secondary bile acid, formed by bacterial dehydroxylation of primary bile acids in the intestine.

►Nuclear Receptor Regulation of Hepatic Cytochrome P450 Enzymes

Local Anaesthetics

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Definition

Local anaesthetics are drugs that reversibly interrupt impulse propagation in peripheral nerves thus leading to autonomic nervous system blockade, analgesia, anaesthesia and motor blockade in a desired area of the organism.

Mechanism of Action

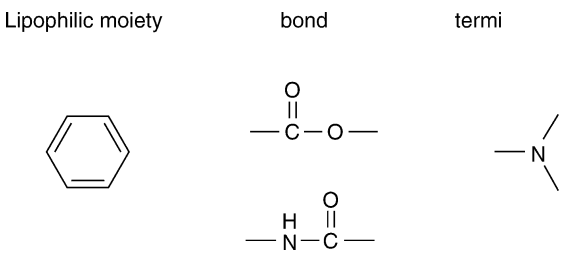
Impulse propagation in the ▶peripheral nervous system depends on the interplay between ion channels selective to potassium and sodium. Briefly, few voltage insensitive potassium channels allow diffusion of positively charged potassium ions from the internal side of the axon to the exterior. This leaves a negative charge at the internal side that is called resting potential and amounts to approximately −80 mV over the ▶axonal membrane. Upon a small depolarisation of the membrane, ▶voltage–gated Na^+ channels open (activate) and conduct positively charged sodium ions from the exterior to the interior of the axon that depolarises the membrane to about +60 mV (action potential). After a few milliseconds the channels close spontaneously (inactivation) terminating the action potential and thus giving it an impulse like character. The action potential spreads electrotonically to neighbouring sodium channels that also open to produce an action potential. In this way, the impulse is propagated along the nerve to (afferent) or from (efferent) the central nervous system. Local anaesthetics inhibit ionic current through voltage gated sodium channels in a concentration dependent and reversible manner therefore directly blocking the impulse propagation process in either direction [1]. The interaction between the local anaesthetic molecule and the sodium channel is complex. The binding affinity is low for resting channels, but dramatically increases when the channel is activated. Thus, at high stimulus frequency sodium current block increases (use-dependent block). Use-dependent block is not important for conduction block during local anaesthesia since very high concentrations are reached locally producing complete and sufficient block. However, when local anaesthetics such as lidocaine are applied intravenously, lower systemic concentrations may already induce use-dependent block and thus reduce excitability in electrically active cells. This mechanism is important for the successful use of lidocaine as an antiarrhythmic or analgesic drug.

The putative binding site for local anaesthetic molecules at the sodium channel has been identified as two amino acids in the sixth membrane-spanning segment of domain IV [2]. This binding site is located directly underneath the channel pore and can only be reached from the internal side of the membrane. Because local anaesthetics are applied exterior to the nerve fibre, they have to penetrate the axonal membrane before they can bind to the channel.

Besides sodium channels, other ion channels such calcium- and potassium channels as well as certain ligand-gated channels are affected by local anaesthetics. However, this plays only a minor role for nerve block but may have more impact on adverse effects induced by systemical concentrations of these drugs.

Structure Activity Relation

Local anaesthetics comprise a lipophilic and a hydrophilic portion separated by a connecting hydrocarbon chain (Fig. 1). The hydrophilic group is mostly a tertiary amine such as diethylamine; the lipophilic group is usually an unsaturated aromatic ring such as xylidine or para-aminobenzoic acid. The lipophilic portion is essential for anaesthetic potency whereas the hydrophilic portion is required for water solubility. Both groups are important for binding the drug molecule to the sodium channel. The connecting hydrocarbon chain usually contains an ester or an amide, in rare cases also an ether, and separates the lipophilic and hydrophilic portions of the molecule in an ideal distance for binding to the channel. Local anaesthetics are classified by the nature of this bond into ester or amide local anaesthetics. This bond is also important for metabolism of the drugs and to adverse reactions such as allergic reactions. Clinically relevant local anaesthetics have an efficacy of 100%, i.e. at high concentrations they completely abolish the sodium current. Their blocking potencies range from a few micromolar to millimolar half-maximal blocking concentration and are highly dependent on lipid solubility. To a smaller amount potency also depends on the structure of the molecule and on the type of bonding



Local Anaesthetics. Figure 1 Common structure of local anaesthetics. A lipophilic moiety on the left, an aliphatic spacer containing an ester or amide bond in the middle and an amine group on the right are the typical structural elements for local anaesthetic drugs.

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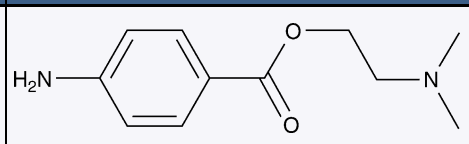
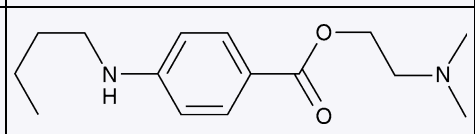
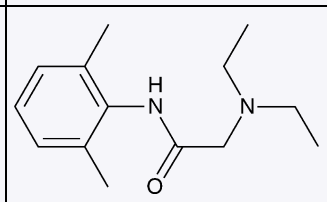
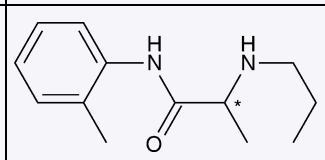
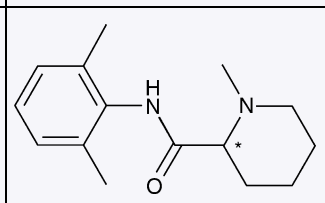
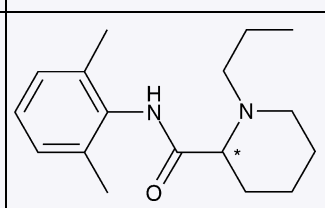
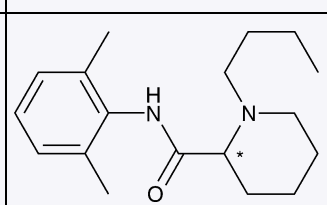
(Table 1). Further determinants of blocking potency are the membrane potential and the state in which the sodium channel is in (resting, activated, inactivated). The tertiary amine group can be protonated giving most local anaesthetics a pK_a of about eight so that a larger amount of the drug is in the hydrophilic form when injected into tissue with physiological pH. However, only the unprotonated lipophilic form is able to penetrate the

Local Anaesthetics. Table 1 Examples of clinically used local anaesthetics

Name	Structure	Relative potency	Mean blocking duration [h]	Lipid solubility log[P]	Molwt. [g/mol]	pKa
Procaine		1	0.5–1	1.92	236	9.1
Tetracaine		8	2–3	3.73	264	8.6
Lidocaine		2	1–2	2.26	234	8.2
Prilocaine		2	1–2	2.11	220	7.9
Mepivacaine		2	1,5–2	1.95	246	7.9
Ropivacaine		6	3–5	2.90	274	8.2
Bupivacaine		8	4–6	3.41	288	8.2

The ending “caine” stems from cocaine, the first clinically employed local anaesthetic. Procaine and tetracaine are ester-linked substances, the others are amides. Amide bonded local anaesthetics usually contain two i’s in their name, ester-bonded only one. In the structure drawings, the lipophilic portion of the molecule is depicted at the left, the amine at the right. The asterisk marks the chiral centre of the stereoisomeric drugs. Lipid solubility is given as the logarithm of the water:octanol partition coefficient, log(P).

axonal membrane, which is required before binding to the sodium channel can occur. In a very acidic environment, like inflamed tissue, local anaesthetics are highly protonated, therefore cannot penetrate the axonal membrane and have little effect.

Toxicity

Local anaesthetics interfere with all voltage-gated sodium channel isoforms in an organism and thus with all electrically excitable cells in organs such as brain, heart and muscle. The major unwanted effects of local anaesthetics are thus disturbances of brain and heart function occurring during systemically high concentrations after overdosing or after accidental intra-vascular injection. Cerebral convulsions, general anaesthesia as well as dysrhythmias up to asystolic heart failure or ventricular fibrillation are feared as rare but most harmful complications. Other adverse effects can be allergic reactions. This occurs especially with ester-bonded local anaesthetics because their metabolism produces para-aminobenzoic acid that may serve as a hapten. As a special case, the local anaesthetic prilocaine is metabolised to o-toluidine, which may induce methemoglobinemia, especially in patients that have a glucose-6-phosphatase deficiency. The ►S-stereoisomers of the piperidine local anaesthetics bupivacaine and ropivacaine have now been introduced into clinical practise to reduce side effects. Their blocking potencies are minimally lower compared to their R-counterparts but their therapeutic index is wider.

Clinical Use

Local anaesthetics are mainly employed to induce regional anaesthesia and analgesia to allow surgical procedures in a desired region of the organism. Nerve block result in autonomic nervous system blockade, analgesia, anaesthesia and motor blockade. The patient normally stays awake during surgical procedures under regional anaesthesia, but regional anaesthesia can also be combined with general anaesthesia to reduce the requirement of narcotics and analgesic drugs. Local anaesthetics have to be injected locally into the circumference of a peripheral nerve, which gives sufficiently high concentrations to achieve conduction block. Certain injection techniques and procedures such as single nerve block as well as spinal, ►epidural, or intravenous regional anaesthesia have evolved to achieve the desired nerve block. It is also possible to place a catheter adjacent to a nerve and continuously apply local anaesthetics to receive long-term analgesia. Low concentrations of lipophilic local anaesthetics may be used for differential nerve block, i.e. only block of sympathetic and nociceptive fibres whereas somatosensory and motor fibres are less affected. Differential nerve block for example is useful in labour analgesia or for analgesia after surgical procedures of the extremities.

After local anaesthetic injection, onset of nerve block and duration depends mainly on lipid solubility and on the region in where the drug is injected. In some formulations adrenaline is added to prolong the blocking action by inducing regional vasoconstriction and hereby reduce absorption and metabolisation.

The amide local anaesthetic lidocaine may also be used as an antiarrhythmic for ventricular tachycardia and exrasystoles after injection into the blood circulation. Drugs with high lipid solubility such as bupivacaine cannot be used for these purposes because their prolonged binding to the channel may induce dysrhythmias or asystolic heart failure [3]. Systemically applied lidocaine has also been used successfully in some cases of ►neuropathic pain syndromes [4]. Here, electrical activity in the peripheral nervous system is reduced by used-dependent but incomplete sodium channel blockade.

►General Anaesthetics
►Voltage-dependent Na^+ Channels

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Locus

A specific region within a DNA sequence.

►Pharmacogenomics

Locus Ceruleus

The locus ceruleus is a structure located on the floor of the fourth ventricle in the rostral pons. It contains more than 50% of all noradrenergic neurons in the brain, and projects to almost all areas of the central nervous system.

The locus ceruleus is important for the regulation of attentional states and autonomic nervous system activity. It has also been implicated in the autonomic and stress-like effects of opiate withdrawal. A noradrenergic pathway originating from the locus ceruleus which descends into the spinal cord is part of the descending inhibitory control system, which has an inhibitory effect on nociceptive transmission in the dorsal horn.

Long-Chain Fatty Acids

Long-chain fatty acids (LCFAs) are aliphatic compounds with a terminal carboxyl group and with a chain length greater than 12 carbon atoms (e.g., lauric acid). Very long-chain fatty acids are fatty acids with more than 18 carbon atoms (e.g., stearic acid).

►Fatty Acid Transport Proteins

Long-QT Syndromes

Long-QT syndromes (LQTS) are potentially fatal inherited cardiac arrhythmias characterized by prolonged or delayed ventricular repolarization, manifested on the electrocardiogram as a prolongation of the QT interval. LQTS can be caused by mutations of at least six genes including KCNQ1 for LQT1, KCNH2 for LQT2, SCN5A encoding a cardiac sodium channel for LQT3, KCNE1 for LQT5 and MiRP1 for LQT6. LQT4 is linked to the mutations located in chromosome 4q25–27. Blockade of HERG channels is the most commonly identified drug-induced LQT.

►K^+ Channels
►Voltage-dependent Na^+ Channels

Long-Term Depression

Long-term depression (LTD) is a synaptic plasticity phenomenon that corresponds to a decrease in the synaptic strength (decrease in the post-synaptic response observed for the same stimulation of the pre-synaptic terminals) observed after a specific stimulation of the afferent fibres. This decreased response is still observed hours after its induction.

►Metabotrophic Glutamate Receptors

Long-Term Potentiation

Synonyms
LTP

Definition
Long-term potentiation (LTP) is a synaptic plasticity phenomenon that corresponds to an increase in the synaptic strength (increase in the post-synaptic response observed for the same stimulation of the presynaptic terminals) observed after a high frequency stimulation (tetanus) of the afferent fibres. This increased response is still observed hours and even days after the tetanus. The phenomenon is often observed at glutamatergic synapses and involves, in most cases, the activation of the *N*-methyl D-aspartate (NMDA) subtype of ionotropic glutamate receptors.

►Metabotropic Glutamate Receptors
►Nicotinic Receptors
►Ionotropic Glutamate Receptors

Loop Diuretics

►Diuretics

Low-density-lipoprotein-cholesterol

LDL is the major carrier of cholesterol to the periphery and supplies the cholesterol essential for the integrity of nerve tissue, steroid hormone synthesis, and cell membranes. The association between elevated plasma cholesterol carried in LDL and the risk of coronary heart disease has been well established. LDL is also sometimes called the “bad” cholesterol.

►Lipoprotein Metabolism

Low-density Lipoprotein Receptor Gene Family

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Synonyms

Low-density lipoprotein receptor-related proteins; LRP

Definition

The low-density lipoprotein (►LDL) receptor gene family encompasses a group of structurally related endocytic receptors that are expressed in many mammalian and nonmammalian cell types. The receptors mediate cellular binding and uptake of a variety of diverse macromolecules including lipoproteins and lipid-carrier-complexes, proteases and protease inhibitors, signaling factors, as well as viruses and toxins. Gene family members play crucial roles in many biological processes ranging from lipid homeostasis, to signal transduction, to cell migration.

Basic Characteristics

Receptor-Mediated Endocytosis

Endocytic receptors are cell surface proteins that transport macromolecules into cells through a process known as receptor-mediated ►endocytosis. In this process, a receptor on the cell surface binds a specific ligand from the extracellular space, internalizes via specialized in-tucked regions of the plasma membrane called clathrin-coated pits, and moves to an intracellular vesicle (►endosome) to discharge its cargo. From endosomal compartments, ligands are further directed to lysosomes for catabolism, while the unliganded receptor returns to the cell surface to initiate the next round of endocytosis. Endocytic receptors serve to regulate the concentration of ligands in the extracellular space and to deliver them to cells in need of these metabolites. Binding of some ligands to endocytic receptor may also trigger intracellular signaling cascades involved in regulation of important physiological processes such as embryonic patterning, neuronal cell migration, or synaptic plasticity.

Structural Organization of the LDL Receptor Gene Family

Much of our knowledge of the structure and function of endocytic receptors is based on the analysis of the LDL receptor gene family. Members of this extended gene family can be found in a variety of species ranging from roundworms to insects, to vertebrates. Ten receptors exist in mammalian organisms, all of which share common structural motifs required for receptor-mediated endocytosis (Fig. 1). Among other modules, their extracellular domains are composed of clusters of ►complement-type repeats, the site of ligand binding, as well as β-propellers, essential for pH-dependent release of ligands in endosomes. The cytoplasmic tails harbor recognition sites for cytosolic adaptor proteins involved in protein trafficking and signal transduction. All ectodomains share significant sequence similarity in line with the ability of the receptors to bind an overlapping spectrum of ligands. In contrast, their cytoplasmic domains are unique, indicating distinct cellular fates for macromolecules internalized by individual receptors [1].

Functions of the LDL Receptor Gene Family

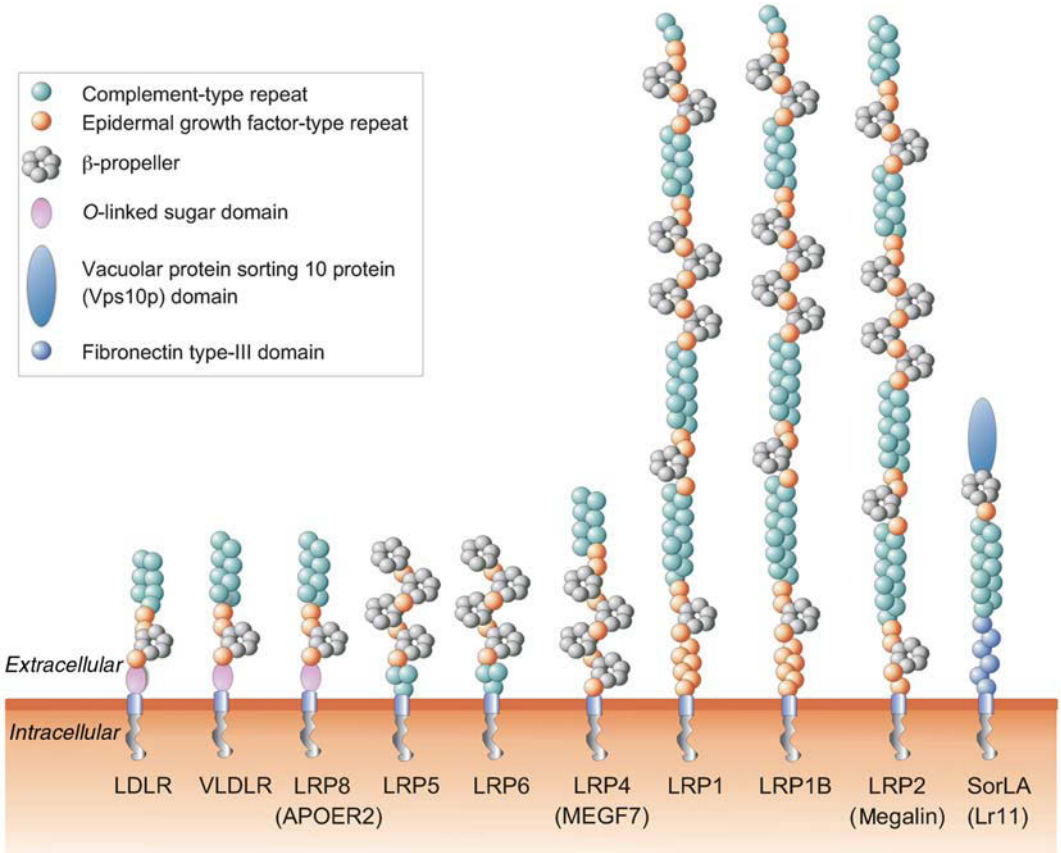
Transgenic animal models with spontaneous or induced receptor gene defects have been instrumental in elucidating the physiological roles of the LDL receptor gene family. In addition, a number of human diseases have been identified that are caused by sporadic or inherited forms of receptor deficiency (Table 1).

LDL Receptor

The LDL receptor is the archetype receptor of the gene family. It is the major endocytic receptor responsible for cellular uptake of cholesterol-rich ►lipoproteins from the circulation and extracellular body fluids. It binds lipoprotein particles that contain the ►apolipoproteins B-100 (APOB-100) or APOE, and mediates their endocytic uptake. Particles internalized via the LDL receptor are delivered to lysosomal degradation where the apolipoproteins are broken down into amino acids while the lipids are released into the cytosol for further metabolism. The uptake of lipoproteins by the LDL receptor has a dual role in lipid homeostasis. It delivers cholesterol required for maintenance of cellular functions (e.g., formation of membranes, synthesis of steroid hormones, and bile acids), and it regulates the concentration of this lipid in the circulation. The importance of the latter function is underscored by pathological abnormalities observed in patients with LDL receptor gene defects; a syndrome designated familial hypercholesterolemia (►FH). In humans with FH, lack of LDL receptor activity results in massive increase in circulating LDL. The mean plasma cholesterol levels are elevated approximately twofold in individuals with heterozygous and fourfold in patients with homozygous gene defects. As a consequence of fulminate ►hypercholesterolemia, FH patients suffer from premature atherosclerosis and coronary artery disease. To date more than 150 different mutations have been identified in the human LDL receptor gene.

VLDLR and APOER2

Very low-density lipoprotein receptor (►VLDLR) and APOE receptor-2 (►APOER2) are two gene family members with redundant functions. They are expressed in neurons of the developing brain and act as cell



Low-density Lipoprotein Receptor Gene Family. Figure 1 The LDL receptor gene family. The figure depicts the structural organization of mammalian members of the LDL receptor gene family. APOER2 (apolipoprotein E receptor 2); LDLR (low-density lipoprotein receptor); LRP (LDL receptor-related protein); MEGF7 (multiple epidermal growth factor repeat-containing protein 7); sorLA (sorting protein-related receptor); VLDLR (very low-density lipoprotein receptor). Designations in brackets indicate alternative names for the respective receptors.

Low-density Lipoprotein Receptor Gene Family. Table 1 Human diseases of the LDL receptor gene family

Receptor	Type of mutation	Disease
LDL receptor	Loss-of-function (familial, autosomal dominant)	Familial hypercholesterolemia (impaired clearance of LDL)
VLDL receptor	Loss-of-function (familial, autosomal recessive)	Autosomal recessive cerebellar hypoplasia (ataxia, mental retardation)
LRP5	Loss-of-function (familial, autosomal recessive)	Osteoporosis-pseudoglioma-syndrome (reduced bone mass)
	Gain-of-function (familial, autosomal dominant)	High-bone-mass trait (increased osteogenic activity)
LRP6	Missense mutation (familial, autosomal dominant)	Autosomal dominant early coronary artery disease (hyperlipidemia, hypertension, diabetes)
LRP1B	Loss-of-function (sporadic)	Esophageal squamous cell carcinoma, nonsmall-cell lung cancer
LRP2/ megalin	Loss-of-function (familial, autosomal recessive)	Donnai-Barrow syndrome (brain malformation, renal tubular deficiency, diaphragmatic hernia)
SorLA	Polymorphisms (sporadic)	Late-onset Alzheimer's disease

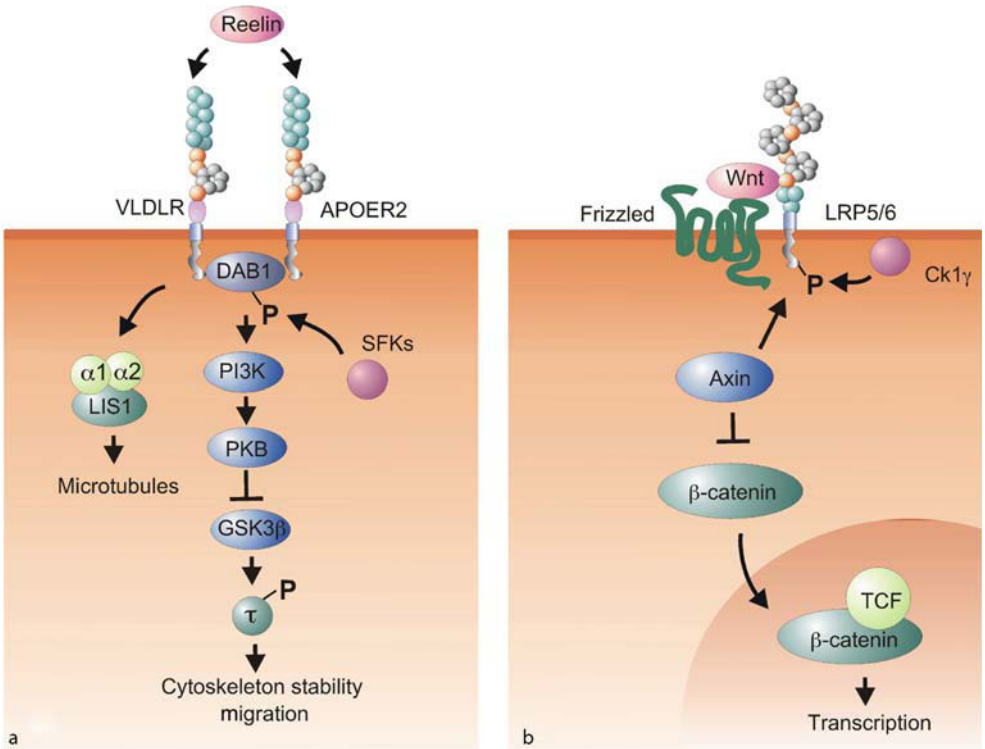
surface receptor for ►reelin, a secreted guidance factor for newborn neurons that migrate to their proper position in the laminating neocortex and cerebellum. Binding of Reelin to VLDLR and APOER2 initiates an intracellular signaling cascade that involves the cytoplasmic adaptor disabled-1 (►DAB1) bound to the receptor tails, and that ultimately leads to reorganization of the cytoskeleton and regulation of cell migration (Fig. 2a) [2]. Receptor gene defects in humans (Table 1) and in animal models result in abnormal layering of neurons in the brain, and in severe neuronal dysfunction (cerebellar dysplasia, ataxia).

LRP5/6

These receptors are integral components of the ►wingless (Wnt) signaling cascade by acting as coreceptors to ►frizzled. Simultaneous binding of Wnt ligands to LRP5/6 and frizzled results in signal transduction through the canonical Wnt signaling pathway (Fig. 2b) [3]. The function of LRP6 seems most relevant for early embryonic patterning events, as loss of receptor function in fruit fly, *Xenopus*, and mouse causes aberrant pattern formation and early embryonic lethality. In contrast, LRP5 activity is mainly required for regulation of bone formation as judged from loss-of-function and gain-of-function syndromes in humans that result in low or high-bone-mass traits, respectively (Table 1).

LRP4

LRP4 is another receptor of the LDL receptor gene family involved in regulation of embryonic patterning, mainly controlling formation of limb structures. Loss of receptor activity in gene targeted mice or spontaneous mutation in bovine cause abnormal limb development and



Low-density Lipoprotein Receptor Gene Family. Figure 2 Signal transduction by members of the LDL receptor gene family. (a) The reelin signaling pathway in migrating neurons is initiated by binding of the guidance factor to its cognate receptors VLDLR and APOER2. Binding induces clustering of DAB1 on the cytoplasmic receptor tails, activating SRC family tyrosine kinases (SFKs) that in turn phosphorylate the adaptor. Phosphorylated DAB1 activates phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB). PKB inhibits the activity of glycogen synthase kinase 3β (GSK3β), resulting in reduced phosphorylation of Tau (τ) and stabilization of microtubules. Lissencephaly 1 (LIS1) is another interaction partner of phosphorylated DAB1. It associates with α-subunits to a multimeric protein complex that also regulates microtubule dynamics (modified from [2]). (b) As part of the canonical Wnt signaling pathway, Wnt ligands bind to LRP5/6 and Frizzled resulting in phosphorylation of the LRP5/6 tail by membrane-associated casein kinase 1γ (CK1γ). Phosphorylation recruits scaffold protein Axin to the LRP tail domain, preventing Axin-induced destruction of β-catenin. Stabilized β-catenin enters the nucleus to interact with high-mobility group protein TCF to induce target gene transcription.

polysyndactyly. The molecular mechanisms of LRP4 actions remain unclear at present, but may also involve signaling through embryonic morphogen pathways.

LRP1 and LRP1B

In hepatocytes, LRP1 serves as backup pathway to the LDL receptor in hepatic clearance of ►chylomicron remnants, lipoproteins that transport dietary lipids from the intestine to the liver. In vascular smooth muscle cells, LRP1 likely acts as signaling receptor that suppresses the activity of the platelet-derived growth factor (►PDGF) receptor. Loss of local LRP1 expression in mouse models causes enhanced activation of the PDGF receptor pathway, resulting in smooth muscle cell proliferation and in marked susceptibility to atherosclerotic lesion formation [4]. LRP1B is also known as LRP-DIT (deleted-in-tumors) because of its original description as a gene frequently inactivated in lung cancer cell lines. A possible role as tumor suppressor gene remains yet to be established. LRP1B may share some functional redundancy with LRP1 as suggested from the close sequence similarity and overlap in expression pattern of both receptors.

LRP2

LRP2 (megalin, gp330) is the largest member of the LDL receptor gene family. Inherited *Lrp2* gene defects are the cause of Donnai-Barrow syndrome, characterized by brain malformation, renal dysfunction, and diaphragmatic hernia. Rather than acting as lipoprotein receptor such as the LDL receptor and LRP1, LRP2 functions as endocytic receptor for cellular uptake of lipophilic vitamins and steroid hormones bound to plasma carrier proteins [1]. Most notably, a role for the receptor in retrieval of filtered 25-OH vitamin D_3 bound to the vitamin D binding protein (►DBP) from the glomerular filtrate is appreciated. Renal uptake of vitamin/DBP complexes prevents uncontrolled urinary loss of this essential metabolite and it delivers to renal proximal tubular cells the precursor for conversion into 1,25-$(OH)_2$ vitamin D_3, the active hormone that controls systemic calcium and bone metabolism. Loss of receptor function results in excessive urinary excretion of vitamin D_3 metabolites, and consequently, in vitamin D deficiency and osteomalacia (softening of the bones).

SorLA

SorLA is a chimeric receptor that harbors protein modules not present in other members of the LDL receptor gene family (Fig. 1). As well as acting as endocytic receptor for extracellular ligands (such as PDGF), sorLA also regulates the intracellular trafficking of target proteins between secretory and endocytic compartments. In particular, the receptor acts as sorting receptor for the amyloid precursor protein (►APP), the etiologic agent in ►Alzheimer's disease. In neurons, SorLA directs APP into intracellular compartments less favorable for proteolytic processing, thereby impairing breakdown of APP to the amyloid-β peptide, the principle component of senile plaques (a hallmark of Alzheimer's disease). Polymorphisms in the human *SorLA* gene are among the most important genetic risk factors of late-onset Alzheimer's disease, likely by determining the expression levels of this protective factor in the brain of individuals [5].

Drugs

Currently no drugs directly modulating the LDL receptor family are known. The possible use of drugs targeting the LDL receptor family or downstream signaling proteins may be derived from Table 1.

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Low-density Lipoprotein Receptor-related Proteins

►Low-Density Lipoprotein Receptor Gene Family

Low Molecular Mass GTPases

►Small GTPases

Low Voltage-activated (LVA) Ca^{2+} Channels

►Voltage-dependent Ca^{2+} Channels

LPL

►Lipoprotein Lipase

LPS

►Lipopolysaccharide

LRP

►Low-Density Lipoprotein Receptor Gene Family

LRP5 and LRP6

LRP5 and 6 are closely related genes in humans and mice that are structurally distinct from other *LRP* family members. A single *Drosophila* ortholog exists and is called *arrow*. The proteins are single-pass transmembrane receptors with an extracellular domain comprising four amino-terminal epidermal growth factor (EGF)-like repeats and three low density lipoprotein (LDL) receptor type A repeats. They have a relatively short proline-rich intracellular domain. Genetic evidence in *Drosophila* as well as loss-of-function and overexpression evidence in vertebrates support the conclusion that arrow and LRP5/6 are essential coreceptors with the Fz proteins for Wnt/β-catenin-dependent signaling.

►Wnt Signaling
►Low-Density Lipoprotein Receptos Gene Family

LTD

►Long Term Depression

LTP

►Long Term Potentiation

Luteinizing Hormone

►Contraceptives

Lymphangiogenesis

Lymphangiogenesis is the growth of lymphatic vessels, which is critically controlled by the interaction of VEGF-C and VEGF-D with the receptor VEGF-R3 on lymphatic endothelial cells.

►Angiogenesis and Vascular Morphogenesis

Lymphocytes

Lymphocytes are specialized white blood cells that play a crucial role in an immune response.They can be T lymphocytes, which can directly target and destroy defective cells, or B lymphocytes, which produce antibodies directed against specific antigens. Both T and B lymphocytes produce a variety of cytokines to augment and amplify the immune response.

►Immune Defense

Lymphokines

►Cytokines
►Growth Factors

Lysine Acetylation of Histones

►Histone Acetylation

Lysipressin

►Vasopressin/Oxytocin

Lysolipid Mediators

►Lysophospholipids

Lysophosphatidic Acid

Lysophosphatidic acid (LPA) is the prototype of a group of bioactive *lysophospholipids* that act on specific G-protein-coupled receptors to mediate a wide variety of cellular functions.

►Lysophospholipids

Lysophosphatidylcholine

Synonym
LPC

Definition
Potential signaling phospholipid derived from phosphatidylcholine by phospholipase action. No receptors known.

►Proton-Sensing GPCRs
►Lysophospholipids

Lysophospholipids

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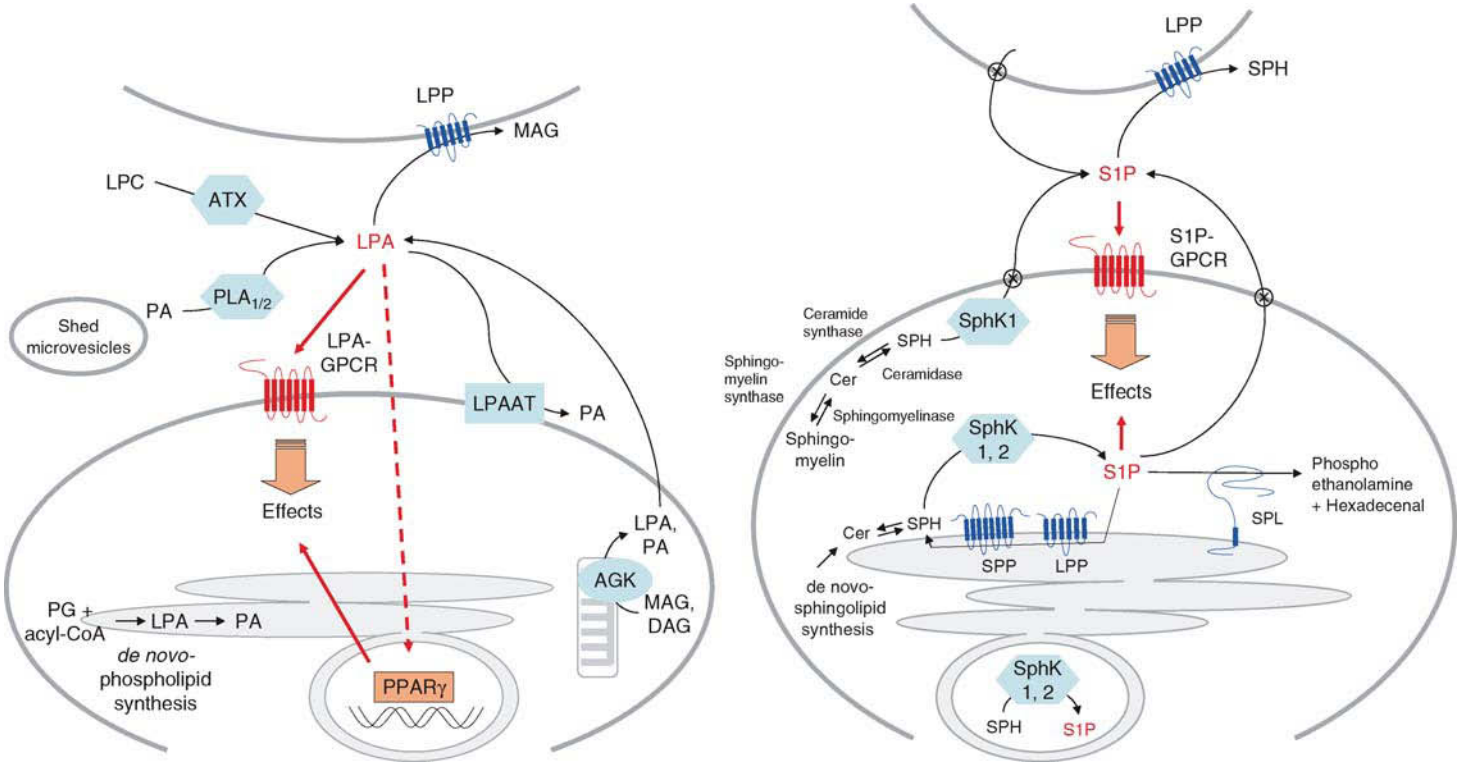
Synonyms
Lysosphingolipids and lysoglycerophospholipids; Lysolipid mediators

Definition
Lysophospholipids are small bioactive lipid molecules characterized by a single carbon chain and a polar head group. Two subgroups can be distinguished: molecules containing the sphingoid base backbone (lysosphingolipids) and molecules containing the glycerol backbone (lysoglycerophospholipids). The lysolipid structure renders these lipids more hydrophilic and versatile than their corresponding phospholipids. Lysophospholipids act as extracellular mediators activating specific ►G-protein-coupled receptors (GPCRs), although some of them additionally play a role in intracellular signal transduction. ►Sphingosine-1-phosphate (S1P) and ►lysophosphatidic acid (LPA) have been characterized in greatest detail so far, and the concept of lysophospholipids as a group of mediators is supported by the high homology between S1P- and LPA-specific GPCRs. Other, less well characterized lysophospholipids are sphingosylphosphorylcholine (SPC), lysophosphatidylcholine (LPC), psychosine (galactosylsphingosine) and glucopsychosine (glucosylsphingosine); the latter two are lacking a phosphate group. Lysophospholipids are auto- or paracrine regulators of cell growth and survival, migration and chemotaxis, cytoskeletal architecture, cell–cell-contacts and adhesion, Ca^{2+} homoeostasis and Ca^{2+}-dependent functions. By regulating these cellular functions, lysophospholipids play a role in angiogenesis, lymphocyte trafficking, development of the nervous system, cancer growth and metastasis, inflammation and arteriosclerosis. Relatives of the lysophospholipid family are platelet-activating factor and the ►endocannabinoids, arachidonyl ethanolamide and 2-arachidonyl glycerol.

Basic Characteristics
Metabolism and Occurrence
S1P is formed from sphingosine by ►sphingosine kinases (SphKs). Degradation of S1P occurs either reversibly by lipid phosphate phosphohydrolases (LPPs) and S1P phosphatases (SPPs), or irreversibly by S1P lyase (SPL) (Fig. 1). The localization of S1P production is highly important since S1P plays a role both as extracellular mediator and as intracellular



Lysophospholipids. Figure 1 Metabolism of S1P (right panel) and LPA (left panel). S1P is formed by sphingosine kinases (SphKs) and degraded by S1P lyase (SPL), S1P phosphatases (SPPs) and non-specific lipid phosphate phosphatases (LPPs). S1P is in equilibrium with sphingosine (SPH) and ceramide (Cer). Shown is also the sphingomyelin cycle which contributes to regulation of ceramide levels. SphK1 occurs cytosolic as well as membrane-bound and can translocate upon cellular stimulation to the plasma membrane or to intracellular sites. SphK2 is a cytosolic enzyme and has not been found at the plasma membrane. Both SphKs have been observed in the nucleus. S1P, depending on the localization where it is generated, can act on so far unknown intracellular target sites, or it can be excreted, for example by the ATP-binding cassette transporter, ABCC1 (⊗), and act on S1P-GPCRs. LPPs are integral membrane proteins with their catalytic activity directed to the extracellular space or to the luminal side of organelles, regulating extra- and intracellular levels of LPA and S1P, respectively. SPPs and SPL are endoplasmic reticulum proteins with their catalytic sites directed towards the lumen of endoplasmic reticulum and the cytosol, respectively. Both contribute to regulation of intra- and extracellular S1P levels. A major source of extracellular LPA is cleavage of lysophospholipids, predominantly lysophosphatidylcholine (LPC), by a lysophospholipase D named autotaxin (ATX). LPA can furthermore be generated by deacylation of phosphatidic acid (PA), catalysed by phospholipases A_1 or A_2. For this, phosphatidic acid has to be transferred to the outer membrane leaflet as it is the case in shed microvesicles. Recently, a mitochondrial acylglycerol kinase was identified that phosphorylated monoacylglycerol (MAG) and diacylglycerol (DAG), thereby producing LPA and PA generation, respectively. LPA is not only a GPCR agonist, but also activates the transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ). Degradation of LPA occurs by dephosphorylation, catalysed by LPPs, or by acylation, catalysed by LPA acyl transferases (LPAAT).

second messenger (Fig. 1). Intracellular formation of S1P by SphKs is regulated by many diverse stimuli, among them GPCR agonists, ►growth factors, ►cytokines or depolarization. Some stimuli induce a translocation of SphK1 from the cytosol to the plasma membrane, which appears to enable S1P secretion and so-called "inside-out signalling". Many cell types including mast cells and tumour cells can release S1P. ATP-binding cassette (ABC) transporters have shown to be involved in this process. S1P is furthermore stored in platelets and released upon platelet activation. Accordingly, it has been found in serum in higher concentrations (~0.5–0.8 μM) than in plasma (~0.2–0.4 μM). Plasma S1P is bound to albumin and ►lipoproteins, mainly high-density lipoproteins (HDL). Tissue S1P levels are significantly lower, and thus there is a plasma/tissue S1P gradient.

LPA, i.e. monoacyl-glycerol-3-phosphate, can be formed and degraded by multiple metabolic pathways (Fig. 1). Depending on the precursor molecule and respective pathway, the fatty acid chain in LPA differs in length, degree of saturation and position (sn-1 or sn-2), which has an influence on biological activity. LPA analogs with ether-bound alkyl or alkenyl chains are quantitatively less abundant than those with ester-bound acyl chains. Extracellular LPA is generated to a large part by a lysophospholipase D named ►autotaxin. Autotaxin is an extracellular enzyme that acts as autocrine motility factor of tumour cells. LPA production by autotaxin is essential for embryonic development in mice and apparently contributes most of LPA in plasma. Alternatively, LPA can be produced from surface-exposed phosphatidic acid by phosphatidic acid-selective PLA_1 or secretory type-II PLA_2. Like S1P, LPA occurs in plasma (~200 nM), is formed during coagulation, and present in serum in micromolar concentrations. Differences in the fatty acid composition of plasma- and serum-LPA suggest that the sources of these LPA pools were different. LPA is produced by many cell types, e.g. fibroblasts, adipocytes and tumour cells.

Lysophospholipid Receptors

An overview of lysophospholipid GPCR is presented in Table 1. Presently best characterized are the receptors of the endothelial differentiation gene (EDG)

Lysophospholipids. Table 1 G-protein-coupled lysophospholipid receptors

+Receptor	Endogenous ligands	Main tissue distribution	Signalling	Main biological effects
$S1P_1$	S1P (nM) SPC (μM)	Ubiquitous	$G_{i/o}$	Migration ↑, proliferation, survival, cell–cell-contacts, angiogenesis, lymphocyte trafficking
$S1P_2$		Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	Migration ↓, vascular development, differentiation of vascular smooth muscle cells
$S1P_3$		Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	Heart rate ↓, vascular development, NO-dependent vasorelaxation
$S1P_4$		Lymphoid and haematopoietic tissue	$G_{i/o}$, $G_{12/13}$	Proliferation and cytokine secretion in T-cells ↓
$S1P_5$		Brain, white matter tracts	$G_{i/o}$, $G_{12/13}$	Proliferation, cell rounding
LPA_1	LPA	Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	Proliferation, survival, neurite retraction, brain development
LPA_2		Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	Proliferation, survival
LPA_3		Ubiquitous	$G_{i/o}$, G_q	Implantation and embryo spacing in mice

Receptors	Activators		Main biological effects
$P2Y_9$/GPR23 (LPA_4)	LPA		
GPR63 (LPA_5)			Stress fibre formation, neurite retraction
GPR3, GPR6, GPR12	High constitutive activity S1P or SPC		Meiotic arrest of rodent oocytes, increase in synaptic contacts in rat cortical neurons
OGR1	SPC	Proton	Inhibition of proliferation, osteoclastogenesis
GPR4	SPC, LPC		Migration, angiogenesis, impairment of endothelial barrier function
G2A	LPC, SPC		Migration, apoptosis, suppression of autoimmunity
TDAG8	Psychosine		Formation of multinuclear cells, apoptosis

family, $S1P_{1–5}$ and $LPA_{1–3}$. They had originally been named after EDG-1, now $S1P_1$, which was detected in 1990 as an orphan GPCR upregulated during the differentiation of endothelial cells. According to an IUPHAR committee, these receptors are now named after the primary natural ligand and numbered in order of identification. $S1P_{1–3}$ and $LPA_{1–3}$ are widely expressed and often found co-expressed within a single cell type. $S1P_4$ is predominantly expressed in lymphatic and haematopoietic tissues and $S1P_5$ in brain white matter and skin. The EDG family receptors couple via $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$ proteins to inhibition of ►adenylyl cyclase, stimulation of phospholipase C and increase in $[Ca^{2+}]_i$, stimulation or inhibition of ►mitogen-activated protein kinases (ERK, JNK and p38) and cell growth, activation of Akt and survival, activation or inhibition of Rho and Rac and rearrangement of the cytoskeleton, and finally stimulation or inhibition of cell migration (for details, see Biological Actions).

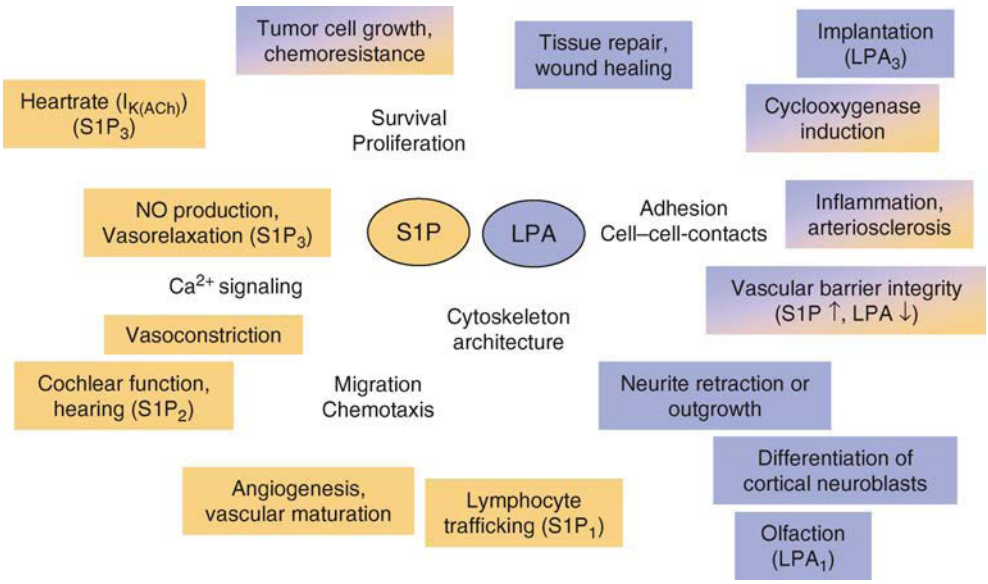
Much less is known about other lysophospholipid receptors. LPA_4 and LPA_5 couple via G_s to activation of adenylyl cyclase. GPR3, GPR6 and GPR12 have a high constitutive activity, thereby stimulating adenylyl cyclase, but appear to be further activated by S1P and/or SPC. GPR6 and GPR12 are strongly expressed in mouse brain, and SPC increased synaptic contacts in GPR12-expressing embryonic cortical neurons. GPR3 and GPR12 are expressed in mouse oocytes and mediate a signal that maintains the oocytes in meiotic arrest, a process that is dependent on high cAMP levels. A role for lipid ligands in meiotic arrest is likely since spontaneous in vitro maturation of rodent oocytes was delayed by SPC and S1P.

Another group of putative lysophospholipid GPCR is highly controversial. OGR1, GPR4, G2A and TDAG8 form a cluster of homologous GPCRs that are candidate high-affinity receptors for SPC, LPC and psychosine (Table 1). SPC and LPC are the phosphocholine-containing analogs of S1P and LPA, respectively. Both lipids are normal constituents of plasma and serum. SPC acts also as a low-affinity or partial agonist at $S1P_{1–5}$. Contradictory studies have shown that the receptors of the OGR1 cluster can be regulated by lysophospholipids and/or by protons. Recent studies revealed that OGR1 was strongly induced during differentiation of bone marrow mononuclear cells into osteoclasts, and knockdown of OGR1 attenuated osteoclastogenesis. Endogenous GPR4 was required for SPC-stimulated migration of endothelial cells and endothelial tube formation. G2A and TDAG8 appear to play a functional role in migration and apoptosis of immune cells.

Finally, it has to be mentioned that LPA also has an intracellular target site, which is the nuclear transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ). LPA competes for thiazolidinedione binding and activates PPARγ-dependent gene transcription. Thereby, LPA induced neointima formation in a rat carotid artery model.

Biological Actions

Cellular responses to S1P and LPA can be classified as growth-related (stimulation of cell proliferation and



Lysophospholipids. Figure 2 Functional roles of S1P and LPA.

survival, protection from apoptosis), cytoskeleton-dependent (shape change, migration, adhesion, chemotaxis) and Ca^{2+}-dependent (contraction, secretion). Some cellular responses are regulated in an opposite manner by different receptors. For example, the $S1P_1$ receptor mediates cell migration and chemotaxis, thereby regulating for example angiogenesis and lymphocyte trafficking. Growth factors such as platelet-derived growth factor need the $S1P_1$ receptor for signalling migration. On the contrary, this must be "and inhibits migration". $S1P_2$ promotes cellular stress fibre formation and inhibits migration. Furthermore, the $S1P_1$ receptor does not increase $[Ca^{2+}]_i$ but rather inhibits $[Ca^{2+}]_i$ increases by other agonists, while $S1P_2$ and $S1P_3$ mediate $[Ca^{2+}]_i$ increases in response to S1P.

S1P is an important mediator in the cardiovascular system (Fig. 2). S1P stimulates proliferation, survival and migration of endothelial cells and induces formation of adherens junction assembly, decrease in vascular permeability and differentiation of endothelial cells into capillary-like networks. Expression of the S1P1 receptor within endothelial cells is required for vasculogensis. Mice lacking this receptor die during embryonic development from haemorrhages caused by a failure of pericytes to migrate around newly formed capillaries. S1P and LPA induce smooth muscle cell contraction and can cause vasoconstriction. S1P, via endothelial $S1P_3$ receptors, can also cause NO-dependent vasorelaxation. A lack of the $S1P_2$ receptor results in profound deafness in mice, apparently because the cochlear spiral artery does not appropriately contract in the absence of $S1P_2$. The $S1P_3$ receptor mediates $I_{K(ACh)}$ activation in atrial myocytes, leading to bradycardia. However, systemic cardiovascular parameters such as blood pressure or heart rate are marginally affected by intravenous S1P, consistent with a role of S1P as a local rather than systemic mediator. The importance of LPA in vascular development is illustrated by the phenotype of autotaxin deficiency, which is characterized by vascular defects early in development. LPA promotes surface expression of leukocyte adhesion molecules in endothelial cells. In contrast to S1P, LPA enhances vascular permeability. Of the two platelet-derived lysophospholipids, LPA but not S1P stimulates platelet aggregation. S1P, LPA and other lysolipids have been detected in lipoproteins and play a role in atherosclerosis. The major part of lipoprotein-bound S1P is associated with HDL, and many of HDL's beneficial vascular effects are mediated by S1P and S1P-GPCR. In contrast, LPA is produced during mild oxidation of low-density lipoproteins (LDL) and accumulates in atherosclerotic plaques where it acts proinflammatory and promotes thrombus formation.

The $S1P_1$ receptor and the gradient between lymphatic tissue (low S1P levels) and plasma (high S1P levels) are essential for lymphocyte trafficking. Deletion of $S1P_1$, as well as pharmacological downregulation of $S1P_1$ (see Drugs), inhibit lymphocyte emigration from lymphatic tissues. Inhibition of S1P lyase leads to elevated S1P tissue levels and also to lymphopenia. It appears likely that the $S1P_1$ receptor on the lymphocyte surface senses the S1P gradient and mediates lymphocyte trafficking.

LPA receptors play an important role in the nervous system (Fig. 2). The LPA_1 receptor was originally cloned from cerebral cortical neuroblasts and is highly expressed in the neurogenic ventricular zone of the embryonic cerebral cortex. In the adult nervous system, it is predominantly found in myelinating cells, i.e. oligodendrocytes and Schwann cells. Schwann cell morphology, adhesion and survival are regulated by LPA. LPA_1 and LPA_2 receptors mediate neuronal cell rounding and neurite retraction, while LPA_3 appears to stimulate neurite outgrowth. Deletion of the LPA_1 receptor in mice caused ~50% neonatal lethality. The surviving pups had a reduced size, craniofacial dysmorphism and impaired suckling behaviour. The defect in suckling behaviour caused malnutrition of the pups, leading to growth retardation and death, and was attributed to a defective olfaction. Deletion of LPA_2 in mice did not lead to obvious defects, while LPA_3 deficiency was accompanied by ~50% reduced litter sizes. This was caused by downregulation of cyclooxygenase-2 (►cyclooxygenases) in LPA_3-deficient uteri during preimplantation, which delayed implantation and disturbed embryo spacing. The mice that were born were grossly normal.

Both S1P and LPA stimulate proliferation and migration of fibroblasts, while they inhibit proliferation and induce migration and differentiation of keratinocytes. LPA promotes wound healing in vivo. Thus, S1P and LPA interact in tissue repair. Many other tissues are responsive to S1P or LPA, including the respiratory tract (contraction of airway smooth muscle cells and cytokine release by S1P), kidney (diuresis by S1P), adipose tissue (preadipocyte proliferation by LPA), and others. Finally, S1P and LPA promote tumour cell growth, chemoresistance and metastasis (Fig. 2). The two mediators are produced by several tumour cells and occur for example in malignant ascites. The antiapoptotic action of S1P and LPA may protect the cells from chemotherapy or radiation. Furthermore, S1P and LPA stimulate tumour cell motility and invasiveness, although it has to be noted that motility of certain cancer cells (e.g. melanoma cells) is inhibited by S1P. S1P might also play a role in tumour angiogenesis. Its importance for tumour progression is further illustrated by the antitumour activity of SphK inhibitors and an anti-S1P antibody (see Drugs).

In summary, the lysophospholipids are local mediators that regulate development, tissue regeneration and homoeostasis, but also play a role in inflammation, arteriosclerosis and cancer.

Drugs

In the field of S1P pharmacology, there is currently a novel immunosuppressive (►immunosuppressive agents) in clinical development for multiple sclerosis. FTY720 is a prodrug that, after being phosphorylated, acts as an agonist with low nanomolar affinity on $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$, but not on $S1P_2$ receptors. SphK2 is required for effective phosphorylation of FTY720 in vivo. The immunosuppressive action of FTY720 is caused basically by activation and subsequent internalization of the $S1P_1$ receptor, which needs to be expressed on the lymphocyte surface to enable sensing of the plasma/tissue S1P gradient and to promote emigration of lymphocytes from lymphatic tissues. Since FTY720 does not generally impair T- and B-cell proliferation and functions, it presents a novel mode of immunosuppressive action, which might be useful in multiple sclerosis as well as transplantation or autoimmune diabetes. Activation of S1P receptor subtypes other than $S1P_1$ causes further, desired and undesired effects. For example, the bradycardia that occurs during the first days of FTY720 treatment is mediated by activation of $S1P_3$. Animal models show further effects of FTY720: it reduced the damage caused by ischemia-reperfusion in liver and kidney, inhibited angiogenesis, impeded tumour growth in a mouse model of melanoma, induced endothelial NO synthase activation and vasodilatation (via $S1P_3$) and reduced vascular permeability in vivo.

Other compounds acting on S1P-GPCR are only in the experimental stage. Several specific $S1P_1$ agonists have been described. SEW2871, that has been identified by high-throughput screening and lacks a phosphate group, induces lymphopenia in mice (in agreement with the role of $S1P_1$ in lymphocyte trafficking) but no bradycardia (in agreement with a lack of an effect on $S1P_3$). KRP-203 needs to be phosphorylated, like FTY720, but then activates specifically $S1P_1$ and produces effects similar to those of SEW2871. Interestingly, the $S1P_1$ receptor has a high constitutive activity which was reduced by an inverse agonist for $S1P_1$ (SB649146). A recently described $S1P_1$ antagonist did not induce lymphopenia but reversed immunosuppression induced by $S1P_1$ agonist, and furthermore enhanced capillary leakage. A putative $S1P_3$ receptor antagonist, BML-241, was identified by searching a three-dimensional compound database with a pharmacophore model of S1P, however, BML-241 has appears to have other, GPCR-independent effects. A specific $S1P_2$ receptor antagonist, JTE-013, has widely been used to analyse $S1P_2$ signalling pathways. This compound prevented for example $S1P_2$-mediated inhibition of cell migration and contraction of smooth muscle cells. Recently, a series of aryl amide compounds was presented which were more or less receptor subtype-selective. The lead compound, VPC23019, was a competitive antagonist at $S1P_1$ and $S1P_3$. K_i values for VPC23019 in radioligand binding assays at $S1P_1$ and $S1P_3$ were in the low nanomolar range, and VPC23019 inhibited S1P-induced migration and Ca^{2+} mobilization. Small structural changes converted the molecule into an agonist. Furthermore, VPC23019 as all compounds of this series behaved as agonist at $S1P_4$ and $S1P_5$, but had no activity at $S1P_2$. Recently, an anti-S1P antibody was described. This antibody retarded the proliferation of various transplanted mouse tumours. Furthermore, it reduced tumour-associated angiogenesis and the ability of S1P to protect tumour cells from apoptosis. An antitumour activity was also observed with SphK inhibitors.

LPA–GPCR pharmacology is at the experimental stage. Dioctylglycerol pyrophosphate (DGPP 8:0) acts as a competitive antagonist preferentially at human LPA_3 receptors and inhibits at higher concentrations LPA_1, but not LPA_2. DGPP 8:0 specifically inhibited LPA-induced platelet activation. Furthermore, it blocked platelet activation by mildly oxidized low density lipoproteins and by homogenates of lipid-rich core isolated from soft arteriosclerotic plaques, illustrating the importance of LPA as a component of arteriosclerotic plaques. Fatty alcohol phosphates (FAP) with carbon chain lengths between 10 and 14 activate LPA_2 and inhibit LPA_3 in a competitive manner. Another LPA–GPCR antagonist is Ki16425, which was identified by high-throughput screening of 150,000 compounds for inhibition of LPA-induced $[Ca^{2+}]_i$ increases. This compound inhibited LPA_1 and LPA_3 at slightly lower concentrations than LPA_2, but was highly specific for LPA-GPCR. A similar preference for LPA_1 and LPA_3 was observed with VPC12249. O-methylphosphothionate (OMPT), a LPA_3-selective agonist, aggravated ischemia-reperfusion injury in mouse kidney, while the $LPA_{1/3}$ antagonist, VPC12249, reduced the kidney damage. Since most compounds that interact with LPA–GPCRs, except Ki16425, have a phosphate group that can be subject to dephosphorylation, a novel strategy focuses on metabolically stabilized LPA analogues such as phosphonates, phosphorothioates, phosphonothioates and fluorophosphonates. These compounds affect LPA–GPCRs as well as LPA metabolizing enzymes.

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Lysosphingolipids and Lysoglycerophospholipids

►Lysophospholipids