16 Purines

OVERVIEW

In addition to their role in the energy economy of the cell, purine nucleosides and nucleotides function as extracellular chemical mediators subserving a wide range of functions. In this chapter we describe the mechanisms responsible for their synthesis and release, the drugs that act through purinergic signalling pathways and the receptors that transduce these effects.

INTRODUCTION

Nucleosides (especially adenosine) and nucleotides (especially ADP and ATP) will already be familiar to you because of their crucial role in DNA/RNA synthesis and energy metabolism, but it may come as a surprise to learn that they also function extracellularly as signalling molecules that produce a wide range of unrelated pharmacological effects.

The finding, in 1929, that adenosine injected into anaesthetised animals caused bradycardia, hypotension, vasodilatation and inhibition of intestinal movements, foreshadowed the current interest in purines. But the true origins of the field can really be traced to the crucial observations in 1970 by Burnstock and his colleagues that provided strong evidence that ATP is a neurotransmitter (see Ch. 2). After a period during which this radical idea was treated with scepticism, it has become clear that the 'purinergic' signalling system is not only of ancient evolutionary origin but participates in many physiological control mechanisms, including the regulation of coronary blood flow and myocardial function (Chs 21 and 22), platelet aggregation and immune responses (Chs 17 and 24), as well as neurotransmission in both the central and peripheral nervous system (Chs 12 and 39).

The full complexity of purinergic control systems, and their importance in many pathophysiological mechanisms, is only now emerging, and the therapeutic relevance of the various receptor subtypes is still being unravelled. As a result there is an increasing interest in purine pharmacology and the prospect of developing 'purinergic' drugs for the treatment of pain and a variety of other disorders, particularly of thrombotic and respiratory origin. There is no doubt that such drugs will assume growing significance but, recognising that the overall picture is far from complete, we will focus our discussion in this chapter on a few prominent areas.

Figure 16.1 summarises the mechanisms by which purines are stored, released and interconverted, and the main receptor types on which they act.

PURINERGIC RECEPTORS

Purines exert their biological actions through three families of receptors. Table 16.1 lists these and summarises what is currently known about their signalling systems, their endogenous ligands and antagonists of pharmacological interest. It should be noted, however, that the action of drugs and ligands at purinergic receptors can be confusing. In part, this is because nucleotides are rapidly degraded by ecto-enzymes and there is also evidence of interconversion by phosphate exchange. Thus ATP may produce effects at all three receptor subclasses depending upon the extent of its enzymatic conversion to ADP, AMP and adenosine.

The three main families of purine receptor are:

- Adenosine receptors (A_1, A_{2A}, A_{2B} and A_3), formerly known as P1 receptors. These are G protein-coupled receptors that act through adenylyl cyclase/cAMP, or by direct effects on Ca^{2+} and K^+ channels, as described in Ch 3.
- P2Y metabotropic receptors ($P2Y_{1-14}$), which are G protein-coupled receptors that utilise either phospholipase C activation or cAMP as their signalling system (see Ch. 3); they respond to various adenine nucleotides, generally preferring ATP over ADP or AMP. Some also recognise pyrimidines such as UTP.
- P2X ionotropic receptors ($P2X_{1-7}$) which are multimeric (in many cases heteromeric) ATP-gated cation channels.

The subtypes in each family are distinguished on the basis of their molecular structure as well as their agonist and antagonist selectivity. The P2Y group is particularly problematic: several receptors have been cloned on the basis of homology with other family members, but their ligands have yet to be identified (in other words they are 'orphan receptors'). In addition, since some members of this group also recognise pyrimidines such as UTP and UDP as well as purines, they are sometimes classed as pyrimidinoceptors. However, little is currently known about the role of pyrimidines in cell signalling.

With the exception of adenosine, **caffeine** and **theophylline**, which act at adenosine receptors, and antagonists such as **clopidogrel**, **prasugrel** and **ticagrelor,** which act at platelet $P2Y_{12}$ receptors, there are so far few therapeutic agents that act on purinergic receptors. We will therefore confine this account to some prominent and interesting aspects of purinergic pharmacology; the reading list provides further information.

ADENOSINE AS A MEDIATOR

The simplest of the purines, adenosine, is found in biological fluids throughout the body. It exists free in the cytosol of all cells and is transported in (active transport against a concentration gradient) and out mainly by a membrane transporter (of which there are several types). Little is known about the way in which this is controlled but the extracellular concentrations are usually quite low

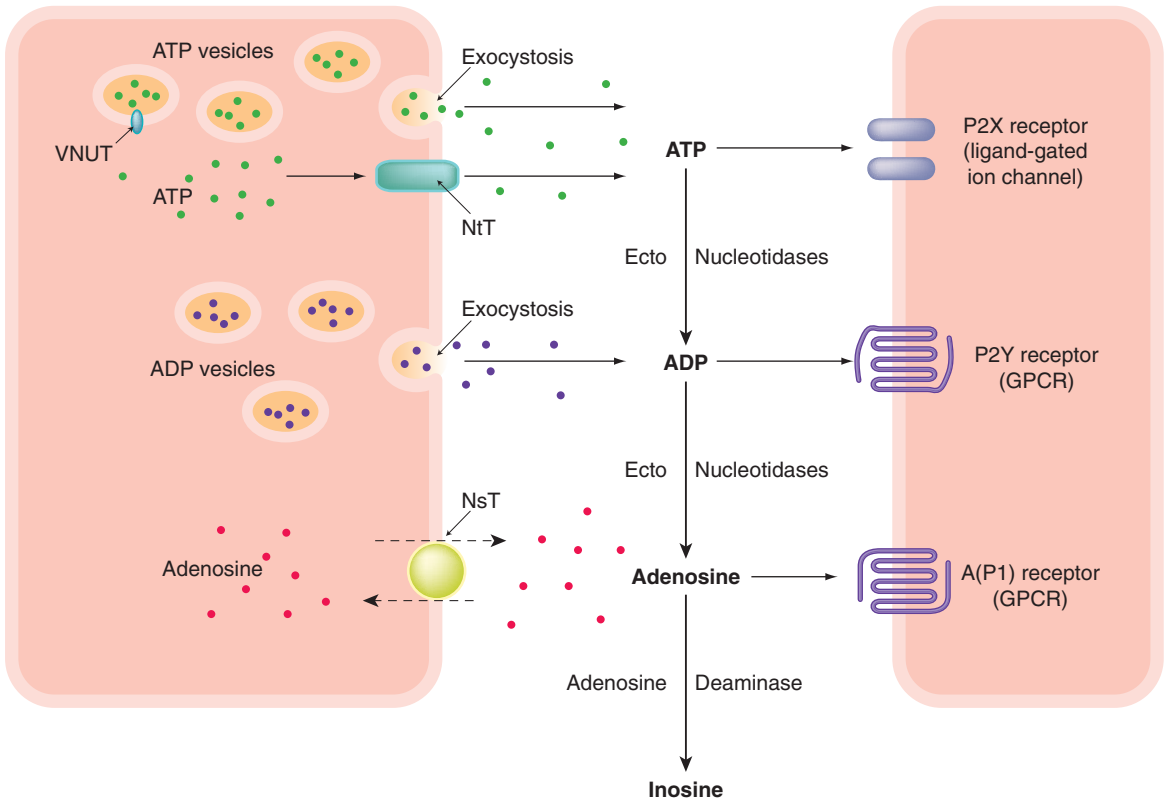

Fig. 16.1 Purines as mediators. ATP (and, in platelets, ADP) may be present in the cytosol of cells (and released following cellular damage) or concentrated into vesicles by the vesicular nucleotide transporter (VNUT). Nucleotides may be released by exocytosis or through membrane channels or transporters (NtT). Once released, ATP can be converted to ADP and to adenosine by the action of ectonucleotidases. Adenosine is present in the cytosol of all cells and is taken up and released via a specific membrane transporter(s) (NsT). Adenosine itself can be hydrolysed to inosine by the enzyme adenosine deaminase. ATP acts upon the P2X receptors (ligand-gated ion channels) and also upon P2Y receptors (GPCRs), the principal target for ADP. Adenosine itself acts on A receptors (also called P1 receptors), which are also GPCRs. Chapter 4 contains more details of exocytotic and other secretory mechanisms.

Purines as mediators

- *Adenosine* acts through A_1, A_{2A}, A_{2B} and A_3 G protein receptors, coupled to inhibition or stimulation of adenylyl cyclase. Adenosine receptors are blocked by methylxanthines such as **caffeine** and **theophylline**.
  - *Adenosine* affects many cells and tissues, including smooth muscle and nerve cells. It is not a conventional transmitter but may be important as a local hormone and 'homeostatic modulator'.
  - Important sites of action include the heart and the lung. **Adenosine** is very short-acting and is sometimes used for its antidysrhythmic effect.
  - *ADP* acts through the $P2Y_{1–14}$ 'metabotropic' G protein-receptor family. These are coupled to cAMP or PLCβ.
  - Important sites of action include platelets where ADP released from granules promotes aggregation by acting on the PY_{12} receptor. This is antagonised by the drugs **clopidogrel**, **prasugrel** and **ticagrelor**.
- *ATP* is stored in vesicles and released by exocytosis or through membrane channels. Cytoplasmic ATP may be released when cells are damaged. It also functions as an intracellular mediator, inhibiting the opening of membrane potassium channels.
  - ATP acts on P2X receptors: these are ligand-gated ion channels. It can also act on P2Y receptors.
  - **Suramin** blocks the ATP actions at most receptors.
  - Important sites of ATP action include the CNS, peripheral and central pathways and inflammatory cells.
  - ATP is rapidly converted to ADP and adenosine when released yielding products that may act on other purinergic receptors.

compared with intracellular levels. Adenosine in tissues comes partly from this intracellular source and partly from extracellular hydrolysis of released ATP or ADP (Fig. 16.1). Drugs such as **dipyridamole** block the transporter thereby indirectly increasing the concentration of extracellular adenosine. Adenosine can be inactivated by *adenosine deaminase* yielding *inosine* providing yet another level of control of this biologically active molecule, and another potential drug target.

Virtually all cells express one or more adenosine receptors and so adenosine produces many pharmacological effects, both in the periphery and in the CNS. Based on its

Table 16.1 Purinergic receptors

Receptor subtype	Mechanism	Principal endogenous ligands	Notes
Adenosine (also called P1)			
A_1	G protein-coupled ($G_{i/o}$) Lowers cAMP	Adenosine (high affinity)	Caffeine, theophylline (antagonists)
A_{2A}	G protein-coupled (G_s) Raises cAMP		
A_{2B}	G protein-coupled (G_s) Raises cAMP	Adenosine (low affinity)	
A_3	G protein-coupled ($G_{i/o}$) Lowers cAMP		
P2Y 'metabotropic'[a]			
$P2Y_1$	G protein coupled (mainly $G_{q/11}$). Activates PLCβ mobilises Ca^{2+} Sometimes alters cAMP	ATP (antagonist or partial agonist) ADP (agonist)	Suramin (antagonist)
$P2Y_2$		UTP and ATP	Suramin (antagonist)
$P2Y_4$		ATP, GTP, UTP (partial agonists)	Pyrimidinoceptor
$P2Y_6$		UDP	Pyrimidinoceptor
$P2Y_{11}$		ATP > ADP	Suramin (antagonist)
$P2Y_{12}$	G protein-coupled (mainly $G_{i/o}$) Reduces cAMP	ADP>ATP	Platelet ADP receptor. Clopidigrel, prasugrel and ticagrelor (potent antagonists)
$P2Y_{13}$		ADP	Suramin, PPADS
$P2Y_{14}$		UDP-glucose	UDP
P2X 'ionotropic'			
$P2X_1$ $P2X_2$ $P2X_3$ $P2X_4$ $P2X_5$ $P2X_6$ $P2X_7$	Receptor-gated cation-selective ion channels	ATP	Suramin (antagonist)

[a]Only functional human receptors are listed. The missing numbers in the sequence indicate that these receptors have been cloned, but their ligands have not yet been identified. A further family of related receptors that binds extracellular cAMP ($CAR_{1–4}$) is omitted as little is known about their biology. PPADS: pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid.

ability to minimise the metabolic requirements of cells, one of its functions may be as an 'acute' defensive agent that is released immediately when tissue integrity is threatened (e.g. by coronary or cerebral ischaemia; see Chs 21 and 40). Under less extreme conditions, variations in adenosine release may play a role in controlling blood flow and (through effects on the carotid bodies) respiration, matching them to the metabolic needs of the tissues.

ADENOSINE AND THE CARDIOVASCULAR SYSTEM

Adenosine inhibits cardiac conduction and it is likely that all four of the adenosine receptors are involved in this effect. Because of this action, adenosine is used therapeutically, being given as an intravenous bolus injection to terminate supraventricular tachycardia (Ch. 21). Because of its short duration of action (it is destroyed or taken up within a few seconds of intravenous administration) it is considered safer than alternatives such as β-adrenoceptor antagonists or **verapamil**. Longer-lasting analogues have been discovered that also show greater receptor selectivity. Adenosine uptake is blocked (and thus its action prolonged) by dipyridamole, a vasodilator and antiplatelet drug (see Ch. 24).

ADENOSINE AND ASTHMA

Adenosine receptors are found on all the cell types involved in asthma (Ch. 28) and the overall pharmacology is complex. For example, activation of the A_{2A} subtype exerts a largely protective and anti-inflammatory effect, but acting through its A_1 receptor, adenosine promotes mediator release from mast cells, and causes enhanced mucus secretion, bronchoconstriction and leukocyte activation. Methylxanthines, especially analogues of theophylline (Ch. 28), are adenosine receptor antagonists. Theophylline has been

used for the treatment of asthma and part of its beneficial activity may be ascribed to its antagonism of the A_1 receptor; however, methylxanthines also increase cAMP by inhibiting phosphodiesterase, which underwrites some of their pharmacological actions independently of adenosine receptor antagonism. Certain derivatives of theophylline are claimed to show greater selectivity for adenosine receptors over phosphodiesterase.

Activation of the A_{2B} receptor also promotes mast cell mediator release, while the role of the A_3 receptor has yet to be fully elucidated. Recent thinking therefore suggests that an antagonist of the A_1 and A_{2B} receptor or an agonist of the A_{2A} receptor would represent a significant therapeutic advance (see Brown et al., 2008; Burnstock et al., 2012).

ADENOSINE IN THE CNS

Acting through A_1 and A_{2A} receptors, adenosine has an inhibitory effect on many CNS neurons, and the stimulation experienced after consumption of methylxanthines such as caffeine (see Ch. 48) occurs partly as a result of blocking this action.

ADP AS A MEDIATOR

ADP is usually stored in vesicles in cells. It exerts its direct biological effects predominantly through the P2Y family of receptors but once released it can be converted to adenosine by ectonucleotidases, of which there are several different types.

ADP AND PLATELETS

The secretory vesicles of blood platelets store both ATP and ADP in high concentrations, and release them when the platelets are activated (see Ch 24). One of the many effects of ADP is to promote platelet aggregation, so this system provides positive feedback – an important mechanism for controlling this process. The receptor involved is $P2Y_{12}$. Clopidogrel, prasugrel, and ticagrelor, are $P2Y_{12}$ antagonists and are important therapeutic agents for preventing arterial thromboembolic disorders (Ch. 24).

ATP AS A MEDIATOR

ATP exerts its action primarily through the P2X receptors. The extracellular domain of these multimeric receptors can bind three molecules of ATP. When activated, the receptor gates the cation-selective ion channels that trigger ongoing intracellular signalling. Some other actions of ATP in mammals are mediated through the P2Y receptors. **Suramin** (a drug originally developed to treat trypanasome infections) and an experimental compound *PPADS* (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid) antagonise ATP and have broad-spectrum inhibitory activity at most P2X receptors. Suramin may additionally antagonise P2Y receptors.

ATP is present in all cells in millimolar concentrations and is released if the cells are damaged (e.g. by ischaemia). The mechanism of release can be through exocytosis of vesicles containing ATP or through *pannexin* or *connexin* channels in the cell membrane. In addition, dying cells may release ATP, which may serve as a 'danger signal' alerting immune cells to potential tissue damage (see Ch. 6).

ATP released from cells is rapidly dephosphorylated by a range of tissue-specific nucleotidases, producing ADP and adenosine (Fig. 16.1), both of which may produce further receptor-mediated effects. The role of intracellular ATP in regulating membrane potassium channels to control vascular smooth muscle (Ch. 22) and insulin secretion (Ch. 31), is quite distinct from this transmitter function.

ATP AS A NEUROTRANSMITTER

The idea that such a workaday metabolite as ATP might be a member of the neurotransmitter elite was resisted for a long time, but is now firmly established. ATP is a transmitter in the periphery, both as a primary mediator and as a co-transmitter in noradrenergic nerve terminals. $P2X_2$, $P2X_4$ and $P2X_6$ are the predominant receptor subtypes expressed in neurons. $P2X_1$ predominates in smooth muscle.

ATP is contained in synaptic vesicles of both adrenergic and cholinergic neurons, and it accounts for many of the actions produced by stimulation of autonomic nerves that are not caused by acetylcholine or noradrenaline (see Ch. 12). These effects include the relaxation of intestinal smooth muscle evoked by sympathetic stimulation, and contraction of the bladder produced by parasympathetic nerves. Burnstock and his colleagues have shown that ATP is released on nerve stimulation in a Ca^{2+}-dependent fashion, and that exogenous ATP, in general, mimics the effects of nerve stimulation in various preparations. ATP may function as a conventional 'fast' transmitter in the CNS and in autonomic ganglia, or as an inhibitory presynaptic transmitter.

Adenosine, produced following hydrolysis of ATP, exerts presynaptic inhibitory effects on the release of excitatory transmitters in the CNS and periphery.

ATP IN NOCICEPTION

ATP causes pain when injected (for example) subdermally, as a result of activation of $P2X_2$ and/or $P2X_3$ heteromeric receptors in afferent neurons involved in the transduction of nociception (see Ch. 42). The pain can be blocked by aspirin (see Ch. 26) suggesting the involvement of prostaglandins. There is a current upsurge of interest in the potential role of purinergic receptors (mainly P2Y and P2X receptors), in various aspects of nociceptive pain transmission and in particular the development of neuropathic pain, which is difficult to treat (see Ch. 42). Interestingly, purinergic receptors are found not just on neurons, but also on glial cells, suggesting a role for these 'support' cells in modulating the chain of nociceptive transmission. It has been suggested that both types of receptors could be useful targets for analgesic and anti-migraine drugs (Tsuda et al., 2012; Magni & Ceruti, 2013).

Oddly, perhaps, the same receptors seem to be involved in taste perception on the tongue.

ATP IN INFLAMMATION

ATP is released from stimulated, damaged or dying cells and P2X receptors are widely distributed on cells of the immune system; P2Y receptors less so. Acting through these receptors, ATP can regulate neutrophil and phagocyte chemotaxis and provoke the release from

macrophages and mast cells of cytokines and other mediators of the inflammatory response. Mice in which the $P2X_7$ receptor is deleted show a reduced capacity to develop chronic inflammation. Purinergic signalling also plays an important role in T-cell signalling.

Adenosine, may also exert anti-inflammatory effects and **methotrexate**, a useful anti-inflammatory drug (see Ch. 26) may owe some of its actions to the release of adenosine. A good account of the role of autocrine signalling in the immune system is given by Junger (2011).

FUTURE PROSPECTS

While there are only a few current drugs that act through purinergic receptors, the area as a whole holds promise for future therapeutic exploitation. Other disease areas not mentioned above, which seem particularly promising in this respect are gastrointestinal disorders (Burnstock, 2008; Antonioli et al., 2013) and regulation of bone remodelling (Gartland et al., 2012).

REFERENCES AND FURTHER READING

(A note of caution: the nomenclature of these receptors has changed several times and this can make for difficulties when reading some older papers. For the latest version of the nomenclature, always refer to www.guidetopharmacology.org/)

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17 Local hormones 1: histamine and the biologically active lipids

OVERVIEW

In Chapter 6 we discussed the function of cellular players in host defence and alluded to the crucial role of soluble chemical regulators of inflammation. In this chapter, and the next, we take a closer look at these substances. We begin with some small molecule mediators. While also having a physiological role, these are pressed into service by the host defence mechanism when necessary, and are therefore important targets for anti-inflammatory drug action.

INTRODUCTION

The growth of pharmacology as a discipline was attended by the discovery of many biologically active substances. Many initially attracted attention as uncharacterised smooth muscle contracting (or relaxing) 'factors', which appeared in blood or tissues during particular physiological or pathological events. Sometimes, these factors were identified comparatively quickly but others resisted analysis for many years and the development of a particular area was often tied to progress in analytical methodology. For example, 5-HT (Ch. 15) and histamine, which are quite simple compounds, were identified soon after their biological properties were described. On the other hand, structural elucidation of the more complex prostaglandins, which were first discovered in the 1930s, had to await the development of the mass spectrometer some 30 years later. Peptide and protein structures took even longer to solve. Substance P (11 amino acids) was also discovered in the 1930s, but was not characterised until 1970 when peptide sequencing techniques had been developed. By the 1980s, molecular biology had greatly enhanced our analytical proficiency. Endothelin (21 residues) for example, was discovered, fully characterised, synthesised and cloned within about a year, the complete information being published in a single paper (Yanagisawa et al., 1988).

WHAT IS A 'MEDIATOR'?

Like regular hormones, such as thyroxine (Ch. 34) or insulin (Ch. 31), a *local hormone* is simply a chemical messenger that conveys information from one cell to another.[1] Hormones such as thyroxine and insulin are released from a single endocrine gland, circulate in the blood and, in this manner, can affect other 'target' tissues. In contrast, local hormones are usually produced by local cells and operate in the immediate microenvironment. The distinction is not actually clear-cut. For example one of the 'classical' hormones, hydrocortisone, is normally released by the adrenal gland but can, it transpires, also be produced and act locally in some tissues. Conversely, some cytokines (see Ch. 18), usually described as local hormones, whilst being produced locally, can circulate in the blood and produce systemic, as well as local, effects.

When, in response to a stimulus of some kind, a local hormone produces a particular biological effect (such as contraction of smooth muscle in response to allergen challenge), it is said to be a *mediator* of this response. Traditionally, a putative mediator[2] had to satisfy certain criteria before gaining official recognition. Dale, in the 1930s, proposed a set of five rules to establish the credentials of mediators and these guidelines have been used as a point of reference ever since. Originally formulated as a test for putative neurotransmitters, these criteria cannot easily be applied to mediators of other responses and have been modified on several occasions.

Currently, the experimental criteria that establish a substance as a mediator are:

- that it is released from local cells in sufficient amounts to produce a biological action on the target cells within an appropriate time frame
- that application of an authentic sample of the mediator reproduces the original biological effect
- that interference with the synthesis, release or action (e.g. using receptor antagonists, enzyme inhibitors, 'knock-down' or 'knock out' techniques) ablates or modulates the original biological response.

HISTAMINE

In a classic study, Sir Henry Dale and his colleagues demonstrated that a local anaphylactic reaction (a type I or 'immediate hypersensitivity reaction' such as the response to egg albumin in a previously sensitised animal; see Ch. 6) was caused by antigen–antibody reactions in sensitised tissue, and found that histamine mimicked this effect both *in vitro* and *in vivo*. Later studies confirmed that histamine is present in tissues, and released (along with other mediators) during anaphylaxis.

SYNTHESIS AND STORAGE OF HISTAMINE

Histamine is a basic amine formed from histidine by histidine decarboxylase. It is found in most tissues but is present in high concentrations in tissues exposed to the

[1]The term 'autocrine' is sometimes used to denote a local mediator that acts on the cell from which it is released, whereas a 'paracrine' mediator acts on other neighbouring cells.

[2]To add to the lexicographical confusion that already exists over hormones and mediators, another word 'bioregulator' has recently crept into use. As this portmanteau term could cover just about any biologically active substance, it is not much use for our purposes.

outside world (lungs , skin and gastrointestinal tract). At the cellular level, it is found largely in mast cells (approximately 0.1–0.2 pmol/cell) and basophils (0.01 pmol/cell), but non-mast cell histamine occurs in 'histaminocytes' in the stomach and in histaminergic neurons in the brain (see Ch. 39). In mast cells and basophils, histamine is complexed in intracellular granules with an acidic protein and a high-molecular-weight heparin termed macroheparin.

HISTAMINE RELEASE

Histamine is released from mast cells by exocytosis during inflammatory or allergic reactions. Stimuli include complement components C3a and C5a (see Ch. 6), which interact with specific surface receptors, and the combination of antigen with cell-fixed immunoglobulin (Ig)E antibodies. In common with many secretory processes (Ch. 4), histamine release is initiated by a rise in cytosolic [Ca^{2+}]. Various basic drugs, such as morphine and tubocurarine, release histamine, as does **compound 48/80**, an experimental tool often used to investigate mast cell biology. Agents that increase cAMP formation (e.g. β-adrenoceptor agonists; see Ch. 14) inhibit histamine secretion. Replenishment of secreted histamine by mast cells or basophils is a slow process, which may take days or weeks, whereas turnover of histamine in the gastric histaminocyte is very rapid. Histamine is metabolised by histaminase and/or by the methylating enzyme imidazole *N*-methyltransferase.

HISTAMINE RECEPTORS

Four types of histamine receptor have been identified, $H_{1\text{-}4}$. All are G protein-coupled receptors that modulate cAMP to effect downstream signalling. Splice variants of H_3 and H_4 receptors have been reported. All four are implicated in the inflammatory response in some capacity. A good account of the role of histamine in inflammation has been given by Jutel et al. (2009).

Selective antagonists at H_1, H_2 and H_3 receptors include mepyramine, cimetidine and thioperamide, respectively. Selective agonists for H_2 and H_3 receptors are, respectively, dimaprit and (R)-methylhistamine. Histamine H_1 antagonists are the principal antihistamines used in the treatment or prevention of inflammation (notably allergic inflammation such as hay fever). Other clinical uses of subtype antagonists may be found in Chapters 28, 39 and 48. The pharmacology of H_4 receptors is less well developed.

ACTIONS

Smooth muscle effects. Histamine, acting on H_1 receptors, contracts the smooth muscle of the ileum, bronchi, bronchioles and uterus. The effect on the ileum is not as marked in humans as it is in the guinea pig (this tissue remains the *de facto* standard preparation for histamine bioassay). Histamine reduces air flow in the first phase of bronchial asthma (see Ch. 28 and Fig. 28.3).

Cardiovascular effects. Histamine dilates human blood vessels and increases permeability of postcapillary venules, by an action on H_1 receptors, the effect being partly endothelium-dependent in some vascular beds. It also increases the rate and the output of the heart by action on cardiac H_2 receptors.

Gastric secretion. Histamine stimulates the secretion of gastric acid by action on H_2 receptors. In clinical terms, this is the most important action of histamine, because it is implicated in the pathogenesis of peptic ulcer. It is considered in detail in Chapter 30.

Effects on skin. When injected intradermally, histamine causes a reddening of the skin, accompanied by a weal with a surrounding flare. This mimics the *triple response* to scratching of the skin, described by Sir Thomas Lewis over 80 years ago. The reddening reflects vasodilatation of the small arterioles and precapillary sphincters, and the weal the increased permeability of the postcapillary venules. These effects are mainly mediated through activation of H_1 receptors. The flare is an axon reflex: stimulation of sensory nerve fibres evokes antidromic impulses through neighbouring branches of the same nerve, releasing vasodilators such as calcitonin gene-related peptide (CGRP; see Chs 18 and 26). Histamine causes intense itch if injected into the skin or applied to a blister base, because it stimulates sensory nerve endings through an H_1-dependent mechanism. H_1 antagonists are used to control itch caused by allergic reactions, insect bites, etc.

Despite the fact that histamine release is manifestly capable of reproducing many of the inflammatory signs and symptoms, H_1 antagonists do not have much clinical utility in the acute inflammatory response *per se*, because other mediators are more important. Histamine is, however, important in type I hypersensitivity reactions such as allergic rhinitis and urticaria. Other significant actions of histamine in inflammation include effects on B and T cells, modulating the acquired immune response (Jutel et al., 2009).

Histamine

- Histamine is a basic amine, stored in mast cell and basophil granules, and secreted when C3a and C5a interact with specific membrane receptors or when antigen interacts with cell-fixed IgE.
- Histamine produces effects by acting on H_1, H_2, H_3 or H_4 receptors on target cells.
- The main actions in humans are:
  - stimulation of gastric secretion (H_2)
  - contraction of most smooth muscle, except blood vessels (H_1)
  - cardiac stimulation (H_2)
  - vasodilatation (H_1)
  - increased vascular permeability (H_1).
- Injected intradermally, histamine causes the 'triple response': *reddening* (local vasodilatation), *wheal* (increased permeability of postcapillary venules) and *flare* (from an 'axon' reflex in sensory nerves releasing a peptide mediator).
- The main pathophysiological roles of histamine are:
  - as a stimulant of gastric acid secretion (treated with H_2-receptor antagonists)
  - as a mediator of type I hypersensitivity reactions such as urticaria and hay fever (treated with H_1-receptor antagonists)
  - CNS functions (see Ch. 39).

The use of H_1 antagonists in these and other conditions is dealt with in Chapter 26.

EICOSANOIDS

GENERAL REMARKS

The term *eicosanoid* refers to a group of mediators that are generated from fatty acid precursors as required, and are not stored preformed in cells. They are implicated in the control of many physiological processes, are among the most important mediators and modulators of the inflammatory reaction (Figs 17.1, 17.2) and are a very significant target for drug action.

Interest in eicosanoids first arose in the 1930s after reports that semen contained a lipid substance, apparently originating from the prostate gland, which contracted uterine smooth muscle. Later, it became clear that *prostaglandin* (as the factor was named[3]) was not a single substance but a whole family of compounds that could be generated from 20-carbon unsaturated fatty acid precursors by virtually all cells.

STRUCTURE AND BIOSYNTHESIS

In mammals, the main eicosanoid precursor is arachidonic acid (5,8,11,14-eicosatetraenoic acid), a 20-carbon unsaturated fatty acid containing four unsaturated double bonds (hence the prefix *eicosa-*, referring to the 20 carbon atoms, and *tetra*-enoic, referring to the four double bonds; see Fig. 17.1). In most cell types, arachidonic acid is esterified in the phospholipid pool, and the concentration of the free acid is low.

The principal eicosanoids are prostaglandins, *thromboxanes* and *leukotrienes*, although other derivatives of arachidonate, for example the *lipoxins* and *resolvins*, are of increasing interest and importance. (The term *prostanoid* will be used here to encompass both prostaglandins and thromboxanes.)

In most instances, the initial and rate-limiting step in eicosanoid synthesis is the liberation of intracellular arachidonate, usually in a one-step process catalysed by the enzyme *phospholipase A*$_2$ (PLA_2; Fig. 17.2). An alternative multi-step process involving phospholipases C or D in conjunction with diacylglycerol lipase is sometimes utilised. Several isoforms of PLA_2 exist, but the most important is probably the highly regulated cytosolic PLA_2 ($cPLA_2$). This enzyme generates not only arachidonic acid (and thus eicosanoids) but also lysoglyceryl-phosphorylcholine (lyso-PAF), the precursor of *platelet activating factor* (PAF), another inflammatory mediator (see Figs 17.1, 17.2).

Cytosolic PLA_2 is activated by phosphorylation and this may be triggered by many stimuli, such as thrombin action on platelets, C5a on neutrophils, bradykinin on fibroblasts and antigen–antibody reactions on mast cells. General cell damage also triggers $cPLA_2$ activation. The free arachidonic acid is metabolised separately (or sometimes jointly) by several pathways, including the following.

- *Fatty acid cyclo-oxygenase* (COX). Two main isoforms exist, COX-1 and COX-2. These are highly homologous enzymes but are regulated in different and tissue-specific ways. They enzymatically combine arachidonic (and some other unsaturated fatty acid) substrates with molecular oxygen to form unstable intermediates, which can subsequently be transformed by other enzymes to different prostanoids.
- *Lipoxygenases*. Several subtypes, which often work sequentially, synthesise leukotrienes, lipoxins or other compounds (Figs 17.1–17.3).

Chapter 26 deals in detail with the way inhibitors of these pathways (including non-steroidal anti-inflammatory drugs [NSAIDs] and glucocorticoids) produce anti-inflammatory effects.

Mediators derived from phospholipids

- The main phospholipid-derived mediators are the eicosanoids (prostanoids and leukotrienes) and platelet-activating factor (PAF).
- The eicosanoids are synthesised from arachidonic acid released directly from phospholipids by phospholipase A_2, or by a two-step process involving phospholipase C and diacylglycerol lipase.
- Arachidonate is metabolised by cyclo-oxygenases (COX)-1 or COX-2 to prostanoids, by 5-lipoxygenase to leukotrienes and, after further conversion, to lipoxins.
- PAF is derived from phospholipid precursors by phospholipase A_2, giving rise to lyso-PAF, which is then acetylated to give PAF.

PROSTANOIDS

COX-1 is present in most cells as a constitutive enzyme. It produces prostanoids that act mainly as homeostatic regulators (e.g. modulating vascular responses, regulating gastric acid secretion). COX-2 is not normally present (at least in most tissues – renal tissue is an important exception) but it is strongly induced by inflammatory stimuli and therefore believed to be more relevant as a target of anti-inflammatory drugs (see Ch. 26). Both enzymes catalyse the incorporation of two molecules of oxygen into two of the unsaturated double bonds in each arachidonate molecule, forming the highly unstable endoperoxides prostaglandin (PG)G_2 and PGH_2 (see Fig. 17.1). The suffix '2' indicates that the product contains only two double bonds. PGG_2 and PGH_2 are rapidly transformed in a tissue specific manner by endoperoxide *isomerase* or *synthase* enzymes to PGE_2, PGI_2 (prostacyclin), PGD_2, $PGF_{2\alpha}$ and thromboxane (TX)A_2, which are the principal bioactive end products of this reaction. The mix of eicosanoids thus produced varies between cell types depending on the particular endoperoxide isomerases or synthases present. In platelets, for example, TXA_2 predominates, whereas in vascular endothelium PGI_2 is the main product. Macrophages, neutrophils and mast cells synthesise a mixture of products. If eicosatrienoic acid (three double bonds) rather than arachidonic acid is the substrate, the resulting prostanoids have only a single double bond, for example PGE_1, while eicosapentaenoic

[3]The name arose through an anatomical error. In some species it is difficult to differentiate the prostaglandin-rich seminal vesicles from the prostate gland which (ironically as we now know) contains virtually none. Nevertheless the name stuck, outlasting the term *vesiglandin*, suggested later, which would have been more appropriate.

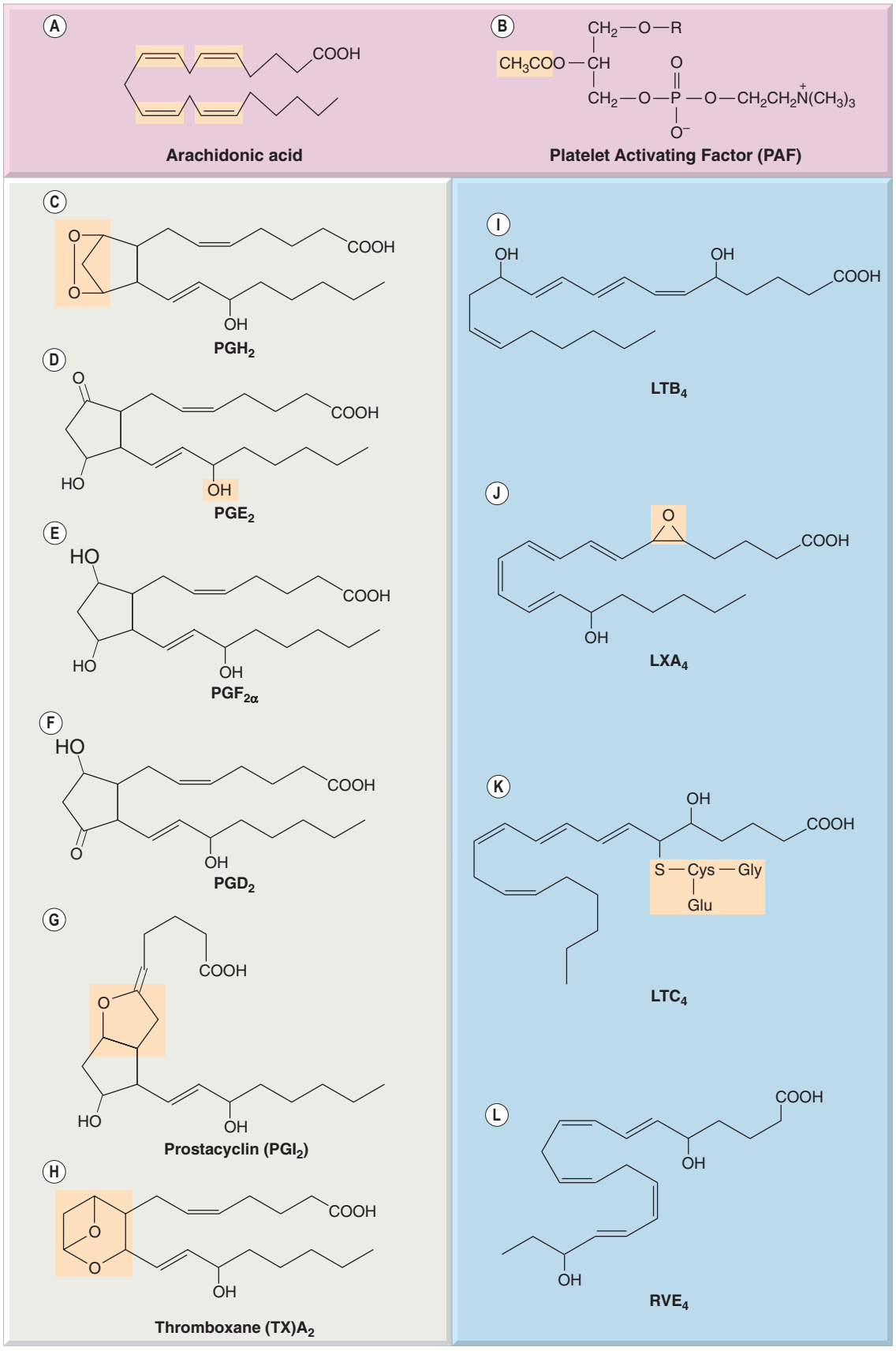

Fig. 17.1 **Some key lipid mediators involved in the host defence response.** [**A**] Arachidonic acid, an important precursor of prostanoids, leukotrienes, lipoxins and resolvins. Note the conjugated double bonds (in shaded box). [**B**] Platelet activating factor (PAF): the location of the acetyl group at C2 is shown in the shaded box. R is a 6- or 8-carbon saturated fatty acid attached by an ether linkage to the carbon backbone. [**C**] Prostaglandin (PG)H_2, one of the labile intermediates in the synthesis of prostaglandins; note unstable ring structure which can spontaneously hydrolyse in biological fluids (in shaded box). [**D**] PGE_2, the 15-hydroxyl group (in shaded box) is crucial for the biological activity of prostaglandins and its removal is the first step in their inactivation. [**E**] and [**F**] $PGF_{2\alpha}$ and PGD_2. [**G**] Prostacyclin (PGI_2); note unstable ring structure (in shaded box). [**H**] Thromboxane (TX)A_2; note unstable oxane structure (in shaded box). [**I**] Leukotriene (LT)B_4. [**J**] Lipoxin (LX)A_4; note unstable and highly reactive oxygen bridge structure (in shaded box). [**K**] Leukotriene (LT)C_4; note conjugated glutathione moiety (in shaded box). [**L**] Resolvin (Rv)E_4.

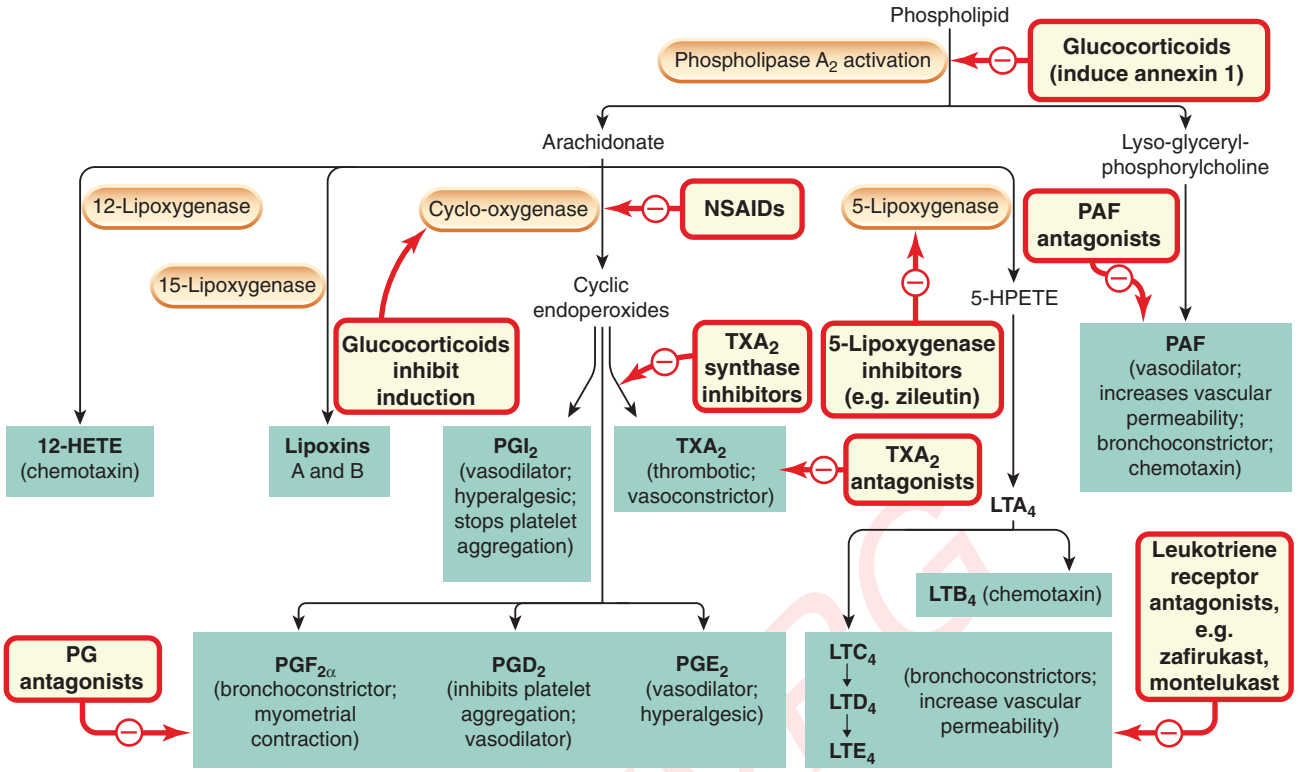

Fig. 17.2 Summary diagram of the inflammatory mediators derived from phospholipids, with an outline of their actions and the sites of action of anti-inflammatory drugs. The arachidonate metabolites are eicosanoids. The glucocorticoids inhibit transcription of the gene for cyclo-oxygenase-2, induced in inflammatory cells by inflammatory mediators. The effects of prostaglandin (PG)E_2 depend on which of the four receptors it activates. HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene; NSAID, non-steroidal anti-inflammatory drug; PAF, platelet-activating factor; PGI_2, prostacyclin; TX, thromboxane.

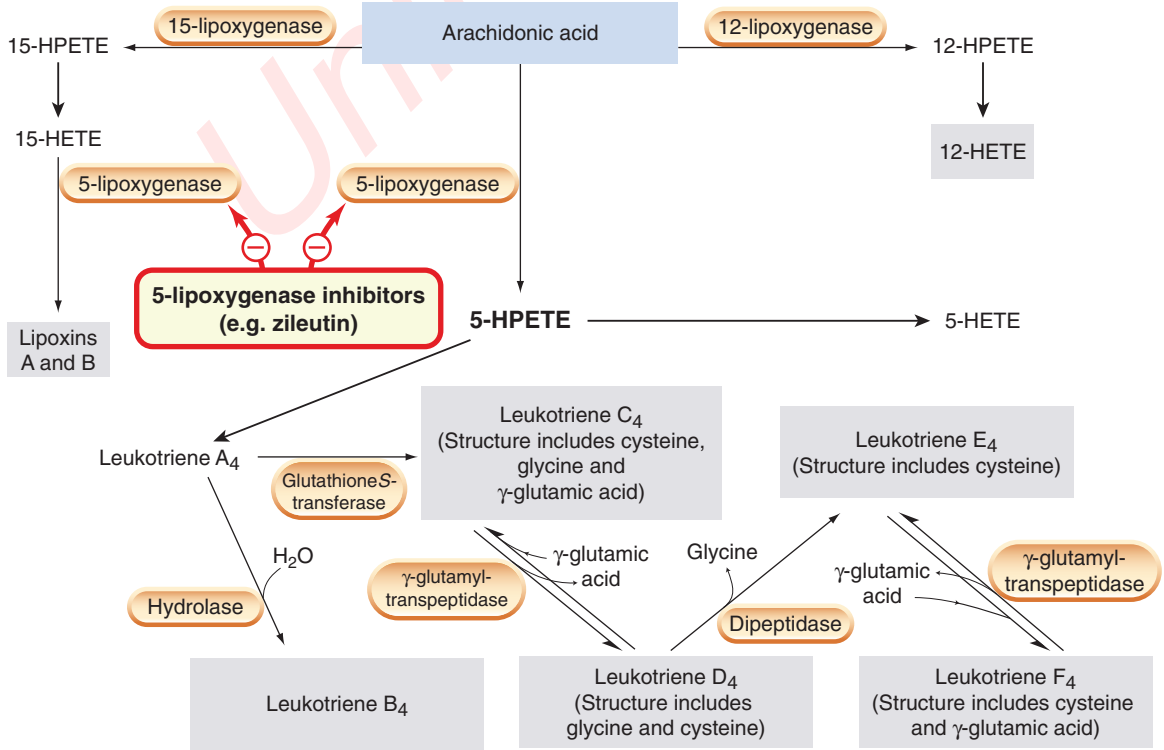

Fig. 17.3 The biosynthesis of leukotrienes from arachidonic acid. Compounds with biological action are shown in grey boxes. HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

acid, which contains five double bonds, yields PGE_3. The latter substrate is significant because it is present in abundance in diets rich in oily fish and may, if present in sufficient amounts, represent a significant fraction of cellular fatty acids. When this occurs, the production of the pro-inflammatory PGE_2 is diminished and, more significantly, the generation of TXA_2 as well. This may partly underlie the beneficial anti-inflammatory and cardiovascular actions that are ascribed to diets rich in this type of marine product (see also Resolvins, below).

The endocannabinoid *anandamide* (see Ch. 19) is an ethanolamine derivative of arachidonic acid and, surprisingly, it can also be oxidised by COX-2 to form a range of *prostamides*. These substances are of increasing interest. They act at prostanoid receptors but often exhibit a unique pharmacology.

CATABOLISM OF THE PROSTANOIDS

This is a multistep process. After carrier-mediated uptake, most prostaglandins are rapidly inactivated by prostaglandin *dehydrogenase* and *reductase* enzymes. These enzymes act on the 15-hydroxyl group (see Fig. 17.1) and the 13-14 double bond, both of which are important for biological activity. The inactive products are further degraded by general fatty acid-oxidising enzymes and excreted in the urine. The dehydrogenase enzymes are present in high concentration in the lung, and 95% of infused PGE_2, PGE_1 or $PGF_{2\alpha}$ is inactivated after a single passage through the lungs, meaning that little normally reaches the arterial circulation. The half-life of most prostaglandins in the circulation is less than 1 minute.

TXA_2 and PGI_2 are slightly different. Both are inherently unstable and decay spontaneously and rapidly (within 30 s and 5 min, respectively) in biological fluids into inactive TXB_2 and 6-keto-$PGF_{1\alpha}$ respectively. Further metabolism occurs, but it is not really relevant to us here.

PROSTANOID RECEPTORS

There are five main classes of prostanoid receptor (Woodward et al., 2011), all of which are typical G protein-coupled receptors (Table 17.1). They are termed DP, FP, IP, EP and TP receptors, respectively, depending on whether their ligands are PGD, PGF, PGI, PGE or TXA species. Some have further subtypes; for example, there are four EP receptors.

ACTIONS OF THE PROSTANOIDS

The prostanoids affect most tissues and exert a bewildering variety of effects.

- PGD_2 causes vasodilatation in many vascular beds, inhibition of platelet aggregation, relaxation of gastrointestinal and uterine muscle, and modification of release of hypothalamic/pituitary hormones. It has a bronchoconstrictor effect through a secondary action on TP receptors.
- $PGF_{2\alpha}$ causes uterine contraction in humans (see Ch. 35), luteolysis in some species (e.g. cattle) and bronchoconstriction in other species (cats and dogs).
- PGI_2 causes vasodilatation, inhibition of platelet aggregation (see Ch. 24), renin release and natriuresis through effects on tubular reabsorption of Na^+.
- TXA_2 causes vasoconstriction, platelet aggregation (see Ch. 24) and bronchoconstriction (more marked in guinea pig than in humans).

Table 17.1 A simplified scheme of prostanoid receptor classification based upon their physiological effects

Receptor	Physiological ligands	Distribution	General physiological effects	Signalling system
IP	$I_2 \gg D_2$	Abundant in cardiovascular system, platelets, neurons and elsewhere	General inhibitory actions: e.g. smooth muscle relaxation, anti-inflammatory and anti-aggregatory effects	G_S ↑ cAMP
DP_1	$D_2 \gg E_2$	Low abundance; vascular smooth muscle, platelets, CNS, airways, the eye		
EP_2	$E_2 > F_{2\alpha}$	Widespread distribution		
EP_4	$E_2 > F_{2\alpha}$	Widespread distribution		
TP	$TxA_2 = H_2 > D_2$	Abundant in cardiovascular system, platelets and immune cells Two subtypes known with opposing actions	General excitatory: e.g. smooth muscle contraction, pro-inflammatory and platelet aggregatory actions	G_q/G_{11} [PLC]* ↑ Ca^{2+}
FP	$F_{2\alpha} > D_2$	Very high expression in female reproductive organs		
EP_1	$E_2 > F_{2\alpha}$	Myometrium, intestine and lung		
EP_3	$E_2 > F_{2\alpha}$	Widespread distribution throughout body; many isoforms with different G protein coupling	General inhibitory actions: e.g. smooth muscle relaxation, anti-inflammatory and anti-aggregatory effects	G_i/G_o ↓ cAMP
DP_2	$D_2 > F_{2\alpha}$	Different structure to other prostanoid receptors. Widely distributed including immune cells		

*PLC may not be involved in EP_1 signalling.
Data derived from Woodward et al., 2011.

PGE_2, the predominant 'inflammatory' prostanoid has the following actions:

- on EP_1 receptors, it causes contraction of bronchial and gastrointestinal smooth muscle
- on EP_2 receptors, it causes bronchodilatation, vasodilatation, stimulation of intestinal fluid secretion and relaxation of gastrointestinal smooth muscle
- on EP_3 receptors, it causes contraction of intestinal smooth muscle, inhibition of gastric acid secretion (see Ch. 30), increased gastric mucus secretion, inhibition of lipolysis, inhibition of autonomic neurotransmitter release and stimulation of contraction of the pregnant human uterus (Ch. 35)
- on EP_4 receptors, it causes similar effects to those of EP_2 stimulation (these were originally thought to be a single receptor). Vascular relaxation is one consequence of receptor activation as is cervical 'ripening'. Some inhibitory effects of PGE_2 on leukocyte activation are probably mediated through this receptor.

A number of clinically useful drugs act at prostanoid receptors. **Misoprostil** is an EP_2/EP_3 agonist used for suppressing gastric acid secretion (see Ch. 30), **bimatoprost**,[4] **latanaprost, taluprost** and **travoprost** are FP agonists used for the treatment of glaucoma (see Ch. 13) and **iloprost** and **epoprostanol** are IP agonists used for the treatment of pulmonary hypertension (see Ch. 22).

THE ROLE OF PROSTANOIDS IN INFLAMMATION

The inflammatory response is inevitably accompanied by the release of prostanoids. PGE_2 predominates, although PGI_2 is also important. In areas of acute inflammation, PGE_2 and PGI_2 are generated by the local tissues and blood vessels, while mast cells release mainly PGD_2. In chronic inflammation, cells of the monocyte/macrophage series also release PGE_2 and TXA_2. Together, the prostanoids exert a sort of yin–yang effect in inflammation, stimulating some responses and decreasing others. The most striking effects are as follows.

In their own right, PGE_2, PGI_2 and PGD_2 are powerful vasodilators and synergise with other inflammatory vasodilators such as histamine and bradykinin. It is this combined dilator action on precapillary arterioles that contributes to the redness and increased blood flow in areas of acute inflammation. Prostanoids do not directly increase the permeability of the postcapillary venules, but potentiate the effects caused by histamine and bradykinin. Similarly, they do not themselves produce pain, but sensitise afferent C fibres (see Ch. 42) to the effects of bradykinin and other noxious stimuli. The anti-inflammatory and analgesic effects of aspirin-like drugs (NSAIDs, see Ch. 26) stem largely from their ability to block these actions.

Prostaglandins of the E series are also pyrogenic (i.e. they induce fever). High concentrations are found in cerebrospinal fluid during infection, and the increase in temperature (attributed to cytokines) is actually finally mediated by the release of PGE_2. NSAIDs exert antipyretic actions (Ch. 26) by inhibiting PGE_2 synthesis in the hypothalamus.

However, some prostaglandins have anti-inflammatory effects which are important during the resolution phase of inflammation. For example, PGE_2 decreases lysosomal enzyme release and the generation of toxic oxygen metabolites from neutrophils, as well as the release of histamine from mast cells.

Prostanoids

- The term *prostanoids* encompasses the prostaglandins and the thromboxanes.
- Cyclo-oxygenases (COX) oxidise arachidonate, producing the unstable intermediates PGG_2 and PGH_2. These are enzymatically transformed to the different prostanoid species.
- There are two main COX isoforms: COX-1, a constitutive enzyme, and COX-2, which is often induced by inflammatory stimuli.
- The principal prostanoids are:
  - PGI_2 (prostacyclin), predominantly from vascular endothelium, acts on IP receptors, producing vasodilatation and inhibition of platelet aggregation.
  - Thromboxane $(TX)A_2$, predominantly from platelets, acts on TP receptors, causing platelet aggregation and vasoconstriction.
  - PGE_2 is prominent in inflammatory responses and is a mediator of fever and pain. Other effects include:
    - at EP_1 receptors: contraction of bronchial and gastrointestinal (GI) tract smooth muscle
    - at EP_2 receptors: relaxation of bronchial, vascular and GI tract smooth muscle
    - at EP_3 receptors: inhibition of gastric acid secretion, increased gastric mucus secretion, contraction of pregnant uterus and of gastrointestinal smooth muscle, inhibition of lipolysis and of autonomic neurotransmitter release.
- $PGF_{2\alpha}$ acts on FP receptors, found in uterine (and other) smooth muscle, and corpus luteum, producing contraction of the uterus and luteolysis (in some species).
- PGD_2 is derived particularly from mast cells and acts on DP receptors, causing vasodilatation and inhibition of platelet aggregation.

LEUKOTRIENES

Leukotrienes (*leuko-* because they are made by white cells, and *-trienes* because they contain a conjugated triene system of double bonds; see Fig. 17.1) are synthesised from arachidonic acid by lipoxygenase-catalysed pathways. These soluble cytosolic enzymes are mainly found in lung, platelets, mast cells and white blood cells. The main enzyme in this group is *5-lipoxygenase*. On cell activation, this enzyme translocates to the nuclear

[4]Some female patients with glaucoma being treated with bimatoprost eye drops were delighted with a side effect of this drug – stimulation of eyelash growth. It wasn't long before a thriving 'off-label' market had been established for its use in beauty spas. Eventually, the FDA licensed a preparation specifically for this cosmetic indication.

Clinical uses of prostanoids

- Gynaecological and obstetric (see Ch. 35):
  - termination of pregnancy: **gemeprost** or **misoprostol** (a metabolically stable prostaglandin (PG)E analogue
  - induction of labour: **dinoprostone** or **misoprostol**
  - postpartum haemorrhage: **carboprost**.
- Gastrointestinal:
  - to prevent ulcers associated with non-steroidal anti-inflammatory drug use: **misoprostol** (see Ch. 30).
- Cardiovascular:
  - to maintain the patency of the ductus arteriosus until surgical correction of the defect in babies with certain congenital heart malformations: **alprostadil** (PGE_1)
  - to inhibit platelet aggregation (e.g. during haemodialysis): **epoprostenol** (PGI_2), especially if **heparin** is contraindicated
  - primary pulmonary hypertension: **epoprostenol** (see Ch. 22).
- Ophthalmic:
  - open-angle glaucoma: **latanoprost** eye drops.

membrane, where it associates with a crucial accessory protein, affectionately termed FLAP (**f**ive-**l**ipoxygenase **a**ctivating **p**rotein). The 5-lipoxygenase incorporates a hydroperoxy group at C5 in arachidonic acid to form *5-hydroperoxytetraenoic acid* (5-HPETE, Fig. 17.3), leading to the production of the unstable leukotriene (LT)A_4. This may be converted enzymatically to LTB_4 and, utilising a separate pathway involving conjugation with glutathione, to the cysteinyl-containing leukotrienes LTC_4, LTD_4, LTE_4 and LTF_4 (also referred to as the *sulfidopeptide leukotrienes*). These cysteinyl leukotrienes are produced mainly by eosinophils, mast cells, basophils and macrophages. Mixtures of these substances constitute the biological activity historically ascribed to *slow-reacting substance of anaphylaxis* (SRS-A), an elusive bronchoconstrictor factor shown many years ago to be generated in guinea-pig lung during anaphylaxis, and consequently predicted to be important in asthma.

LTB_4 is produced mainly by neutrophils. Lipoxins and other active products, some of which have anti-inflammatory properties, are also produced from arachidonate by this pathway (Figs 17.1 and 17.3).

LTB_4 is metabolised by a unique membrane-bound cytochrome P450 enzyme in neutrophils, and then further oxidised to 20-carboxy-LTB_4. LTC_4 and LTD_4 are metabolised to LTE_4, which is excreted in the urine.

LEUKOTRIENE RECEPTORS

Leukotriene receptors are termed BLT (two subtypes) if the ligand is LTB_4, and CysLT (two subtypes) for the cysteinyl leukotrienes. Their signalling mechanisms have not been completely elucidated and there may be further receptors that transduce the effects of these potent mediators.

LEUKOTRIENE ACTIONS

Cysteinyl leukotrienes have important actions on the respiratory and cardiovascular systems, and specific receptors for LTD_4 have been defined on the basis of numerous selective antagonists. The CysLT-receptor antagonists **zafirlukast** and **montelukast** are now in use in the treatment of asthma (see Ch. 28), often with a corticosteroid. Cysteinyl leukotrienes may mediate the cardiovascular changes of acute anaphylaxis. Agents that inhibit 5-lipoxygenase are therefore obvious candidates for anti-asthmatic (see Ch. 28) and anti-inflammatory agents. One such drug, **zileuton**, is available in some parts of the world but has not yet gained a definite place in therapy (see Larsson et al., 2006).

The respiratory system. Cysteinyl leukotrienes are potent spasmogens, causing dose-related contraction of human bronchiolar muscle *in vitro*. LTE_4 is less potent than LTC_4 and LTD_4, but its effect is much longer lasting. All cause an increase in mucus secretion. Given by aerosol to human volunteers, they reduce specific airway conductance and maximum expiratory flow rate, the effect being more protracted than that produced by histamine (Fig. 17.4).

The cardiovascular system. Small amounts of LTC_4 or LTD_4 given intravenously cause a rapid, short-lived fall in blood pressure, and significant constriction of small coronary resistance vessels. Given subcutaneously, they are equipotent with histamine in causing weal and flare. Given topically in the nose, LTD_4 increases nasal blood flow and increases local vascular permeability.

The role of leukotrienes in inflammation. LTB_4 is a potent chemotactic agent for neutrophils and macrophages (see Fig. 6.2). It upregulates membrane adhesion molecule expression on neutrophils, and increases the production of toxic oxygen products and the release of granule enzymes. On macrophages and lymphocytes, it stimulates proliferation and cytokine release. It is found in inflammatory exudates and tissues in many inflammatory conditions, including rheumatoid arthritis, psoriasis and ulcerative colitis.

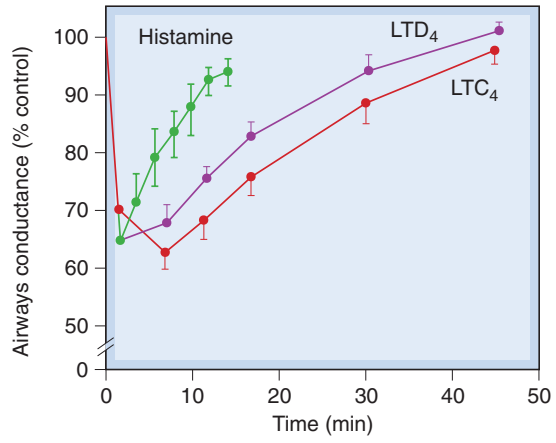

Fig. 17.4 The time course of action on specific airways conductance of the cysteinyl leukotrienes and histamine, in six normal subjects. Specific airways conductance was measured in a constant volume whole-body plethysmograph, and the drugs were given by inhalation. *(From Barnes et al., 1984.)*

The cysteinyl leukotrienes are present in the sputum of chronic bronchitis patients in amounts that are biologically active. On antigen challenge, they are released from samples of human asthmatic lung *in vitro*, and into nasal lavage fluid in subjects with allergic rhinitis. There is evidence that they contribute to the underlying bronchial hyper-reactivity in asthmatics, and it is thought that they are among the main mediators of both the early and late phases of asthma (see Fig. 28.2).

Leukotrienes

- 5-Lipoxygenase oxidises arachidonate to give 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which is converted to leukotriene (LT)A_4. This, in turn, can be converted to either LTB_4 or to a series of glutathione adducts, the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4.
- LTB_4, acting on specific receptors, causes adherence, chemotaxis and activation of polymorphs and monocytes, and stimulates proliferation and cytokine production from macrophages and lymphocytes.
- The cysteinyl leukotrienes cause:
  - contraction of bronchial muscle
  - vasodilatation in most vessels, but coronary vasoconstriction.
- LTB_4 is an important mediator in all types of inflammation; the cysteinyl leukotrienes are of particular importance in asthma.

Di Gennaro and Haeggstrom (2012) have provided a good account of recent thinking on the role of these mediators in inflammation.

LIPOXINS AND RESOLVINS

A recently identified group of trihydroxy arachidonate metabolites termed lipoxins (Figs 17.1, 17.3) are formed by the concerted action of the 5- and the 12- or 15-lipoxygenase enzymes during inflammation. Lipoxins act on polymorphonuclear leukocytes, through a distinct G protein-coupled receptor system (which also recognises other anti-inflammatory factors such as annexin-A_1), to oppose the action of pro-inflammatory stimuli, supplying what might be called 'stop signals' to inflammation (reviewed by Ryan & Godson, 2010). Aspirin (a COX inhibitor, see Ch. 26) stimulates the synthesis of lipoxins because COX-2 can still produce hydroxy fatty acids even when inhibited by aspirin, even though it cannot synthesise prostaglandins. The formation of lipoxins probably contributes to aspirin's anti-inflammatory effects, some of which are not completely explained through inhibition of prostaglandin generation (see Gilroy & Perretti, 2005; Serhan, 2005).

Resolvins, as the name implies, are a series of compounds that fulfil a similar function, but unlike lipoxins, their precursor fatty acid is eicosapentaenoic acid. Fish oils are rich in this fatty acid and it is likely that at least some of their anti-inflammatory benefit is produced through conversion to these highly active species (see Zhang & Spite, 2012, for a recent review of this fascinating area). The leukocyte receptor for resolvins is called Chem 23. Resolvins can counteract inflammatory pain (Xu et al., 2010) and analogues are undergoing trials for the treatment of a variety of inflammatory conditions (Lee & Surh, 2012).

PLATELET-ACTIVATING FACTOR

Platelet-activating factor, also variously termed PAF-acether and AGEPC (acetyl-glyceryl-ether-phosphorylcholine), is a biologically active lipid that can produce effects at exceedingly low concentrations (less than 10_{-10} mol/l) through its G protein-coupled receptor (G_q/G_{11}; stimulates cAMP production). The name is somewhat misleading, however, because PAF has actions on a variety of different target cells, and is believed to be an important mediator in both acute and chronic allergic and inflammatory phenomena.

BIOSYNTHESIS

PAF (Fig. 17.1) is produced by platelets in response to thrombin, and by activated inflammatory cells. It is synthesised from particular phospholipids (acyl-PAF), which have an ether-linked hexadecyl or octadecyl fatty acid at C1, an unsaturated fatty acid such as arachidonic acid ester-linked at C2 and a phosphoryl choline base at C3. The action of PLA_2 on acyl-PAF produces removes the arachidonic acid from C2 leaving *lyso-PAF*, which is then acetylated by an *acetyltransferase* to yield PAF. This, in turn, can be inactivated by an *acetylhydrolase* to lyso-PAF.

ACTIONS AND ROLE IN INFLAMMATION

PAF can reproduce many of the signs and symptoms of inflammation. Injected locally, it produces vasodilatation (and thus erythema), increased vascular permeability and weal formation. Higher doses produce hyperalgesia. It is a potent chemotaxin for neutrophils and monocytes, and recruits eosinophils into the bronchial mucosa in the late phase of asthma (see Fig. 28.3). PAF contracts both bronchial and ileal smooth muscle.

PAF activates PLA_2 and stimulates arachidonate turnover in many cells. In platelets it increases TXA_2 generation, producing shape change and the release of the granule contents. This is important in haemostasis and thrombosis (see Ch. 24).

The anti-inflammatory actions of the glucocorticoids may be caused, at least in part, by inhibition of PAF synthesis (Fig. 17.2). Competitive antagonists of PAF and/or specific inhibitors of *lyso-PAF acetyltransferase* could well be useful anti-inflammatory drugs and/or antiasthmatic agents. The PAF antagonist **lexipafant** is in clinical trial in the treatment of acute pancreatitis (see Leveau et al., 2005). **Rupatidine** is a combined H_1 and PAF antagonist that is available in some parts of the world for treating allergic symptoms, but it is not clear what (if anything) its anti-PAF action adds clinically to its effect as an H_1 antagonist.

CONCLUDING REMARKS

In this chapter we have focused on histamine and lipid mediators. In some species (i.e. rodents) 5-HT (Ch. 15) has pro-inflammatory properties. Other low-molecular-weight factors also have inflammogenic actions, including some purines (Ch. 16) and nitric oxide (Ch. 20).

Platelet-activating factor (PAF)

- PAF precursors are released from activated inflammatory cells by phospholipase A_2. After acetylation, the resultant PAF is released and acts on specific receptors in target cells.
- Pharmacological actions include vasodilatation, increased vascular permeability, chemotaxis and activation of leukocytes (especially eosinophils), activation and aggregation of platelets, and smooth muscle contraction.
- PAF is implicated in bronchial hyper-responsiveness and in the delayed phase of asthma.
- A PAF antagonist, **lexipafant**, is undergoing clinical trial in pancreatitis.

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18 Local hormones 2: peptides and proteins

OVERVIEW

Having discussed small-molecule local hormones in the previous chapter, we now turn our attention to peptides and proteins, which are orders of magnitude larger in molecular terms. This constitutes a very diverse group and, unlike others described in Chapter 17, includes compounds (e.g. cytokines) that seem to be exclusively concerned with host defence. We begin with some general introductory observations on protein and peptide synthesis and secretion. We then discuss bradykinin, neuropeptides, cytokines (interleukins, chemokines and interferons) in more detail. Finally, we conclude with a few remarks on other proteins and peptides that downregulate inflammation.

INTRODUCTION

Despite the fact that several mediators discovered early in the history of our discipline were recognised to be peptides, understanding of their pharmacology was limited until the 1970s when the techniques for purifying, sequencing and synthesising peptides and proteins were first developed. The development of high-performance liquid chromatography and solid-phase peptide synthesis, for example, have greatly accelerated the development of the area and while proteins containing 50 or more amino acids were (and are still) difficult to synthesise chemically, molecular biology techniques have provided a rapid alternative synthetic route. Indeed, the use of recombinant proteins as therapeutic agents – a development driven mainly by the emergent biotechnology industry – is rapidly gaining ground (see Ch. 59).

The use of molecular biology has helped understand peptide and protein pharmacology in many other ways as well. The availability of monoclonal antibodies for radioimmunoassay and immunocytochemistry has solved many quantitative problems. Transgenic animals with peptide or receptor genes deleted, or overexpressed, provide valuable clues about their functions, as has the use of antisense oligonucleotides and siRNA (see also Ch. 59) to silence these genes for experimental purposes. The control of precursor synthesis can be studied indirectly by measuring mRNA, for which highly sensitive and specific assays have been developed. The technique of *in situ* hybridisation enables the location and abundance of the mRNA to be mapped at microscopic resolution.

In summary, the molecular landscape has changed completely. Whereas the discovery of new 'small molecule' mediators has virtually dried up, the discovery of new protein and peptide mediators continues apace. More than 100 cytokines have been discovered since interleukin 2 (IL-2) was first characterised in 1982.

GENERAL PRINCIPLES OF PROTEIN AND PEPTIDE PHARMACOLOGY

STRUCTURE

Peptide and protein mediators generally vary from three to about 200 amino acid residues in length, the arbitrary dividing line between peptides and proteins being about 50 residues. An important difference is that proteins need to adopt a complex folded structure in order to exert their specific function, whereas short peptides are in most cases flexible. Specific residues in proteins and peptides often undergo post-translational modifications, such as *amidation*, *glycosylation*, *acetylation*, *carboxylation*, *sulfation* or *phosphorylation*. They also may contain *intramolecular* disulfide bonds, such that the molecule adopts a partially cyclic conformation, or they may comprise two or more separate chains linked by *intermolecular* disulfide bonds.

Generally speaking, larger proteins adopt restricted conformations that expose functional groups in fixed locations on their surface, which interact with multiple sites on their receptors in 'lock-and-key' mode. To envisage flexible peptides fitting into a receptor site this way is to imagine that you can unlock your front door with a length of cooked spaghetti. These features have greatly impeded the rational design of non-peptide analogues that mimic the action of proteins and peptides at their receptors (peptidomimetics). The use of random screening methods has (somewhat to the chagrin of the rationalists) nevertheless led in recent years to the discovery of many non-peptide *antagonists* – although few *agonists* – for peptide receptors.

TYPES OF PROTEIN AND PEPTIDE MEDIATOR

Protein and peptide mediators that are secreted by cells and act on surface receptors of the same or other cells can be very broadly divided into four groups:

- neurotransmitters (e.g. endogenous opioid peptides, Ch. 42) and neuroendocrine mediators (e.g. vasopressin, somatostatin, hypothalamic releasing hormones, ACTH, LH, FSH and TSH, see Chs 33–35), not discussed further in this chapter)
- hormones from non-neural sources: these comprise plasma-derived peptides, notably angiotensin (Ch. 22) and bradykinin, as well as other hormones such as insulin (Ch. 31), endothelin (Ch. 22), atrial natriuretic peptide (Ch. 21) and leptin (Ch. 32)
- growth factors: produced by many different cells and tissues that control cell growth and differentiation (especially, in adults, in the haemopoietic system; see Ch. 25)
- mediators of the immune system (cytokines, see below).

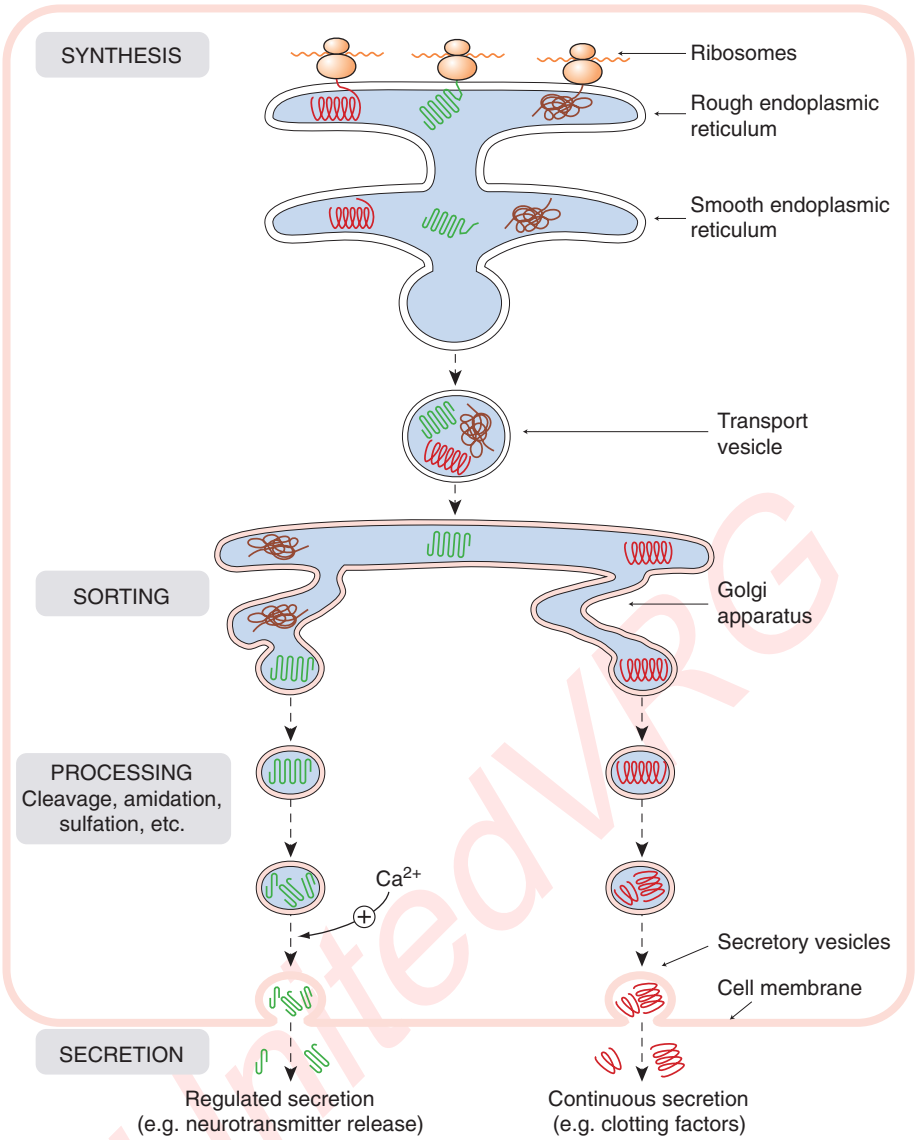


Fig. 18.1 Cellular mechanisms for peptide synthesis and release. Proteins synthesised by ribosomes are threaded through the membrane of the rough endoplasmic reticulum, from where they are conveyed in transport vesicles to the Golgi apparatus. Here, they are sorted and packaged into secretory vesicles. Processing (cleavage, glycosylation, amidation, sulfation, etc.) occurs within the transport and secretory vesicles, and the products are released from the cell by exocytosis. Constitutive secretion (e.g. of plasma proteins and clotting factors by liver cells) occurs continuously, and little material is stored in secretory vesicles. Regulated secretion (e.g. of neuropeptides or cytokines) occurs in response to increased intracellular Ca^{2+} or other intracellular signals, and material is typically stored in significant amounts in secretory vesicles awaiting release.

BIOSYNTHESIS AND REGULATION OF PEPTIDES

Peptide structure is, of course, directly coded in the genome, in a manner that the structure of (say) acetylcholine is not, so intracellular manufacture is a matter of conventional protein synthesis. This often begins with the manufacture of a precursor protein in which the desired peptide sequence is embedded. Specific proteolytic enzymes excise the active peptide, a process of sculpture rather than synthesis. The precursor protein is packaged into vesicles at the point of synthesis, and the active peptide is formed *in situ* ready for release (Fig. 18.1). Thus there is no need for specialised biosynthetic pathways, or for the uptake or recapturing mechanisms, that are important for the synthesis and release of most non-peptide mediators (e.g. 5HT; Ch. 15).

PEPTIDE PRECURSORS

The precursor protein, or *pre-prohormone*, usually 100–250 residues in length, consists of an N-terminal *signal sequence* (peptide), followed by a variable stretch of unknown function, and a peptide-containing region that may contain several copies of active peptide fragments. Often, several different peptides are found within one precursor, but sometimes there are multiple copies of a single peptide.[1]

[1]In the case of the invertebrate *Aplysia*, one protein precursor contains no fewer than 28 copies of the same short peptide.

The *signal sequence*, which is strongly hydrophobic, facilitates insertion of the protein into the endoplasmic reticulum and is then cleaved off at an early stage, yielding the *prohormone*.

The active peptides are usually demarcated within the prohormone sequence by pairs of basic amino acids (Lys-Lys or Lys-Arg), which are cleavage points for the trypsin-like proteases that release the peptides. This *endoproteolytic cleavage* generally occurs in the Golgi apparatus or the secretory vesicles. The enzymes responsible are known as *prohormone convertases*. Scrutiny of the prohormone sequence often reveals likely cleavage points that demarcate previously unknown peptides. In some cases (e.g. CGRP; see below), new peptide mediators have been discovered in this way, but there are many examples where no function has yet been assigned. Whether these peptides are, like strangers at a funeral, waiting to declare their purpose or merely functionless relics, remains a mystery. There are also large stretches of the prohormone sequence of unknown function lying between the active peptide fragments.

The abundance of mRNA coding for particular pre-prohormones, which reflects the level of gene expression, is very sensitive to physiological conditions. This type of *transcriptional control* is one of the main mechanisms by which peptide expression and release are regulated over the medium to long term. Inflammation, for example, increases the expression, and hence the release, of various cytokines by immune cells (see Ch. 16). Sensory neurons respond to peripheral inflammation by increased expression of tachykinins (substance P and neurokinins A and B), which is important in the genesis of inflammatory pain (see Ch. 42).

DIVERSITY WITHIN PEPTIDE FAMILIES

Peptides commonly occur in families with similar or related sequences and actions. For example, the pro-opiomelanocortin (POMC) serves as a source of adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormones (MSH) and β-endorphin, all of which have a role in controlling the inflammatory response (as well as other processes).

GENE SPLICING AS A SOURCE OF DIVERSITY

Diversity of members of a peptide family can also arise by gene splicing or during post-translational processing of the prohormone. Genes contain coding regions (*exons*) interspersed with non-coding regions (*introns*) and when the gene is transcribed, the ensuing RNA (*heterologous nuclear RNA* [*hnRNA*]) is spliced to remove the introns and some of the exons, forming the final mature mRNA that is translated. Control of the splicing process allows a measure of cellular control over the peptides that are produced.

For example, the calcitonin gene codes for calcitonin itself, important in bone metabolism, Ch. 36) and also for a completely dissimilar peptide (calcitonin gene-related peptide, CGRP, involved in migraine pathogenesis, Ch. 15). Alternative splicing allows cells to produce either pro-calcitonin (expressed in thyroid cells) or pro-CGRP (expressed in many neurons) from the same gene. Substance P and neurokinin A are two closely related tachykinins belonging to the same family, and are encoded on the same gene. Alternative splicing results in the production of two precursor proteins; one of these includes both peptides, the other includes only substance P. The ratio of the two varies widely between tissues, which correspondingly produce either one or both peptides.

POST-TRANSLATIONAL MODIFICATIONS AS A SOURCE OF PEPTIDE DIVERSITY

Many peptides, such as tachykinins and ACTH-related peptides (see Ch. 33), must undergo enzymatic amidation at the C-terminus to acquire full biological activity. Tissues may also generate peptides of varying length from the same primary sequence by the action of specific peptidases that cut the chain at different points. For example, pro-cholecystokinin (pro-CCK) contains the sequences of at least five CCK-like peptides ranging in length from 4 to 58 amino acid residues, all with the same C-terminal sequence. CCK itself (33 residues) is the main peptide produced by the intestine, whereas the brain produces mainly CCK-8. The opioid precursor prodynorphin similarly gives rise to several peptides with a common terminal sequence, the proportions of which vary in different tissues and in different neurons in the brain. In some cases (e.g. the inflammatory mediator bradykinin), peptide cleavage occurring after release generates a new active peptide (des-Arg^9-bradykinin), which acts on a different receptor, both peptides contributing differently to the inflammatory response.

PEPTIDE TRAFFICKING AND SECRETION

The basic mechanisms by which peptides are synthesised, packaged into vesicles, processed and secreted are summarised in Fig. 18.1. Two secretory pathways exist, for *constitutive* and *regulated* secretion, respectively. Constitutively secreted proteins (e.g. plasma proteins, some clotting factors) are not stored in appreciable amounts, and secretion is coupled to synthesis. Regulated secretion is, as with many hormones and transmitters, controlled by receptor-activated signals that lead to a rise in intracellular Ca^{2+} (see Ch. 4), and peptides awaiting release are stored in cytoplasmic vesicles. Specific protein–protein interactions appear to be responsible for the sorting of different proteins into different vesicles, and for choreographing their selective release. Identification of the specific 'trafficking' proteins involved in particular secretory pathways may eventually yield novel drug targets for the selective control of secretion.

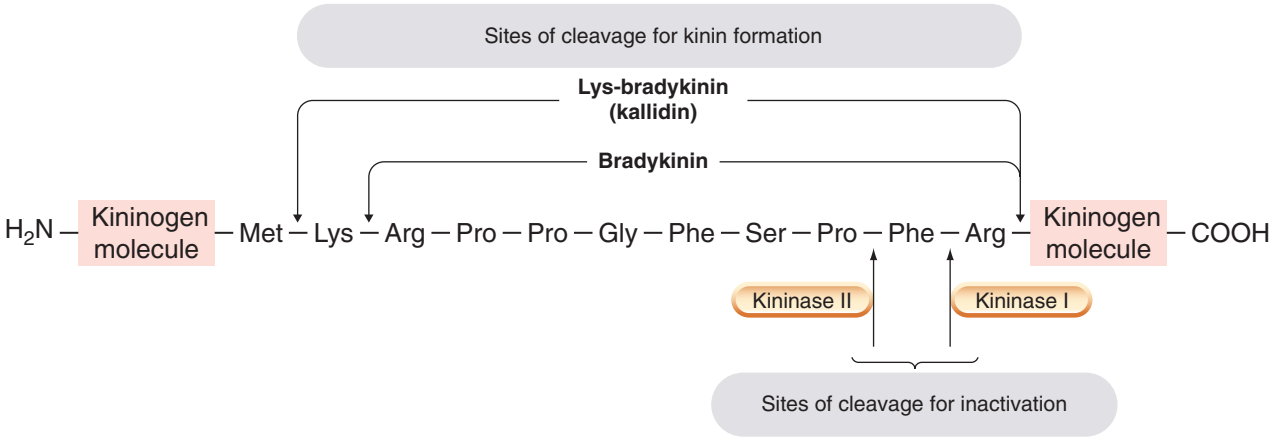
Having described the general mechanisms by which peptides are synthesised, processed and released, we now describe some significant mediators that fall into this category.

BRADYKININ

Bradykinin and lysyl-bradykinin (*kallidin*) are active peptides formed by proteolytic cleavage of circulating proteins termed *kininogens* through a protease cascade pathway (see Fig. 6.1).

SOURCE AND FORMATION OF BRADYKININ

An outline of the formation of bradykinin from high-molecular-weight *kininogen* in plasma by the serine protease *kallikrein* is given in Figure 18.2. Kininogen is a plasma α-globulin that exists in both high- (M_r 110 000) and low- (M_r 70 000) molecular-weight forms. Kallikrein is derived from the inactive precursor *prekallikrein* by the



B_2- receptor antagonist, Hoe 140: D-Arg – Arg – Pro – Hyp – Gly – Thi – Ser – D-Tic – Oic – Arg
B_1- receptor antagonist, des-Arg Hoe 140: D-Arg – Arg – Pro – Hyp – Gly – Thi – Ser – D-Tic – Oic

Fig. 18.2 The structure of bradykinin and some bradykinin antagonists. The sites of proteolytic cleavage of high-molecular-weight kininogen by kallikrein involved in the formation of bradykinin are shown in the upper half of the figure; the sites of cleavage associated with bradykinin and kallidin inactivation are shown in the lower half. The B_2-receptor antagonist icatibant (Hoe 140) has a pA_2 of 9, and the competitive B_1-receptor antagonist des-Arg Hoe 140 has a pA_2 of 8. The Hoe compounds contain unnatural amino acids: Thi, δ-Tic and Oic, which are analogues of phenylalanine and proline.

action of *Hageman factor* (factor XII; see Ch. 24 and Fig. 6.1). Hageman factor is activated by contact with negatively charged surfaces such as collagen, basement membrane, bacterial lipopolysaccharides, urate crystals and so on. Hageman factor, prekallikrein and the kininogens leak out of the vessels during inflammation because of increased vascular permeability, and exposure to negatively charged surfaces promotes the interaction of Hageman factor with prekallikrein. The activated enzyme then 'clips' bradykinin from its kininogen precursor. Kallikrein can also activate the complement system and can convert plasminogen to plasmin (see Fig. 6.1 and Ch. 24).

In addition to plasma kallikrein, there are other kinin-generating isoenzymes found in pancreas, salivary glands, colon and skin. These *tissue kallikreins* act on both high- and low-molecular-weight kininogens and generate mainly kallidin, a peptide with actions similar to those of bradykinin.

METABOLISM AND INACTIVATION OF BRADYKININ

Specific enzymes that inactivate bradykinin and related kinins are called *kininases* (Fig. 18.2). One of these, *kininase II*, is a peptidyl dipeptidase that inactivates kinins by removing the two C-terminal amino acids. This enzyme, which is bound to the luminal surface of endothelial cells, is identical to *angiotensin-converting enzyme* (ACE; see Ch. 22), which cleaves the two C-terminal residues from the inactive peptide angiotensin I, converting it to the active vasoconstrictor peptide angiotensin II. Thus kininase II inactivates a vasodilator and activates a vasoconstrictor. Potentiation of bradykinin actions by ACE inhibitors may contribute to some side effects of these drugs (e.g. cough). Kinins are also metabolised by various less specific peptidases, including a serum carboxypeptidase that removes the C-terminal arginine, generating des-Arg^9-bradykinin, a specific agonist at one of the two main classes of bradykinin receptor.

BRADYKININ RECEPTORS

There are two bradykinin receptors, designated B_1 and B_2. Both are G protein-coupled receptors and mediate very similar effects. B_1 receptors are normally expressed at very low levels but are strongly induced in inflamed or damaged tissues by cytokines such as IL-1. B_1 receptors respond to des-Arg^9-bradykinin but not to bradykinin itself. A number of selective peptide and non-peptide antagonists are known. It is likely that B_1 receptors play a significant role in inflammation and hyperalgesia (see Ch. 42), and antagonists could be used in cough and neurological disorders (Rodi et al., 2005).

B_2 receptors are constitutively present in many normal cells and are activated by bradykinin and kallidin, but not by des-Arg^9-bradykinin. Peptide and non-peptide antagonists have been developed, the best known being the bradykinin analogue **icatibant**, used to treat acute attacks in patients with *hereditary angioedema* (an uncommon disorder caused by deficiency of C1-esterase inhibitor that normally restrains complement activation).

ACTIONS AND ROLE IN INFLAMMATION

Bradykinin causes vasodilatation and increased vascular permeability. Its vasodilator action is partly a result of generation of PGI_2 and release of nitric oxide (NO). It is a potent pain-producing agent at sensory neurons, and its action here is potentiated by prostaglandins (Ch. 17), which are released by bradykinin. Bradykinin also has spasmogenic actions on intestinal, uterine and bronchial smooth muscle in some species. The contraction is slow and sustained in comparison with that produced by tachykinins such as substance P (*brady-* means slow; *tachy-* means rapid).

Although bradykinin reproduces many inflammatory signs and symptoms, its role in inflammation and allergy is not clear, partly because its effects are often component parts of a complex cascade of events triggered by other

mediators. However, excessive bradykinin production contributes to the diarrhoea of gastrointestinal disorders, and in allergic rhinitis it stimulates nasopharyngeal secretion. Bradykinin also contributes to the clinical picture in pancreatitis,[2] although disappointingly B_2 antagonists worsen rather than alleviate this disorder. Physiologically, the release of bradykinin by tissue kallikrein may regulate blood flow to certain exocrine glands, and influence secretions. Bradykinin also stimulates ion transport and fluid secretion by some epithelia, including intestine, airways and gall bladder.

Bradykinin

- Bradykinin (BK) is a nonapeptide 'clipped' from a plasma α-globulin, *kininogen*, by *kallikrein*.
- It is converted by *kininase I* to an octapeptide, $BK_{1–8}$ (des-Arg^9-BK), and inactivated by the removal of an additional amino acid by *kininase II* (angiotensin-converting enzyme) in the lung.
- Pharmacological actions:
  - vasodilatation (largely dependent on endothelial cell nitric oxide and PGI_2)
  - increased vascular permeability
  - stimulation of pain nerve endings
  - stimulation of epithelial ion transport and fluid secretion in airways and gastrointestinal tract
  - contraction of intestinal and uterine smooth muscle.
- There are two main subtypes of BK receptors: B_2, which is constitutively present, and B_1, which is induced in inflammation.
- **Icatibant**, a peptide analogue of BK, is a selective competitive antagonist for B_2 receptors and is used to treat acute attacks of hereditary angioedema.

Other, non-peptide antagonists for both B_1 and B_2 receptors are known, and may be developed for treating inflammatory disorders.

NEUROPEPTIDES

Neuropeptides constitute a large (>100) and diverse family of small to medium-sized peptides. A large number are found in the CNS, the autonomic nervous system, and peripheral sensory neurons, and they are also abundant in many peripheral tissues. They are often released as co-transmitters (Chs 38 and 39), along with non-peptide neurotransmitters.

When released from peripheral endings of nociceptive sensory neurons (see Ch. 42), neuropeptides in some species cause *neurogenic inflammation* (Maggi, 1996). The main peptides involved are *substance P*, *neurokinin A* and *CGRP*. Substance P and neurokinin A are small (about 1100 mw) members of the *tachykinin* family with partly homologous structures, which act on mast cells, releasing histamine and other mediators, and producing smooth muscle contraction, neural activation, mucus secretion and vasodilatation. CGRP is a member of the calcitonin family (37 amino acids in length) shares these properties and is a particularly potent vasodilator. Tachykinins released from the central endings of nociceptive neurons also modulate transmission in the dorsal horn of the spinal cord, affecting sensitivity to pain (see Ch. 42). All these neuropeptides act on specific G protein-coupled receptors to produce their effects.

Neurogenic inflammation is implicated in the pathogenesis of several inflammatory conditions, including the delayed phase of asthma, allergic rhinitis, inflammatory bowel disease and some types of arthritis as well as migraine (Ch. 15 and Pisi et al., 2009). Antagonists at the neurokinin NK_1 receptor such as **aprepitant** and **fosaprepitant** are used to treat emesis, particularly that associated with some forms of cancer chemotherapy (see Ch. 56). Other important members of the neuropeptide family include enkephalins/endorphins (Ch. 42) and orexins (Ch. 39).

CYTOKINES

'Cytokine' is an all-purpose functional term that is applied to protein or polypeptide mediators synthesised and released by cells of the immune system during inflammation. They are crucial for the overall coordination of the inflammatory response. Cytokines act locally by autocrine or paracrine mechanisms. Unlike conventional hormones such as insulin, concentrations in blood and tissues are almost undetectable under normal circumstances, but are massively upregulated (100–1000-fold) during inflammatory episodes. All these mediators are usually active at very low (sub-nanomolar) concentrations.

On the target cell, cytokines bind to and activate specific, high-affinity receptors that, in most cases, are also upregulated during inflammation. Except for *chemokines*, which act on G protein-coupled receptors, most cytokines act on kinase-linked receptors, regulating phosphorylation cascades that affect gene expression, such as the Jak/Stat pathway (Chs 3 and 4).

In addition to their own direct actions on cells, some cytokines amplify inflammation by inducing the formation of other inflammatory mediators. Others can induce receptors for other cytokines on their target cell, or engage in synergistic or antagonistic interactions with other cytokines. Cytokines may be likened to a complex chemical signalling language, with the final response of a particular cell involved being determined by the strength and number of different messages received concurrently at the cell surface.

Various systems for classifying cytokines can be found in the literature, as can a multitude of diagrams depicting complex networks of cytokines interacting with each other and with a range of target cells. No one system of classification does justice to the complexity of cytokine biology. The terminology and nomenclature are horrendous and a comprehensive coverage of this area is beyond the scope of this book. For our purposes of this chapter, however, Table 18.1 lists some of the more significant cytokines and their biological actions. The would-be cytokine aficionado can find further classification tables in Murphy et al. (2011) and the IUPHAR/BPS Guide to Pharmacology.

More than 100 cytokines have been identified. These may be broadly categorised into four main functional groups, namely *interleukins*, *chemokines*, *interferons* and *colony-stimulating factors* (discussed separately in Ch. 25),

[2]A serious and painful condition in which proteolytic enzymes are released from damaged pancreatic cells, initiating cascades that release, among other things, bradykinin.

Table 18.1 Some examples of significant cytokines and their actions

Cytokine	Main cell source	Main target cell or biological effect	Comments
IL-1	Monocyte/macrophages, dendritic and other cells	Regulates cell migration to sites of infection, produces inflammation, fever and pain	Two original subtypes Il-1α and IL-1β, and IL-1ra – a receptor antagonist. Target for anti-inflammatory therapy (Ch. 26)
IL-2	T cells	Stimulates proliferation, maturation and activation of T, B and NK cells	First interleukin to be discovered
IL-4	Th2 cells	Stimulates proliferation, maturation of T and B cells and promotes IgG and E synthesis. Promotes an anti-inflammatory phenotype	A key cytokine in the regulation of the Th2 response (Ch. 26)
IL-5	Th2 cells, mast cells	Important for eosinophil activation. Stimulates proliferation, maturation of B cells and IgA synthesis	Particularly important in allergic disease
IL-6	Monocyte/macrophages and T cells	Pro-inflammatory actions including fever. Stimulation of osteoclast activity	Target for anti-inflammatory drugs (Ch. 26)
IL-8	Macrophages, endothelial cells	Neutrophil chemotaxis, phagocytosis and angiogenesis	C–X–C chemokine (CXCL8)
IL-10	Monocytes and Th2 cells	Inhibits cytokine production and downregulates inflammation	A predominately anti-inflammatory cytokine
IL-17	T cells and others	Stimulates Th17 cells, involved in allergic response and autoimmunity	Several subtypes. Target for anti-inflammatory drugs (Ch. 26)
GM–CSF	Macrophages, T cells, mast cells and others	Stimulates growth of leukocyte progenitor cells. Increases numbers of blood-borne leukocytes	Used therapeutically to stimulate myeloid cell growth (e.g. after bone marrow transplantation)
MIP-1	Macrophages/lymphocyes	Activation of neutrophils and other cells. Promotes cytokine release	C–C chemokine (CCL3). Two subtypes
TGF-β	T cells, monocytes	Induces apoptosis. Regulates cell growth	Three isoforms. Predominately anti-inflammatory action
TNF-α	Mainly macrophages but also many immune and other cells	Kills tumour cells. Stimulates macrophage cytokine expression and is a key regulator of many aspects of the immune response	A major target for anti-inflammatory drugs (Ch. 6)
TNF-β	Th1 cells	Initiates a variety of immune-stimulatory and pro-inflammatory actions in the host defence system	Now often called lymphotoxin α (LTA)
Eotaxin	Airway epithelial and other cells	Activation and chemotaxis of eosinophils. Allergic inflammation	C–C chemokine (CCL11). Three subtypes
MCP-1	Monocytes, osteoblasts/clasts, neurons and other cells	Promotes recruitment of monocytes and T cells to sites of inflammation	C–C chemokine (CC2)
RANTES	T cells	Chemotaxis of T cells. Chemotaxis and activation of other leukocytes	(CCL5)
IFN-α	Leukocytes	Activates NK cells and macrophages. Inhibits viral replication and has antitumour actions	Multiple molecular species
IFN-γ	Th1, NK cells	Stimulates Th1, and inhibits Th2, cell proliferation. Activates NK cells and macrophages	Crucial to the Th1 response (Ch. 6)

GM–CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NK, natural killer (cell); RANTES, regulated on activation normal T cell expressed and secreted; TGF, transforming growth factor; Th, T-helper (cell); TNF, tumour necrosis factor.

but the demarcations are of limited use because many cytokines have multiple roles.

Using biopharmaceuticals (see Ch. 59) to interfere with cytokine action has proved to be a particularly fertile area of drug development: several successful strategies have been adopted, including direct antibody neutralisation of cytokines or the use of 'decoy' receptor proteins that remove the biologically active pool from the circulation. These are explained in detail in Chapters 26 and 59.

INTERLEUKINS AND RELATED COMPOUNDS

The name was originally coined to describe mediators that signalled between leukocytes but, like so much else in the cytokine lexicography, it has become rather redundant, not to say misleading. The primary pro-inflammatory species are *tumour necrosis factor* (TNF)-α and *interleukin 1* (IL-1). The principal members of the latter cytokine group consist of two agonists, IL-1α, IL-1β and, surprisingly, an endogenous IL-1-receptor antagonist (IL-1ra).[3] Mixtures of these are released from macrophages and many other cells during inflammation and can initiate the synthesis and release of a cascade of secondary cytokines, among which are the chemokines. TNF and IL-1 are key regulators of almost all manifestations of the inflammatory response. A long-standing debate about which of the two is really the prime mover of inflammation ended when it was found that this varies according to the disease type. In auto-*immune* disease (e.g. rheumatoid arthritis, where the adaptive immune system is activated), TNF appears to be the predominant influence and blocking its action is therapeutically effective. In auto-*inflammatory* diseases (e.g. gout, where only the innate system is involved), IL-1 seems to be the key mediator (Dinarello et al., 2012). Both TNF-α and IL-1 are important targets for anti-inflammatory biopharmaceuticals (Chs. 26 and 59).

Not all interleukins are pro-inflammatory: some, including *transforming growth factor (TGF)-β*, IL-4, IL-10 and IL-13 are potent anti-inflammatory substances. They inhibit chemokine production, and the responses driven by T-helper (Th) 1 cells, whose inappropriate activation is involved in the pathogenesis of several diseases.

CHEMOKINES

Chemokines are defined as *chemo*attractant cyto*kines* that control the migration of leukocytes, functioning as traffic coordinators during immune and inflammatory reactions. Again, the nomenclature (and the classification) is confusing, because some non-cytokine mediators also control leukocyte movement (C5a, LTB_4, fMet-Leu-Phe, etc; see Fig. 6.2) and many chemokines have more than one name. Furthermore, many chemokines have other actions, causing mast cell degranulation or promoting angiogenesis, for example.

More than 40 chemokines have been identified. They are all highly homologous peptides of 8–10 kDa, which are usually grouped according to the configuration of key cysteine residues in their polypeptide chain. Chemokines with one cysteine are known as *C chemokines*. If there are two adjacent residues they are called *C–C chemokines*. Other members have cysteines separated by one (*C–X–C chemokines*) or three other residues (*C–XXX–C chemokines*).

The C–X–C chemokines (main example IL-8; see Fig. 6.2) act on neutrophils and are predominantly involved in acute inflammatory responses. The C–C chemokines (main examples eotaxin, MCP-1 and RANTES[4]) act on monocytes, eosinophils and other cells, and are involved predominantly in chronic inflammatory responses.

▼ Chemokines generally act through G protein-coupled receptors, and alteration or inappropriate expression of these is implicated in multiple sclerosis, cancer, rheumatoid arthritis and some cardiovascular diseases (Gerard & Rollins, 2001). Some types of virus (herpes virus, cytomegalovirus, pox virus and members of the retrovirus family) can exploit the chemokine system and subvert the host's defences (Murphy, 2001). Some produce proteins that mimic host chemokines or chemokine receptors, some act as antagonists at chemokine receptors and some masquerade as growth or angiogenic factors. The AIDS-causing HIV virus is responsible for the most audacious exploitation of the host chemokine system. This virus has a protein (gp120) in its envelope that recognises and binds T-cell receptors for CD4 and a chemokine co-receptor that allows it to penetrate the T cell (see Ch. 52).

INTERFERONS

So called because they interfere with viral replication, there are three main types of interferon, termed IFN-α, IFN-β and IFN-γ. 'IFN-α' is not a single substance but a family of approximately 20 proteins with similar activities. IFN-α and IFN-β have antiviral activity whereas IFN-α also has some antitumour action. Both are released from virus-infected cells and activate antiviral mechanisms in neighbouring cells. IFN-γ has a role in induction of Th1 responses (Fig. 6.3).

CLINICAL USE OF INTERFERONS

IFN-α is used in the treatment of chronic hepatitis B and C, and has some action against herpes zoster and in the prevention of the common cold. Antitumour action against some lymphomas and solid tumours has been reported. Dose-related side effects, including influenza-like symptoms, may occur. IFN-β is used in patients with the relapsing-remitting form of multiple sclerosis, whereas IFN-γ is used in chronic granulomatous disease, an uncommon chronic disease of childhood in which neutrophil function is impaired, in conjunction with antibacterial drugs (see clinical box below for more details).

THE 'CYTOKINE STORM'

Many cytokines release further cytokines in what is essentially a positive feedback loop. There are times when this feedback system becomes unstable, perhaps as a result of the absence of balancing anti-inflammatory factors. The result can be a massive overproduction of cytokines in response to infection or other injury. This is known as a *cytokine storm* (also called *hypercytokinemia*) and can lead to a particularly dangerous – potentially catastrophic – development called *systemic inflammatory response syndrome* (SIRS; Jaffer et al., 2010). Cytokine storms may be responsible for deaths in septic shock as well as in some pandemic diseases. A tragic case of volunteers suffering

[3]One might have expected evolution to generate more examples of endogenous receptor antagonists as physiological regulators, but apart from IL-1ra, they are only exploited as toxins directed against other species.

[4]MCP, monocyte chemoattractant protein; RANTES, **R**egulated on **A**ctivation **N**ormal **T** cell **E**xpressed and **S**ecreted. (Don't blame us!)

Clinical uses of interferons

- α: Chronic hepatitis B or C (ideally combined with **ribavirin**).
- Malignant disease (alone or in combination with other drugs, e.g. **cytarabine**): chronic myelogenous leukemia (CML), hairy cell leukemia, follicular lymphoma, metastatic carcinoid, multiple myeloma, malignant melanoma (as an adjunct to surgery), myelodysplastic syndrome.
- Conjugation with polyethylene glycol ('pegylation') results in preparations that are more slowly eliminated and are administered intermittently subcutaneously.
- β: Multiple sclerosis (especially the relapsing remitting form of this disease).
- γ: To reduce infection in children with chronic granulomatous disease.

Cytokines

- Cytokines are polypeptides that are rapidly induced and released during inflammation. They regulate the action of inflammatory and immune system cells.
- The cytokine superfamily includes the *interferons*, *interleukins*, *chemokines* and *colony-stimulating factors*.
- Utilising both autocrine or paracrine mechanisms, they exert complex effects on leukocytes, vascular endothelial cells, mast cells, fibroblasts, haemopoietic stem cells and osteoclasts, controlling proliferation, differentiation and/or activation.
- IL-1 and TNF-α are important primary inflammatory cytokines, inducing the formation of other cytokines.
- Chemokines, such as IL-8, are mainly involved in the regulation of cell trafficking.
- Interferons IFN-α and IFN-β have antiviral activity, and **IFN-α** is used as an adjunct in the treatment of viral infections. **IFN-γ** has significant immunoregulatory function and is used in the treatment of multiple sclerosis.

cytokine storms after receiving an experimental drug is related in Ch. 59.

PROTEINS AND PEPTIDES THAT DOWNREGULATE INFLAMMATION

Inflammation is not regulated solely by factors that cause or enhance it: it has become increasingly evident that there is another panel of mediators that function at every step to downregulate inflammation, to check its progress and limit its duration and scope. It is the dynamic balance between these two systems that regulates the onset and resolution of inflammatory episodes, and when this breaks down, may lead also to inflammatory disease or, in extreme cases, to the cytokine storm phenomenon. Some of these are peptidic in nature and we have already encountered IL-1ra, TGF-β and IL-10, which are important negative regulators of inflammation but it transpires that there are two other systems that are significant here because common anti-inflammatory drugs exploit their action.

Annexin-A1 (Anx-A1) is a 37 kDa protein produced by many cells and especially abundant in cells of the myeloid lineage. When released, it exerts potent anti-inflammatory actions, downregulating cell activation, cell transmigration and mediator release. It does this by acting through a G protein-coupled receptor called ALX/FPR2 a member of the formyl peptide receptor family: the same receptor that binds the anti-inflammatory lipoxins (see Ch. 17).

The significance of the Anx-A1 system is that it is activated by anti-inflammatory glucocorticoids (see Ch. 26), which increase Anx-A1 gene transcription and promote its release from cells. Interestingly, the anti-allergic *cromones* (cromoglicate, etc.; see Ch. 28) also promote the release of this protein from cells. Anx-A1 gene 'knockout' studies have shown that this protein is important for restraining the inflammatory response and for its timely resolution. The anti-inflammatory glucocorticoids cannot develop their full inhibitory actions without it. An account of this field is given by Perretti and D'Acquisto (2009).

The *melanocortin* system also plays an important part in regulating inflammation. There are five G protein-coupled melanocortin receptors, $MC_{1\text{-}5}$. Endogenous ligands for these receptors, such as *melanocyte stimulating hormone* (MSH; three types), are derived from the POMC gene, and serve a number of purposes, including regulating the development of a suntan, penile erection and the control of appetite through an action on various MC receptors.

From the point of view of host defence, the MC_3 receptor is the most important. Again, gene deletion studies have highlighted the importance of this receptor in a variety of inflammatory conditions. Interestingly, another product of the POMC gene, ACTH was formerly used as an anti-inflammatory agent but it was thought that its action was secondary to its ability to release endogenous cortisol from the adrenals (an MC_2 action, see Ch. 33). It is now known that it is a ligand at the MC_3 receptor and it is likely that it owes some of its activity to this action.

An account of the importance of this field is given by Patel et al. (2011).

CONCLUDING REMARKS

Even from the superficial sketch presented here and in Chapters 6 and 17, it must be evident that the host defence response is among the most intricate of all physiological responses. Perhaps that is not surprising, given its central importance to survival. For the same reason, it is also understandable that so many different mediators orchestrate its operation. That the activity of many of these mediators can be blocked in experimental models with little or no obvious effect on the initiation and outcome of inflammation points to redundancy amongst the many component systems and goes some way to explaining why, until the advent of highly specific antibody-based therapies for inflammatory conditions (see Chs 26 and 59), our ability to curb the worst ravages of chronic inflammatory disease was so limited.

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19 Cannabinoids

OVERVIEW

Modern pharmacological interest in cannabinoids dates from the discovery that Δ^9-tetrahydrocannabinol (THC) is the main active principle of cannabis, and took off with the discovery of specific cannabinoid receptors – termed CB receptors – and endogenous ligands (endocannabinoids), together with mechanisms for their synthesis and elimination. Drugs that act on this endocannabinoid system have considerable therapeutic potential. Here we consider plant-derived cannabinoids, cannabinoid receptors, endocannabinoids, physiological functions, pathological mechanisms, synthetic ligands and potential clinical applications. More detailed information is given by Kano et al. (2009). The pharmacology of cannabinoids in the central nervous system (CNS) is discussed in Chapters 38, 48 and 49.

PLANT-DERIVED CANNABINOIDS AND THEIR PHARMACOLOGICAL EFFECTS

Cannabis sativa, the hemp plant, has been used for its psychoactive properties for thousands of years (Ch. 48). Its medicinal use was advocated in antiquity, but serious interest resurfaced only in 1964 with the identification of *Δ^9-tetrahydrocannabinol* (THC, see Fig. 19.1) as the main psychoactive component. Cannabis extracts contain numerous related compounds, called cannabinoids, most of which are insoluble in water. The most abundant cannabinoids are THC, its precursor *cannabidiol*, and *cannabinol*, a breakdown product formed spontaneously from THC. Cannabidiol and cannabinol lack the psychoactive properties of THC, but can exhibit anticonvulsant activity and induce hepatic drug metabolism (see Ch. 9).

PHARMACOLOGICAL EFFECTS

THC acts mainly on the central nervous system (CNS), producing a mixture of psychotomimetic and depressant effects, together with various centrally mediated autonomic effects. The main subjective effects in humans consist of the following:

- Sensations of relaxation and well-being, similar to the effect of ethanol but without the accompanying recklessness and aggression. (Insensitivity to risk is an important feature of alcohol intoxication and is often a factor in road accidents. Cannabis users are less accident prone in general – although cannabis does contribute to a significant number of road deaths each year – even though their motor performance is similarly impaired.)
- Feelings of sharpened sensory awareness, with sounds and sights seeming more intense and fantastic.
- These effects are similar to, but usually less pronounced than, those produced by psychotomimetic drugs such as lysergic acid diethylamide (LSD; see Ch. 48). Subjects report that time passes extremely slowly. The alarming sensations and paranoid delusions that often occur with LSD are seldom experienced after cannabis. However epidemiological studies support a connection between heavy cannabis use in adolescence and subsequent psychiatric disorder (Rubino et al., 2012).

Central effects that can be directly measured in human and animal studies include:

- impairment of short-term memory and simple learning tasks – subjective feelings of confidence and heightened creativity are not reflected in actual performance
- impairment of motor coordination (e.g. driving performance)
- catalepsy – the adoption of fixed unnatural postures
- hypothermia
- analgesia
- antiemetic action (see Ch. 30)
- increased appetite (see Ch. 32).

The main peripheral effects of cannabis are:

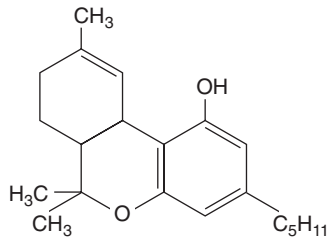
- tachycardia, which can be prevented by drugs that block sympathetic transmission
- vasodilatation, which is particularly marked in superficial blood vessels of the eye (scleral and conjunctival vessels), producing a bloodshot appearance which is characteristic of cannabis smokers
- reduction of intraocular pressure
- bronchodilatation.

PHARMACOKINETIC AND ANALYTICAL ASPECTS

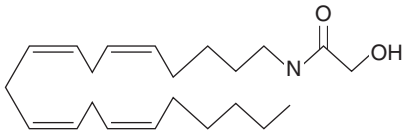
The effect of cannabis, taken by smoking, takes about 1 h to develop fully and lasts for 2–3 h. A small fraction of THC is converted to 11-hydroxy-THC, which is more active than THC itself and probably contributes to the pharmacological effect of smoking cannabis, but most is converted to inactive metabolites that are subject to conjugation and enterohepatic recirculation. Being highly lipophilic, THC and its metabolites are sequestered in body fat, and detectable excretion continues for several weeks after a single dose.

ADVERSE EFFECTS

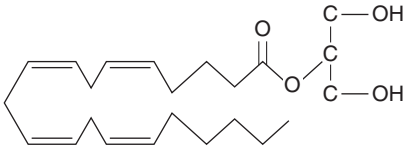
In overdose, THC is relatively safe, producing drowsiness and confusion but not life-threatening respiratory or cardiovascular depression. In this respect, it is safer than most abused substances, particularly opiates and ethanol. Even in low doses, THC and synthetic derivatives such as **nabilone** (licensed for nausea and vomiting caused by cytotoxic chemotherapy) produce euphoria and drowsiness, sometimes accompanied by sensory distortion and hallucinations. These effects, together with legal



Δ^9-Tetrahydrocannabinol (THC)



Anandamide



2 Arachidonoyl glycerol (2-AG)

Fig. 19.1 Structures of Δ^9-tetrahydrocannabinol and two endocannabinoids.

Cannabis

- Main active constituent is Δ^9-tetrahydrocannabinol (THC) + a pharmacologically active 11-hydroxy metabolite.
- Actions on the central nervous system include both depressant and psychotomimetic effects.
- Subjective experiences include euphoria and a feeling of relaxation, with sharpened sensory awareness.
- Objective tests show impairment of learning, memory and motor performance, including impaired driving ability.
- THC also shows analgesic and antiemetic activity, as well as causing catalepsy and hypothermia in animal tests.
- Peripheral actions include vasodilatation, reduction of intraocular pressure and bronchodilatation.
- Cannabinoids are less liable than opiates, **nicotine** or **alcohol** to cause dependence but may have long-term psychological effects.

restrictions on the use of cannabis, have limited the widespread therapeutic use of cannabinoids, although recent regulatory approval in several countries for a cannabis extract as an adjunct in treating spasticitiy in multiple sclerosis may herald an expansion of potential clinical indications, several of which are being investigated.

In rodents, THC produces teratogenic and mutagenic effects, and an increased incidence of chromosome breaks in circulating white cells has been reported in humans. Such breaks are, however, by no means unique to cannabis, and epidemiological studies have not shown an increased risk of fetal malformation or cancer among cannabis users.

TOLERANCE AND DEPENDENCE

Tolerance to cannabis, and physical dependence, occur only to a minor degree and mainly in heavy users. Abstinence symptoms are similar to those of ethanol or opiate withdrawal, namely nausea, agitation, irritability, confusion, tachycardia and sweating, but are relatively mild and do not result in a compulsive urge to take the drug. Psychological dependence does occur with cannabis, but it is less compelling than with the major drugs of addiction (Ch. 49), and it has been argued whether cannabis should be classified as addictive (see Fattore et al., 2008).

CANNABINOID RECEPTORS

Cannabinoids, being highly lipid-soluble, were originally thought to act in a similar way to general anaesthetics. However, in 1988, saturable high-affinity binding of a tritiated cannabinoid was demonstrated in membranes prepared from homogenised rat brain. This led to the identification of specific cannabinoid receptors in brain. These are now termed CB_1 receptors to distinguish them from the CB_2 receptors subsequently identified in peripheral tissues. Cannabinoid receptors are typical members of the family of G protein-coupled receptors (Ch. 3). CB_1 receptors are linked via $G_{i/o}$ to inhibition of adenylyl cyclase and of voltage-operated calcium channels, and to activation of G protein-sensitive inwardly rectifying potassium (GIRK) channels, causing membrane hyperpolarisation (Fig. 19.2). These effects are similar to those mediated by opioid receptors (Ch. 42). CB_1 receptors are located in the plasma membrane of nerve endings and inhibit transmitter release from presynaptic terminals, which is caused by depolarisation and Ca^{2+} entry (Ch. 4). CB receptors also influence gene expression, both directly by activating mitogen-activated protein kinase, and indirectly by reducing the activity of protein kinase A as a result of reduced adenylyl cyclase activity (see Ch. 3).

CB_1 receptors are abundant in the brain, with similar numbers to receptors for glutamate and GABA – the main central excitatory and inhibitory neurotransmitters (Ch. 38). They are not homogeneously distributed, being concentrated in the hippocampus (relevant to effects of cannabinoids on memory), cerebellum (relevant to loss of coordination), hypothalamus (important in control of appetite and body temperature; see Ch. 32 and below), substantia nigra, mesolimbic dopamine pathways that have been implicated in psychological 'reward' (Ch. 49), and in association areas of the cerebral cortex. There is a relative paucity of CB_1 receptors in the brain stem, consistent with the lack of serious depression of respiratory or cardiovascular function by cannabinoids. At a cellular level, CB_1 receptors are mainly localised presynaptically, and inhibit transmitter release as explained in Figure 19.2. Like opioids, they can, however, increase the activity of some neuronal pathways by inhibiting inhibitory connections, including GABA-ergic interneurons in the hippocampus and amygdala.

In addition to their well-recognised location in the CNS, CB_1 receptors are also expressed in peripheral tissues, for example on endothelial cells, adipocytes and peripheral nerves. Cannabinoids promote lipogenesis

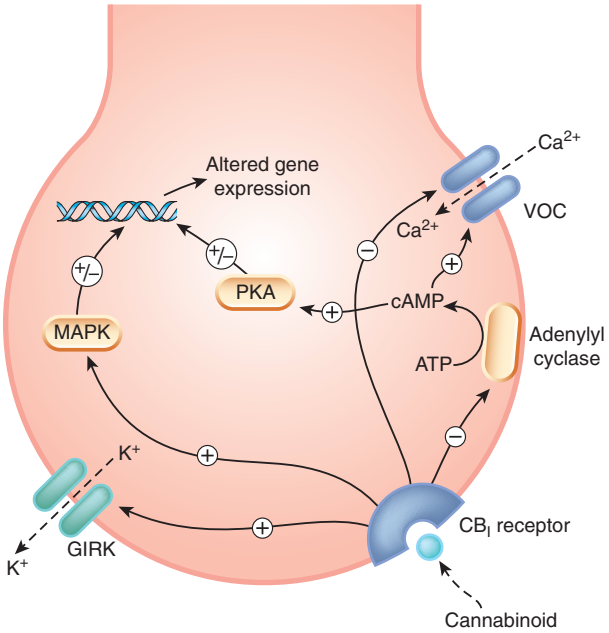


Fig. 19.2 Cellular actions of cannabinoids. CB_1 receptor activation inhibits neurotransmitter release via inhibition of Ca^{2+} entry and hyperpolarisation due to activation of potassium channels. It also alters gene expression. GIRK, G protein-sensitive inward-rectifying potassium channel; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; VOC, voltage-operated calcium channel. *(Redrawn from Devane et al., 1992.)*

through activation of CB_1 receptors, an action that could contribute to their effect on body weight (see DiPatrizio & Piomele, 2012).

The CB_2 receptor has only approximately 45% amino acid homology with CB_1 and is located mainly in lymphoid tissue (spleen, tonsils and thymus as well as circulating lymphocytes, monocytes and tissue mast cells). CB_2 receptors are also present on microglia – immune cells in the CNS which when activated contribute to chronic pain (Ch. 37). The localisation of CB_2 receptors on cells of the immune system was unexpected, but may account for inhibitory effects of cannabis on immune function. CB_2 receptors differ from CB_1 receptors in their responsiveness to cannabinoid ligands (see Table 19.1). They are linked via $G_{i/o}$ to adenylyl cyclase, GIRK channels and mitogen-activated protein kinase similarly to CB_1, but not to voltage-operated calcium channels (which are not expressed in immune cells). So far, rather little is known about their function. They are present in atherosclerotic lesions (see Ch. 22), and CB_2 agonists have antiatherosclerotic effects (Mach & Steffens, 2008).

Some endocannabinoids turned out, surprisingly,[1] to activate vanilloid receptors, ionotropic receptors that stimulate nociceptive nerve endings (see Ch. 42). Other as-yet-unidentified G protein-coupled receptors are also implicated, because cannabinoids exhibit analgesic actions and activate G proteins in the brain of CB_1 knockout mice despite the absence of CB_1 receptors.

[1]Surprising because capsaicin, the active principle of chilli peppers, causes intense burning pain, whereas the endocannabinoid anandamide is associated with pleasure, or even bliss … so perhaps not so surprising after all!

Table 19.1 Definite and possible endocannabinoids

Endocannabinoid	Selectivity
Definite endocannabinoids	
Anandamide	$CB_1 > CB_2$
2-Arachidonoyl glycerol	$CB_1 = CB_2$
Less well established endocannabinoid candidates	
Virodhamine	$CB_2 > CB_1$
Noladin	$CB_1 \gg CB_2$
N-Arachidonoyl dopamine	$CB_1 \gg CB_2$

ENDOCANNABINOIDS

The discovery of specific cannabinoid receptors led to a search for endogenous mediators. The first success was chalked up by a team that screened fractions of extracted pig brain for ability to compete with a radiolabelled cannabinoid receptor ligand (Devane et al., 1992). This led to the purification of N-*arachidonylethanolamide*, an eicosanoid mediator (see Ch. 18), the structure of which is shown in Figure 19.1. This was christened *anandamide*.[2] Anandamide not only displaced labelled cannabinoid from synaptosomal membranes in the binding assay, but also inhibited electrically evoked twitches of mouse vas deferens, a bioassay for psychotropic cannabinoids (Fig. 19.3). A few years later, a second endocannabinoid, *2-arachidonoyl glycerol* (2-AG, Fig. 19.1), was identified, and more recently three further endocannabinoid candidates with distinct CB_1/CB_2 (see Fig. 19.1) receptor selectivities have been added to the list (Table 19.1). Endocannabinoids are made 'on demand' like eicosanoids (see Ch. 18), rather than being presynthesised and stored for release when needed.

BIOSYNTHESIS OF ENDOCANNABINOIDS

Biosynthesis of anandamide and of 2-AG is summarised in Figure 19.4. A fuller account of biosynthesis and degradation is given by Di Marzo (2008).

▼ Anandamide is formed by a distinct phospholipase D (PLD) selective for *N*-acyl-phosphatidylethanolamine (NAPE) but with low affinity for other membrane phospholipids, and known as NAPE-PLD. NAPE-PLD is a zinc metallohydrolase that is stimulated by Ca^{2+} and also by polyamines. Selective inhibitors for NAPE-PLD are being sought. The precursors are produced by an as-yet-uncharacterised but Ca^{2+}-sensitive transacylase that transfers an acyl group from the *sn*-1 position of phospholipids to the nitrogen atom of phosphatidylethanolamine.

2-AG is also produced by hydrolysis of precursors derived from phospholipid metabolism. The key enzymes are two *sn*-1-selective diacylglycerol lipases (DAGL-α and DAGL-β), which belong to the family of serine lipases. Both these enzymes, like NAPE-PLD, are Ca^{2+} sensitive, consistent with intracellular Ca^{2+} acting as the physiological stimulus to endocannabinoid synthesis. The DAGLs are located in axons and presynaptic axon terminals during development, but postsynaptically in dendrites and cell bodies of adult neurons, consistent with a role for 2-AG in neurite growth, and with a role as a retrograde mediator (see p. 235) in adult brain.

[2]From a Sanskrit word meaning 'bliss' + amide.

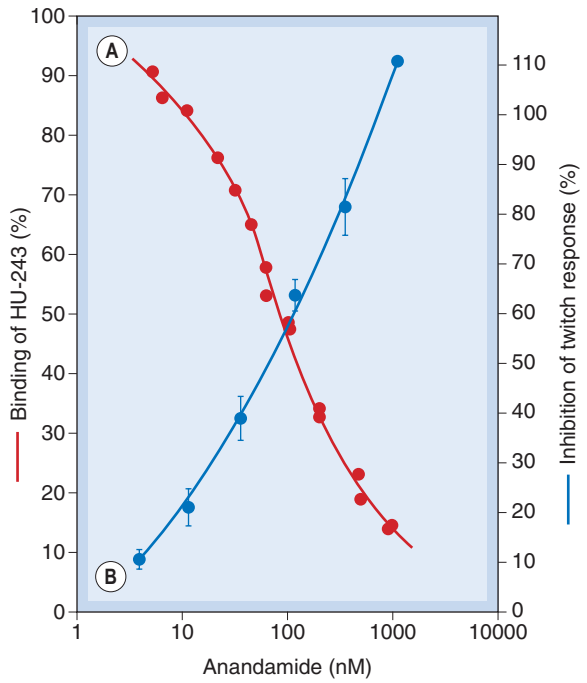


Fig. 19.3 Anandamide as an endocannabinoid. Anandamide is an endogenous cannabinoid. [**A**] Competitive inhibition of tritiated HU-243 (a cannabinoid receptor ligand) binding to synaptosomal membranes from rat brain by natural anandamide (red circles, left hand ordinate axis). [**B**] Inhibition of vas deferens twitch response (a bioassay for cannabinoids) by natural anandamide (blue symbols, right hand ordinate). Note the similarity between the binding and bioactivity. *(Redrawn from Devane et al., 1992.)*

Little is known as yet about the biosynthesis of the more recent endocannabinoid candidates noladin, virodhamine and *N*-arachidonoyl dopamine. pH-dependent non-enzymatic interconversion of virodhamine and anandamide is one possibility, and could result in a switch between CB_2- and CB_1-mediated responses (see Table 19.1).

TERMINATION OF THE ENDOCANNABINOID SIGNAL

Endocannabinoids are rapidly taken up from the extracellular space. Being lipid-soluble, they diffuse through plasma membranes down a concentration gradient. There is also evidence for a saturable, temperature-dependent, facilitated transport mechanism for anandamide and 2-AG, dubbed the 'endocannabinoid membrane transporter', for which selective uptake inhibitors (e.g. UCM-707) have been developed. Pathways of endocannabinoid metabolism are summarised in Figure 19.4. The key enzyme for anandamide is a microsomal serine hydrolase known as fatty acid amide hydrolase (FAAH). FAAH converts anandamide to arachidonic acid plus ethanolamine and also hydrolyses 2-AG, yielding arachidonic acid and glycerol.

The phenotype of FAAH 'knockout' mice gives some clues to endocannabinoid physiology; such mice have an increased brain content of anandamide and an increased pain threshold. Selective inhibitors of FAAH have analgesic and anxiolytic properties in mice (see Ch. 44 for an explanation of how drugs are tested for anxiolytic properties in rodents). In contrast to anandamide, brain content of 2-AG is not increased in FAAH knockout animals, indicating that another route of metabolism of 2-AG is likely to be important. Other possible routes of metabolism include esterification, acylation and oxidation by cyclooxygenase-2 to prostaglandin ethanolamides ('prostamides'), or by 12- or 15-lipoxygenase (see Ch. 18).

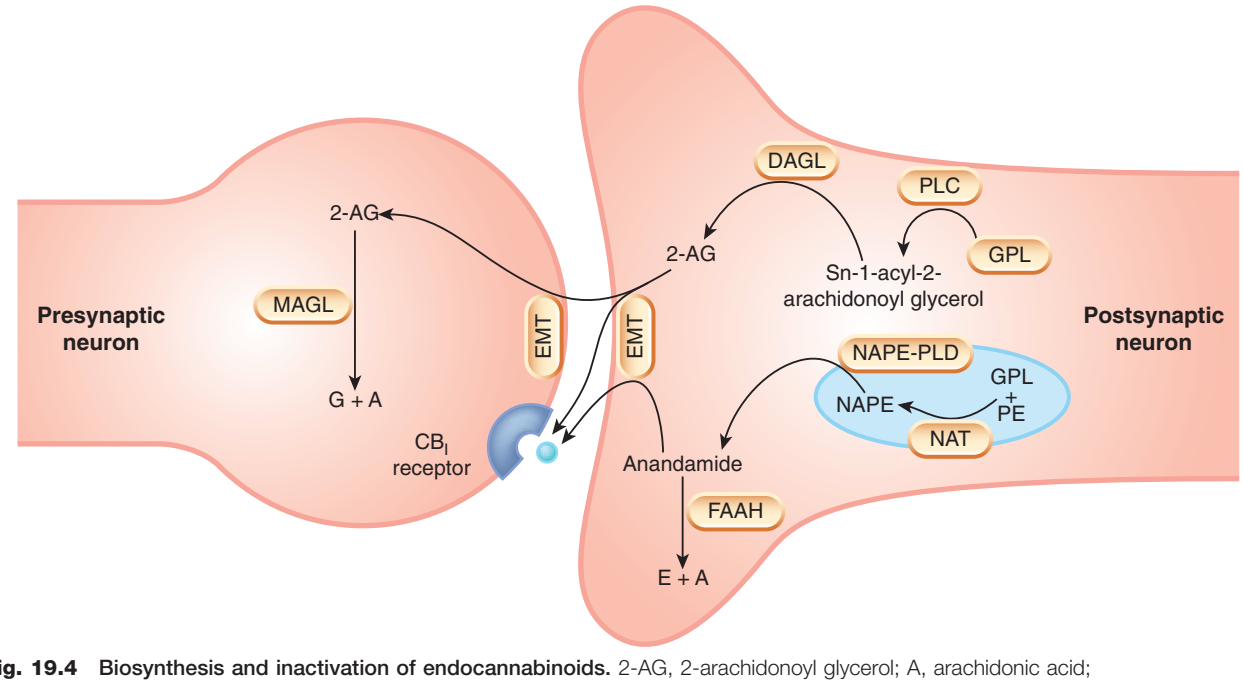


Fig. 19.4 Biosynthesis and inactivation of endocannabinoids. 2-AG, 2-arachidonoyl glycerol; A, arachidonic acid; DAGL, diacylglycerol lipase; E, ethanolamine; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; GPL, glycerophospholipid; MAGL, monoacyl glycerol lipase; NAPE, *N*-acyl-phosphatidylethanolamine; NAPE-PLD, *N*-acyl phosphatidylethanolamine-specific phopholipase D; NAT, *N*-acyl-transferase; PE, phosphatidylethanolamine; PLC, phospholipase C.

PHYSIOLOGICAL MECHANISMS

Stimuli that release endocannabinoids, leading to activation of CB_1 receptors and the linkage to downstream events including behavioural or psychological effects, are incompletely defined. Increased intracellular Ca^{2+} concentration is probably an important cellular trigger because, as mentioned on p. 233, Ca^{2+} activates NAPE-PLD and other enzymes involved in endocannabinoid biosynthesis.

Activation of CB receptors is implicated in a phenomenon known as *depolarisation-induced suppression of inhibition* (DSI). DSI occurs in hippocampal pyramidal cells; when these are depolarised by an excitatory input, this suppresses the GABA-mediated inhibitory input to the pyramidal cells, implying a retrograde flow of information from the depolarised pyramidal cell to inhibitory axons terminating on it. Such a reverse flow of information from post- to presynaptic cell is a feature of other instances of neuronal plasticity, such as 'wind-up' in nociceptive pathways (Fig. 42.3) and long-term potentiation in the hippocampus (Fig. 38.7). DSI is blocked by the CB_1 antagonist rimonabant. The presynaptic location of CB_1 receptors and cellular distributions of the DAGL and MAGL enzymes (Fig. 19.4) fit nicely with the idea that the endocannabinoid 2-AG could be a 'retrograde' messenger in DSI (see Fig. 39.8).

Neuromodulatory actions of endocannabinoids could influence a wide range of physiological activities, including nociception, cardiovascular, respiratory and gastrointestinal function. Interactions of endocannabinoids with hypothalamic hormones are believed to influence food intake and reproductive function. Mouse models lacking CB receptors support important and balanced roles of endocannabinoid signalling in male and female fertility and are implicated in spermatogenesis, fertilisation, preimplantation development of the early embryo, implantation and postimplantation growth of the embryo (see Battista et al., 2012). Effects of endocannabinoids on food intake are of particular interest, because of the importance of obesity (Ch. 32).

PATHOLOGICAL INVOLVEMENT

There is evidence, both from experimental animals and from human tissue, that endocannabinoid signalling is abnormal in various neurodegenerative diseases (see Ch. 40). Other diseases where abnormalities of cannabinoid signalling have been reported in human tissue as well as experimental models include hypotensive shock (both haemorrhagic and septic; see Ch. 22), advanced cirrhosis of the liver (where there is evidence that vasodilatation is mediated by endocannabinoids acting on vascular CB_1 receptors – see Bátkai et al., 2001), miscarriage (see Battista et al., 2012) and malignant disease. It seems likely that in some disorders endocannabinoid activity is a compensatory mechanism limiting the progression of disease or occurrence of symptoms, whereas in others it may be 'too much of a good thing' and actually contribute to disease progression. Consequently, there may be a place in therapeutics for drugs that potentiate or inhibit the cannabinoid system (see Di Marzo & Petrosino, 2007, for a fuller discussion).

The endocannabinoid system

- Cannabinoid receptors (CB_1, CB_2) are G protein-coupled ($G_{i/o}$).
- Activation of CB_1 inhibits adenylyl cyclase and calcium channels, and activates potassium channels, inhibiting synaptic transmission.
- The peripheral receptor (CB_2) is expressed mainly in cells of the immune system.
- Selective agonists and antagonists have been developed.
- Endogenous ligands for CB receptors are known as endocannabinoids. They are eicosanoid mediators (see Ch. 18).
- The best-established endocannabinoids are anandamide and 2-arachidonoyl glycerol (2-AG), which have many roles, including functioning as 'retrograde' mediators passing information from postsynaptic to presynaptic neurons.
- The main enzyme that inactivates anandamide is fatty acid amide hydrolase (FAAH).
- A putative 'endocannabinoid membrane transporter' may transport cannabinoids from postsynaptic neurons, where they are synthesised, to the synaptic cleft, where they access CB_1 receptors, and into presynaptic terminals, where 2-AG is metabolised.
- FAAH 'knockout' mice have an increased brain content of anandamide and an increased pain threshold; selective inhibitors of FAAH have analgesic and anxiolytic properties, implicating endocannabinoids in nociception and anxiety.

SYNTHETIC CANNABINOIDS

Cannabinoid receptor agonists were developed in the 1970s in the hope that they would prove useful non-opioid/non-NSAID analgesics (cf. Chs 42 and 26, respectively, for limitations of opioids and NSAIDs), but adverse effects, particularly sedation and memory impairment, were problematic. Nevertheless, one such drug, **nabilone**, is sometimes used clinically for nausea and vomiting caused by cytotoxic chemotherapy if this is unresponsive to conventional antiemetics (Ch. 30). Furthermore synthetic cannabinoid agonists (e.g. spice) have been used as legal 'highs'. There were more than 20 of these introduced in the UK in 2012–13 in an attempt to circumvent the law on cannabis possession. The cloning of CB_2 receptors, and their absence from healthy neuronal brain cells, led to the synthesis of CB_2-selective agonists in the hope that these would lack the CNS-related adverse effects of plant cannabinoids. Several such drugs are being investigated for possible use in inflammatory and neuropathic pain.

The first selective CB_1 receptor antagonist, **rimonabant**, also has inverse agonist properties in some systems. It was licensed in Europe for treating obesity, and there were hopes that it would help promote abstinence from tobacco, but it was withdrawn because it caused psychiatric problems including depression. Synthetic inhibitors of endocannabinoid uptake and/or metabolism have shown potentially useful effects in animal models of pain, epilepsy, multiple sclerosis, Parkinson's disease, anxiety and diarrhoea.

CLINICAL APPLICATIONS

Clinical uses of drugs that act on the cannabinoid system remain controversial, but in both the UK and the USA cannabinoids have been used as antiemetics and to

encourage weight gain in patients with chronic disease such as HIV-AIDS and malignancy. Cannabis extract (**sativex**) is used to treat spasticity in patients with multiple sclerosis (see Borgelt et al., 2013). Adverse events were generally mild at the doses used – see UK MS Research Group (2003). Endocannabinoids have been implicated in shock and hypotension in liver disease (Malinowska et al., 2008), and modulation of this system is a potential therapeutic target. Other potential clinical uses are given in the clinical box below.

In addition to central CB_1 receptors, hepatocyte CB_1 receptors also implicated in obesity and in non-alcoholic fatty liver disease and research on selective peripheral antagonists continues (Klumpers et al., 2013).

Potential and actual clinical uses of cannabinoid agonists and antagonists

Cannabinoid agonists and antagonists are undergoing evaluation for a wide range of possible indications, including the following.

- Agonists:
  - glaucoma (to reduce pressure in the eye)
  - nausea/vomiting associated with cancer chemotherapy
  - cancer and AIDS (to reduce weight loss)
  - neuropathic pain
  - head injury
  - Tourette's syndrome (to reduce tics – rapid involuntary movements that are a feature of this disorder)
  - Parkinson's disease (to reduce involuntary movements caused as an adverse effect of **levodopa**; see Ch. 40).
- Antagonists:
  - obesity
  - tobacco dependence
  - drug addiction
  - alcoholism.

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Nitric oxide and related mediators

20

OVERVIEW

Nitric oxide (NO) is a ubiquitous mediator with diverse functions. It is generated from L-arginine by nitric oxide synthase (NOS), an enzyme that occurs in endothelial, neuronal and inducible isoforms. In this chapter, we concentrate on general aspects of NO, especially its biosynthesis, degradation and effects. We touch on evidence that it can act as a circulating as well as a local mediator, and conclude with a brief consideration of the therapeutic potential of drugs that act on the L-arginine/NO pathway. Other gaseous mediators (carbon monoxide, hydrogen sulfide)[1] are described briefly: while they have yet to yield therapeutic drugs, their pathways are tempting drug targets.

INTRODUCTION

Nitric oxide, a free radical gas, is formed in the atmosphere during lightning storms. Less dramatically, but with far-reaching biological consequences, it is also formed in an enzyme-catalysed reaction between molecular oxygen and L-arginine. The convergence of several lines of research led to the realisation that NO is a key signalling molecule in the cardiovascular and nervous systems, and that it has a role in host defence.

A physiological function of NO emerged when biosynthesis of this gas was shown to account for the *endothelium-derived relaxing factor* described by Furchgott & Zawadzki (1980) (see Figs 20.1 and 20.2). NO is the endogenous activator of soluble guanylyl cyclase, leading to the formation of cyclic GMP (cGMP), an important 'second messenger' (Ch. 3) in many cells, including neurons, smooth muscle, monocytes and platelets. Nitrogen and oxygen are neighbours in the periodic table, and NO shares several properties with O_2, in particular a high affinity for haem and other iron–sulfur groups. This is important for activation of guanylyl cyclase, which contains a haem group, for the inactivation of NO by haemoglobin and for the regulation of diffusion of NO from endothelial cells (which express the alpha chain of haemoglobin) to vascular smooth muscle.

The role of NO in specific settings is described in other chapters: the endothelium in Chapter 22, the autonomic nervous system in Chapter 12, as a chemical transmitter and mediator of excitotoxicity in the central nervous system (CNS) in Chapters 37–39, and in the innate mediator-derived reactions of acute inflammation and the immune response in Chapter 17. Therapeutic uses of organic nitrates and of nitroprusside (NO donors) are described in Chapters 21 and 22.

BIOSYNTHESIS OF NITRIC OXIDE AND ITS CONTROL

Nitric oxide synthase (NOS) enzymes are central to the control of NO biosynthesis. There are three isoforms: an *inducible* form (iNOS or NOS2 expressed in macrophages and Kupffer cells, neutrophils, fibroblasts, vascular smooth muscle and endothelial cells in response to pathological stimuli such as invading microorganisms) and two *constitutive* forms, which are present under physiological conditions in endothelium (eNOS or NOS3)[2] and in neurons (nNOS or NOS1).[3] The constitutive enzymes generate small amounts of NO, whereas NOS2 produces much greater amounts, both because of its high activity and because of its abundance, at least in pathological states associated with cytokine release.

▼ All three NOS isoenzymes are dimers. They are structurally and functionally complex, bearing similarities to the cytochrome P450 enzymes (described in Ch. 9) that are so important in drug metabolism. Each isoform contains iron protoporphyrin IX (haem), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (H_4B) as bound prosthetic groups. They also bind L-arginine, reduced nicotinamide adenine dinucleotide phosphate (NADPH) and calcium–calmodulin. These prosthetic groups and ligands control the assembly of the enzyme into the active dimer. NOS3 is doubly acylated by *N*-myristoylation and cysteine palmitoylation; these post-translational modifications lead to its association with membranes in the Golgi apparatus and in *caveolae*, specialised cholesterol-rich microdomains in the plasma membrane derived from the Golgi apparatus. In the caveolae, NOS3 is held as an inactive complex with *caveolin*, the main membrane protein of caveolae. Dissociation from caveolin activates the enzyme.

The nitrogen atom in NO is derived from the terminal guanidino group of L-arginine. NOS enzymes combine oxygenase and reductase activities. The oxygenase domain contains haem, while the reductase domain binds calcium–calmodulin. In pathological states, the enzyme can undergo structural change leading to electron transfer between substrates, enzyme co-factors and products becoming 'uncoupled', so that electrons are transferred to molecular oxygen, leading to the synthesis of superoxide anion (O_2^-) rather than NO. This is important, as superoxide anion reacts with NO to form a toxic product (peroxynitrite anion; see p. 241).

L-Arginine, the substrate of NOS, is usually present in excess in endothelial cell cytoplasm, so the rate of production of NO is determined by the activity of the enzyme

[1]The pure substances (NO, CO and H_2S) are gases at room temperature and usual atmospheric pressure, and when pure NO is administered therapeutically (see p. 242 and clinical box, p. 244) it is in the form of a gas; when formed endogenously, the gases are of course dissolved in intra- and extracellular fluids.

[2]NOS3 is not restricted to endothelium. It is also present in cardiac myocytes, renal mesangial cells, osteoblasts and osteoclasts, airway epithelium and, in small amounts, platelets so the term eNOS may be somewhat misleading.

[3]It is possible that some of the NO made in healthy animals under basal conditions is derived from the action of NOS2, just as the inducible form of cyclo-oxygenase is active under basal conditions (Ch. 18) – whether this is because there is some NOS2 expressed even when there is no pathology, or because there is enough 'pathology' in healthy mammals, for example gut microflora, to induce it, is a moot point.

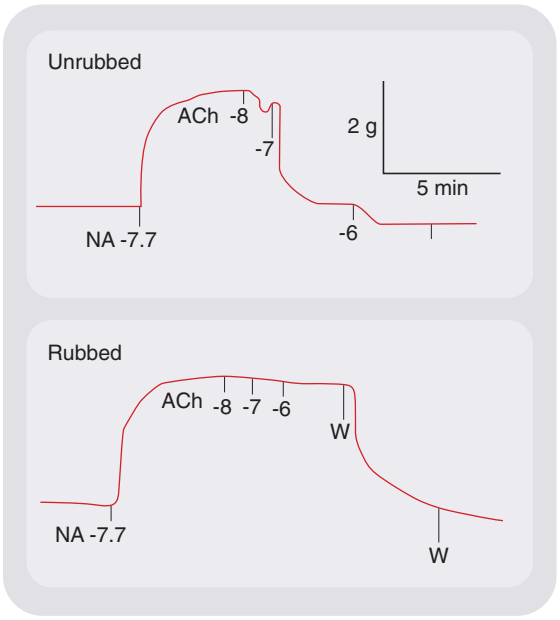

Fig. 20.1 Endothelium-derived relaxing factor. Acetylcholine (ACh) relaxes a strip of rabbit aorta precontracted with noradrenaline (NA) if the endothelium is intact ('unrubbed': upper panel), but not if it has been removed by gentle rubbing ('rubbed': lower panel). The numbers are logarithms of molar concentrations of drugs. *(From Furchgott & Zawadzki, 1980.)*

rather than by substrate availability. Nevertheless, very high doses of L-arginine can restore endothelial NO biosynthesis in some pathological states (e.g. hypercholesterolaemia) in which endothelial function is impaired. Possible explanations for this paradox include:

- compartmentation: i.e. existence of a distinct pool of substrate in a cell compartment with access to NOS, which can become depleted despite apparently plentiful total cytoplasmic arginine concentrations
- competition with endogenous inhibitors of NOS such as *asymmetric dimethylarginine* (ADMA; see p. 242 and Fig. 20.4), which is elevated in plasma from patients with hypercholesterolaemia
- reassembly/reactivation of enzyme in which transfer of electrons has become uncoupled from L-arginine as a result of an action of supraphysiological concentrations of L-arginine.

The activity of constitutive isoforms of NOS is controlled by intracellular calcium–calmodulin (Fig. 20.3). Control is exerted in two ways:

1. Many endothelium-dependent agonists (e.g. acetylcholine, bradykinin, substance P) increase the cytoplasmic concentration of calcium ions $[Ca^{2+}]_i$; the consequent increase in calcium–calmodulin activates NOS1 and NOS3.
2. Phosphorylation of specific residues on NOS3 controls its sensitivity to calcium–calmodulin; this can alter NO synthesis in the absence of any change in $[Ca^{2+}]_i$.

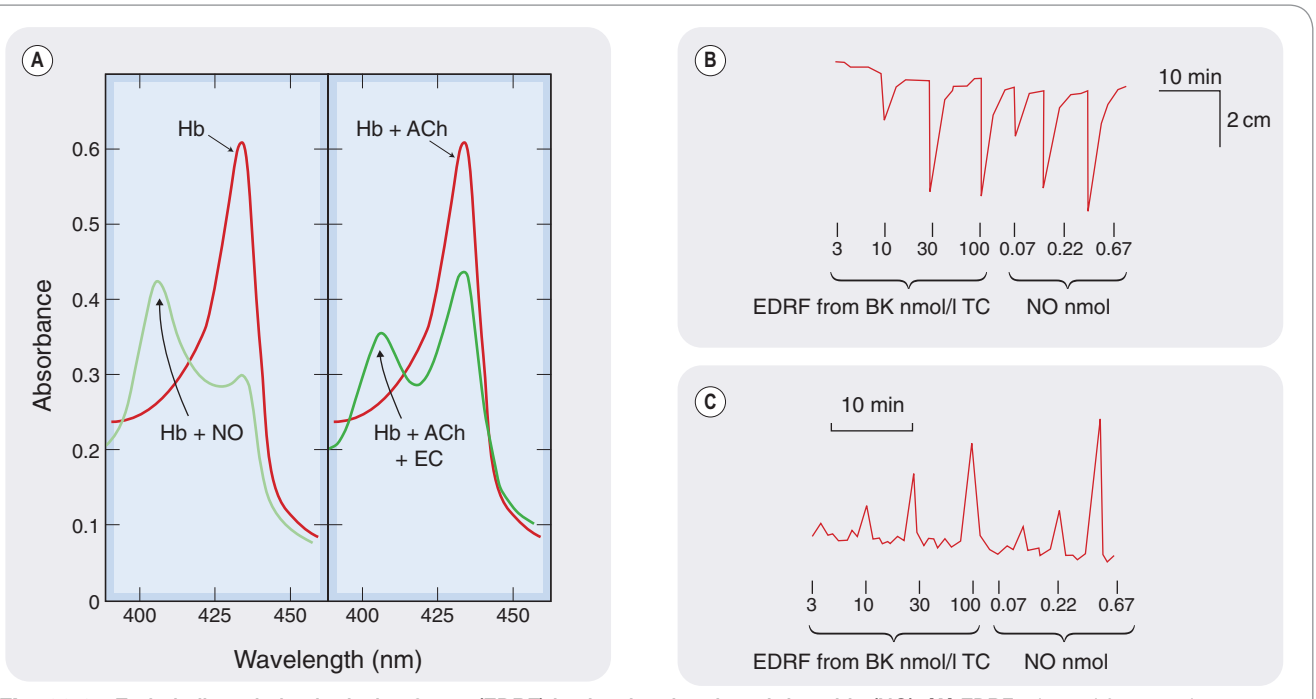

Fig. 20.2 Endothelium-derived relaxing factor (EDRF) is closely related to nitric oxide (NO). [**A**] EDRF released from aortic endothelial cells (EC) by acetylcholine (ACh) (right panel) has the same effect on the absorption spectrum of deoxyhaemoglobin (Hb) as does authentic NO (left panel). [**B**] EDRF is released from a column of cultured endothelial cells by bradykinin (BK 3–100 nmol) applied through the column of cells (TC) and relaxes a de-endothelialised precontracted bioassay strip, as does authentic NO (upper trace). [**C**] A chemical assay of NO based on chemiluminescence shows that similar concentrations of NO are present in the EDRF released from the column of cells as in equiactive authentic NO solutions. *(From Ignarro LJ, Byrns RE, Buga GM, et al 1987 Circ Res 61, 866–879; and Palmer RMJ, Ferrige AG, Moncada S et al 1987 Nature 327, 524–526.)*

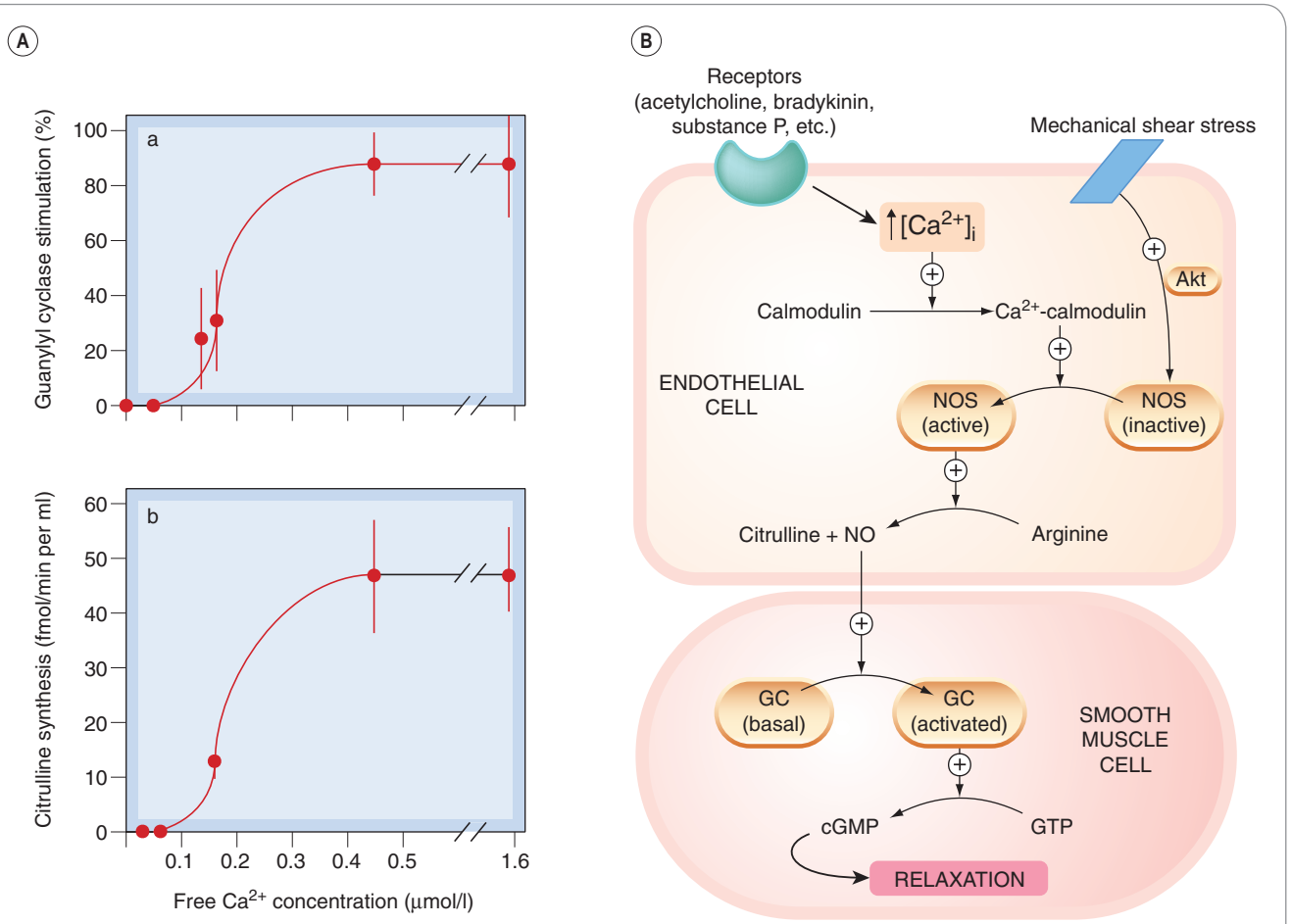

Fig. 20.3 Control of constitutive nitric oxide synthase (NOS) by calcium–calmodulin. [**A**] Dependence on Ca^{2+} of nitric oxide (NO) and citrulline synthesis from L-arginine by rat brain synaptosomal cytosol. Rates of synthesis of NO from L-arginine were determined by stimulation of guanylyl cyclase (GC) (upper panel) or by synthesis of [^{3}H]-citrulline from L-[^{3}H]-arginine (lower panel). [**B**] Regulation of GC in smooth muscle by NO formed in adjacent endothelium. Akt is a protein kinase that phosphorylates NOS, making it more sensitive to calcium–calmodulin. *(Panel [A] from Knowles RG et al. 1989 Proc Natl Acad Sci U S A 86, 5159–5162.)*

Shear stress is an important physiological stimulus to endothelial NO synthesis in resistance vessels. This is sensed by endothelial mechanoreceptors and transduced via a serine–threonine protein kinase called Akt (also known as protein kinase B). Agonists that increase cAMP in endothelial cells (e.g. β_2-adrenoceptor agonists) increase NOS3 activity, but via protein kinase A-mediated phosphorylation[4] whereas protein kinase C *reduces* NOS3 activity by phosphorylating residues in the calmodulin-binding domain, thereby reducing the binding of calmodulin. Insulin increases NOS3 activity via tyrosine kinase activation (and also increases the expression of NOS1 in diabetic mice).

In contrast to constitutive NOS isoforms, the activity of NOS2 is effectively independent of $[Ca^{2+}]_i$, being fully activated even at the low values of $[Ca^{2+}]_i$ present under resting conditions. The enzyme is induced by bacterial lipopolysaccharide and/or inflammatory cytokines, notably interferon-γ, the antiviral effect of which can be explained by this action. Tumour necrosis factor-α and interleukin-1 do not alone induce NOS2, but they each synergise with interferon-γ in this regard (see Ch. 17). Induction of NOS2 is inhibited by glucocorticoids and by several cytokines, including transforming growth factor-β. There are important species differences in the inducibility of NOS2, which is less readily induced in human than in mouse cells.

[4]As explained in Chapter 4, β_2 agonists also act directly on smooth muscle cells, causing relaxation via cAMP.

DEGRADATION AND CARRIAGE OF NITRIC OXIDE

Nitric oxide reacts with oxygen to form N_2O_4, which combines with water to produce a mixture of nitric and nitrous acids. Nitrite ions are oxidised to nitrate by oxyhaemoglobin. These reactions are summarised as follows:

$$2NO + O_2 \rightarrow N_2O_4 \quad (20.1)$$

$$N_2O_4 + H_2O \rightarrow NO_3^- + NO_2^- + 2H^+ \quad (20.2)$$

$$NO_2^- + HbO \rightarrow NO_3^- + Hb \quad (20.3)$$

Low concentrations of NO are relatively stable in air, because the rate of reaction in (equation 20.1) depends on

Nitric oxide: synthesis, inactivation and carriage

- Nitric oxide (NO) is synthesised from L-arginine and molecular O_2 by nitric oxide synthase (NOS).
- NOS exists in three isoforms: inducible (NOS2), and constitutive 'endothelial' (NOS3, which is not restricted to endothelial cells) and neuronal (NOS1) forms. NOSs are dimeric flavoproteins, contain tetrahydrobiopterin and have homology with cytochrome P450. The constitutive enzymes are activated by calcium–calmodulin. Sensitivity to calcium–calmodulin is controlled by phosphorylation of specific residues on the enzymes.
- NOS2 is induced in macrophages and other cells by interferon-γ.
- NOS1 is present in the central nervous system (see Chs 37–40) and in autonomic nerves (see Ch. 12).
- NOS3 is present in platelets and other cells in addition to endothelium.
- NO diffuses to sites of action in neighbouring cells. This is regulated by the redox state of haemoglobin alpha which is present in the myoendothelial junctions that act as diffusion corridors across the internal elastic lamina (and in other cells): signalling can occur when the haem is in the Fe^{3+} state, but is stopped – like at a red traffic light – when haem is in the Fe^{2+} state.
- NO is inactivated by combination with the haem of haemoglobin or by oxidation to nitrite and nitrate, which are excreted in urine; it is also present in exhaled air, especially in patients with inflammatory lung diseases such as bronchitis.
- NO can react reversibly with cysteine residues (e.g. in globin or albumin) to form stable nitrosothiols; as a result, red cells can act as an O_2-regulated source of NO. NO released in this way escapes inactivation by haem by being exported via cysteine residues in the anion exchange protein in red cell membranes.

the square of the NO concentration, so small amounts of NO produced in the lung escape degradation and can be detected in exhaled air. Exhaled NO is increased in patients with lung diseases such as bronchitis, and is used as a biomarker of airway inflammation (Ch. 28). In contrast, NO reacts very rapidly with even low concentrations of superoxide anion (O_2^-) to produce peroxynitrite anion ($ONOO^-$), which is responsible for some of its toxic effects.

▼ Haem has an affinity for NO > 10000 times greater than for oxygen. In the absence of oxygen, NO bound to haem is relatively stable, but in the presence of oxygen NO is converted to nitrate and the haem iron (Fe^{2+}) oxidised to methaemoglobin (Fe^{3+}).

Endothelium-derived NO acts locally on underlying vascular smooth muscle or on adherent monocytes or platelets. The internal elastic lamina of small arteries is a layer of elastic fibres between the endothelium and the smooth muscle, which represents a barrier to diffusion. It is penetrated by myoendothelial junctions where endothelial and smooth muscle cells kiss, forming a corridor along which NO can diffuse. Recently it has been found that haemoglobin alpha is concentrated in these junctions and acts as a redox-sensitive stop/go signal. When the haem iron is in the oxidised Fe^{3+} state (methaemoglobin) NO can diffuse along the corridor and into the smooth muscle cell on which it acts; when the haem iron is in the Fe^{2+} state, however, NO is rapidly converted to nitrate and the diffusion pathway is effectively closed to it. Conversion of methaemoglobin to haemoglobin, preventing NO from crossing the barrier, is brought about by the enzyme cytochrome b5 reductase3 (also known as methaemoglobin reductase) – genetic or pharmacological inhibition of this enzyme increases NO bioactivity in small arteries (Straub et al., 2012).

Distinct from the inactivation reaction between NO and haem, a specific cysteine residue in globin combines reversibly with NO under physiological conditions. It is proposed that the resulting *S*-nitrosylated haemoglobin acts as a circulating oxygen-sensitive NO carrier, allowing NO to act as a circulating hormone[3]. Albumin can also be reversibly nitrosylated and could function similarly, as could the inorganic nitrite ion – indeed, foods rich in inorganic nitrate (reduced to nitrite *in vivo* by anaerobic organisms in the mouth) have potential for prevention of vascular disease; see p. 242-243. Evidence supporting the case that NO acts at a distance within the mammalian circulation is reviewed by Singel & Stamler (2005); for a sceptical view, see Schechter & Gladwyn (2003).

EFFECTS OF NITRIC OXIDE

Nitric oxide reacts with various metals, thiols and oxygen species, thereby modifying proteins, DNA and lipids. One of its most important biochemical effects (see Ch. 3) is activation of soluble guanylyl cyclase, a heterodimer present in vascular and nervous tissue as two distinct isoenzymes. Guanylyl cyclase synthesises the second messenger cGMP. NO activates the enzyme by combining with its haem group, and many physiological effects of low concentrations of NO are mediated by cGMP. These effects are prevented by inhibitors of guanylyl cyclase (e.g. 1H-[1,2,4]-oxadiazole-[4,3-α]-quinoxalin-1-one, better known as 'ODQ'), which are useful investigational tools. NO activates soluble guanylyl cyclase in intact cells (neurons and platelets) extremely rapidly, and activation is followed by desensitisation to a steady-state level. This contrasts with its effect on the isolated enzyme, which is slower but more sustained. Guanylyl cyclase contains another regulatory site, which is NO independent. This is activated by **riociguat**, recently licensed for treatment of pulmonary hypertension (see Ch. 22).

Effects of cGMP are terminated by phosphodiesterase enzymes. **Sildenafil** and **tadalafil** are inhibitors of phosphodiesterase type V that are used to treat erectile dysfunction, because they potentiate NO actions in the corpora cavernosa of the penis by this mechanism (see Ch. 35). NO also combines with haem groups in other biologically important proteins, notably cytochrome c oxidase, where it competes with oxygen, contributing to the control of cellular respiration (see Erusalimsky & Moncada, 2007). Cytotoxic and/or cytoprotective effects of higher concentrations of NO relate to its chemistry as a free radical (see Ch. 40). Some physiological and pathological effects of NO are shown in Table 20.1.

BIOCHEMICAL AND CELLULAR ASPECTS

Pharmacological effects of NO can be studied with NO gas dissolved in deoxygenated salt solution. More conveniently, but less directly, various donors of NO, such

Table 20.1 Postulated roles of endogenous nitric oxide

System	Physiological role	Pathological role	
		Excess production	*Inadequate production or action*
Cardiovascular			
Endothelium/vascular smooth muscle	Control of blood pressure and regional blood flow	Hypotension (septic shock)	Atherogenesis, thrombosis (e.g. in hypercholesterolaemia, diabetes mellitus)
Platelets	Limitation of adhesion/aggregation	–	–
Host defence			
Macrophages, neutrophils, leukocytes	Defence against viruses, bacteria, fungi, protozoa, parasites	–	–
Nervous system			
Central	Neurotransmission, long-term potentiation, plasticity (memory, appetite, nociception)	Excitotoxicity (Ch. 39) (e.g. ischaemic stroke, Huntington's disease, AIDS dementia)	–
Peripheral	Neurotransmission (e.g. gastric emptying, penile erection)	–	Hypertrophic pyloric stenosis, erectile dysfunction

as **nitroprusside**, S-*nitrosoacetylpenicillamine* (SNAP) or S-*nitrosoglutathione* (SNOG), have been used as surrogates. This has pitfalls; for example, ascorbic acid potentiates SNAP but inhibits responses to authentic NO.[5]

Nitric oxide can activate guanylyl cyclase in the same cells that produce it, giving rise to autocrine effects, for example on the barrier function of the endothelium. NO also diffuses from its site of synthesis and activates guanylyl cyclase in neighbouring cells. The resulting increase in cGMP affects protein kinase G, cyclic nucleotide phosphodiesterases, ion channels and possibly other proteins. This inhibits the $[Ca^{2+}]_i$-induced smooth muscle contraction and platelet aggregation produced by various agonists. NO also hyperpolarises vascular smooth muscle, as a consequence of potassium-channel activation. NO inhibits monocyte adhesion and migration, adhesion and aggregation of platelets, and smooth muscle and fibroblast proliferation. These cellular effects probably underlie the antiatherosclerotic action of NO (see Ch. 23).

Large amounts of NO (released following induction of NOS or excessive stimulation of NMDA receptors in the brain) cause cytotoxic effects (either directly or via formation of peroxynitrite). These contribute to host defence, but also to the neuronal destruction that occurs when there is overstimulation of NMDA receptors by glutamate (see Chs 38 and 40). Paradoxically, NO is also cytoprotective under some circumstances (see Ch. 40).

VASCULAR EFFECTS (see also Ch. 22)

The L-arginine/NO pathway is tonically active in resistance vessels, reducing peripheral vascular resistance and hence systemic blood pressure. Mutant mice that lack the gene coding NOS3 are hypertensive, consistent with a role for NO biosynthesis in the physiological control of blood pressure. In addition, NO derived from NOS1 is implicated in the control of basal resistance vessel tone in human forearm and cardiac muscle vascular beds (Seddon et al., 2008, 2009). NO may contribute to the generalised vasodilatation that occurs during pregnancy.

NEURONAL EFFECTS (see also Ch. 12)

Nitric oxide is a non-noradrenergic non-cholinergic (NANC) neurotransmitter in many tissues (see Fig. 12.5), and is important in the upper airways, gastrointestinal tract and control of penile erection (Chs 28, 30 and 35). It is implicated in the control of neuronal development and of synaptic plasticity in the CNS (Chs 37 and 39). Mice carrying a mutation disrupting the gene coding NOS1 have grossly distended stomachs similar to those seen in human hypertrophic pyloric stenosis (a disorder characterised by pyloric hypertrophy causing gastric outflow obstruction, which occurs in approximately 1 in 150 male infants and is corrected surgically). NOS1 knockout mice resist stroke damage caused by middle cerebral artery ligation but are aggressive and oversexed (characteristics that may not be unambiguously disadvantageous, at least in the context of natural selection!).

HOST DEFENCE (see Ch. 6)

Cytotoxic and/or cytostatic effects of NO are implicated in primitive non-specific host defence mechanisms against numerous pathogens, including viruses, bacteria, fungi, protozoa and parasites, and against tumour cells. The importance of this is evidenced by the susceptibility of mice lacking NOS2 to *Leishmania major* (to which wild-type mice are highly resistant). Mechanisms whereby NO damages invading pathogens include nitrosylation of nucleic acids and combination with haem-containing enzymes, including the mitochondrial enzymes involved in cell respiration.

[5]Ascorbic acid releases NO from SNAP but accelerates NO degradation in solution, which could explain this divergence.

Actions of nitric oxide

- Nitric oxide (NO) acts by:
  - combining with haem in guanylyl cyclase, activating the enzyme, increasing cGMP and thereby lowering $[Ca^{2+}]_i$
  - combining with haem groups in other proteins (e.g. cytochrome c oxidase)
  - combining with superoxide anion to yield the cytotoxic peroxynitrite anion
  - nitrosation of proteins, lipids and nucleic acids.
- Effects of NO include:
  - vasodilatation, inhibition of platelet and monocyte adhesion and aggregation, inhibition of smooth muscle proliferation, protection against atheroma
  - synaptic effects in the peripheral and central nervous system
  - host defence and cytotoxic effects on pathogens
  - cytoprotection.

THERAPEUTIC ASPECTS

NITRIC OXIDE

Inhalation of high concentrations of NO (as occurred when cylinders of nitrous oxide, N_2O, for anaesthesia were accidentally contaminated) causes acute pulmonary oedema and methaemoglobinaemia, but concentrations below 50 ppm (parts per million) are not toxic. NO (5–300 ppm) inhibits bronchoconstriction (at least in guinea pigs), but the main action of low concentrations of inhaled NO in man is pulmonary vasodilatation. Inspired NO acts preferentially on ventilated alveoli, and could therefore be therapeutically useful in respiratory distress syndrome. This condition has a high mortality and is caused by diverse insults (e.g. infection). It is characterised by intrapulmonary 'shunting' (i.e. pulmonary arterial blood entering the pulmonary vein without passing through capillaries in contact with ventilated alveoli), resulting in arterial hypoxaemia, and by acute pulmonary arterial hypertension. Inhaled NO dilates blood vessels in ventilated alveoli (which are exposed to the inspired gas) and thus reduces shunting. NO is used in intensive care units to reduce pulmonary hypertension and to improve oxygen delivery in patients with respiratory distress syndrome, but it is not known whether this improves long-term survival in these severely ill patients.

NITRIC OXIDE DONORS/PRECURSORS

Nitrovasodilators have been used therapeutically for over a century. The common mode of action of these drugs is as a source of NO (Chs 21 and 22). There is interest in the potential for selectivity of nitrovasodilators; for instance, **glyceryl trinitrate** is more potent on vascular smooth muscle than on platelets, whereas SNOG (see p. 241) selectively inhibits platelet aggregation. It was shown recently that dietary nitrate (contained in beetroot juice) acutely lowers arterial blood pressure in parallel with a rise in plasma nitrite concentration and improved endothelial and platelet function. Interruption of the enterosalivary conversion of nitrate to nitrite prevents the rise in plasma nitrite, blocks the fall in blood pressure and abolishes the inhibitory effect on platelet aggregation (see review by Lidder & Webb, 2013).

INHIBITION OF NITRIC OXIDE SYNTHESIS

▼ Drugs can inhibit NO synthesis or action by several mechanisms. Certain arginine analogues compete with arginine for NOS. Several such compounds, for example N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), have proved of great value as experimental tools. One such compound, ADMA, is approximately equipotent with L-NMMA. It is present in human plasma and is excreted in urine. Its plasma concentration correlates with vascular mortality in patients receiving haemodialysis for chronic renal failure, and is increased in people with hypercholesterolaemia. In addition to urinary excretion, ADMA is also eliminated by metabolism to a mixture of citrulline and methylamine by *dimethylarginine dimethylamino hydrolase* (DDAH), an enzyme that exists in two isoforms, each with a reactive cysteine residue in the active site that is subject to control by nitrosylation. Inhibition of DDAH by NO causes feedback inhibition of the L-arginine/NO pathway by allowing cytoplasmic accumulation of ADMA. Conversely, activation of DDAH could potentiate the L-arginine/NO pathway; see Figure 20.4.

Infusion of a low dose of L-NMMA into the brachial artery causes local vasoconstriction (Fig. 20.5), owing to inhibition of the basal production of NO in the infused arm, probably by inhibiting NOS1 (Seddon et al., 2008), without influencing blood pressure or causing other systemic effects, whereas intravenous L-NMMA causes vasoconstriction in renal, mesenteric, cerebral and striated muscle resistance vessels, increases blood pressure and causes reflex bradycardia.

There is therapeutic interest in selective inhibitors of different isoforms of NOS. Selective inhibitors of NOS2 versus the two constitutive forms have been described (e.g. *N*-iminoethyl-L-lysine), and have potential for the treatment of inflammatory and other conditions in which NOS2 has been implicated (e.g. asthma). 7-Nitroindazole selectively inhibits NOS1, the mechanism of selectivity being uncertain. *S*-methyl-L-thiocitrulline is a potent and

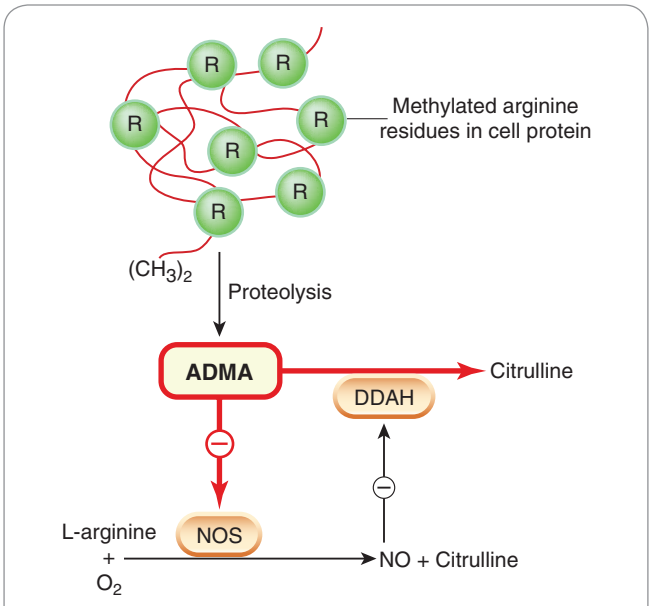

Fig. 20.4 Control of NO synthesis by asymmetric dimethylarginine (ADMA). DDAH, dimethylarginine dimethylamino hydrolase; NO, nitric oxide; NOS, nitric oxide synthase.

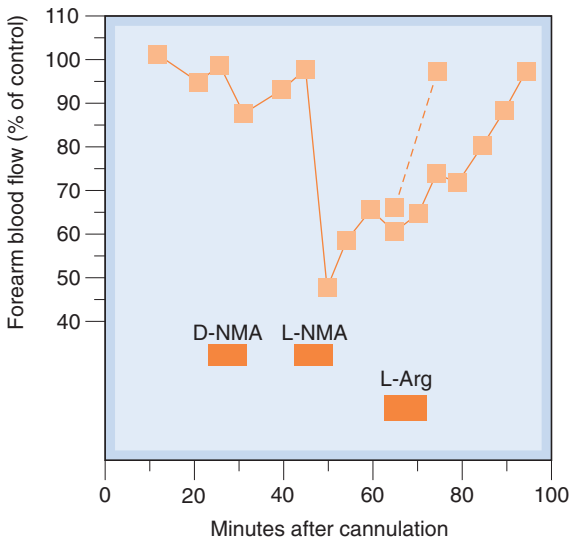

Fig. 20.5 Basal blood flow in the human forearm is influenced by nitric oxide (NO) biosynthesis. Forearm blood flow is expressed as a percentage of the flow in the non-cannulated control arm (which does not change). Brachial artery infusion of the D-isomer of the arginine analogue N^G-monomethyl-L-arginine (D-NMA) has no effect, while the L-isomer (L-NMA) causes vasoconstriction. L-Arginine (L-Arg) accelerates recovery from such vasoconstriction (dashed line). *(From Vallance P, Bhagat K, MacAllister R et al. 1989 Lancet ii, 997–1000.)*

selective inhibitor of human NOS1 (Furfine et al., 1994), and has recently provided new understanding of the importance of NOS1 in control of human resistance vessel tone *in vivo* (Seddon et al., 2008, 2009).

Inhibition of the L-arginine/nitric oxide pathway

- Glucocorticoids inhibit biosynthesis of NOS2.
- Synthetic arginine and citrulline analogues (e.g. **L-NMMA**, **L-NAME**; see text) compete with arginine and are useful experimental tools. Isoform-selective inhibitors include ***S*-methyl-L-thiocitrulline** (selective for NOS1).
- ADMA (asymmetric dimethylarginine) is an endogenous inhibitor of NOS.

NITRIC OXIDE REPLACEMENT OR POTENTIATION

Several means whereby the L-arginine/NO pathway could be enhanced are under investigation. Some of these rely on existing drugs of proven value in other contexts. The hope (as yet unproven) is that, by potentiating NO, they will prevent atherosclerosis or its thrombotic complications or have other beneficial effects attributed to NO. Possibilities include:

- selective NO donors as 'replacement' therapy (see clinical box, p. 244) or to protect against unwanted aspects of the action of another drug (e.g. **naproxinod**, Ch. 26)
- dietary supplementation with L-arginine or inorganic nitrate (see clinical box, p. 244)
- antioxidants (to reduce concentrations of reactive oxygen species and hence stabilise NO; Ch. 22)
- drugs that restore endothelial function in patients with metabolic risk factors for vascular disease (e.g. angiotensin-converting enzyme inhibitors, statins, insulin, oestrogens; Chs 22, 23, 31 and 35)
- β_2-adrenoceptor agonists and related drugs (e.g. **nebivolol**, a β_1-adrenoceptor antagonist that is metabolised to an active metabolite that activates the L-arginine/NO pathway)
- phosphodiesterase type V inhibitors (e.g. **sildenafil**; see clinical box, p. 244 and Ch. 35).

CLINICAL CONDITIONS IN WHICH NITRIC OXIDE MAY PLAY A PART

The wide distribution of NOS enzymes and diverse actions of NO suggest that abnormalities in the L-arginine/NO pathway could be important in disease. Either increased or reduced production could play a part, and hypotheses abound. Evidence is harder to come by but has been sought using various indirect approaches, including:

- analysing nitrate and/or cGMP in urine: these studies are bedevilled, respectively, by dietary nitrate and by membrane-bound guanylyl cyclase (which is stimulated by endogenous natriuretic peptides; see Ch. 21)
- a considerable refinement is to administer [^{15}N]-arginine and use mass spectrometry to measure the enrichment of ^{15}N over naturally abundant [^{14}N]-nitrate in urine
- measuring NO in exhaled air
- measuring effects of NOS inhibitors (e.g. L-NMMA)
- comparing responses to endothelium-dependent agonists (e.g. **acetylcholine**) and endothelium-independent agonists (e.g. **nitroprusside**)
- measuring responses to increased blood flow ('flow-mediated dilatation'), which are largely mediated by NO
- studying histochemical appearances and pharmacological responses *in vitro* of tissue obtained at operation (e.g. coronary artery surgery).

All these methods have limitations, and the dust is far from settled. Nevertheless, it seems clear that the L-arginine/NO pathway is indeed a player in the pathogenesis of several important diseases, opening the way to new therapeutic approaches. Some pathological roles of excessive or reduced NO production are summarised in Table 20.1. We touch only briefly on these clinical conditions, and would caution the reader that not all of these exciting possibilities are likely to withstand the test of time!

Sepsis can cause multiple organ failure. Whereas NO benefits host defence by killing invading organisms, excessive NO causes harmful hypotension. Disappointingly, however, L-NMMA worsened survival in one controlled clinical trial.

Chronic low-grade endotoxaemia occurs in patients with *hepatic cirrhosis*. Systemic vasodilatation is typical in such patients. Urinary excretion of cGMP is increased,

Nitric oxide in pathophysiology

- Nitric oxide (NO) is synthesised under physiological and pathological circumstances.
- Either reduced or increased NO production can contribute to disease.
- Underproduction of neuronal NO is reported in babies with hypertrophic pyloric stenosis. Endothelial NO production is reduced in patients with hypercholesterolaemia and some other risk factors for atherosclerosis, and this may contribute to atherogenesis.
- Overproduction of NO may be important in neurodegenerative diseases (see Ch. 40) and in septic shock (Ch. 22).

and vasodilatation may be a consequence of induction of NOS leading to increased NO synthesis.

Nitrosative stress and nitration of proteins in airway epithelium may contribute to steroid resistance in *asthma*, and the ineffectiveness of glucocorticoids in *chronic obstructive pulmonary disease* (see Ch. 28).

Nitric oxide biosynthesis is reduced in patients with *hypercholesterolaemia* and some other disorders that predispose to atheromatous vascular disease, including cigarette smoking and diabetes mellitus. In hypercholesterolaemia, evidence of blunted NO release in forearm and coronary vascular beds is supported by evidence that this can be corrected by lowering plasma cholesterol (with a statin; see Ch. 24) or by dietary supplementation with L-arginine.

Endothelial dysfunction in diabetic patients with *erectile dysfunction* occurs in tissue from the corpora cavernosum of the penis, as evidenced by blunted relaxation to acetylcholine despite preserved responses to nitroprusside (Fig. 20.6). Vasoconstrictor responses to intra-arterial L-NMMA are reduced in forearm vasculature of insulin-dependent diabetics, especially in patients with traces of albumin in their urine ('microalbuminuria': early evidence of glomerular endothelial dysfunction).

It is thought that failure to increase endogenous NO biosynthesis normally during pregnancy contributes to *eclampsia*. This is a hypertensive disorder that accounts for many maternal deaths and in which the normal vasodilatation seen in healthy pregnancy is lost.

Excessive NMDA receptor activation increases NO synthesis, which contributes to several forms of neurological damage (see Ch. 40).

NOS1 is absent in pyloric tissue from babies with idiopathic hypertrophic pyloric stenosis.

Established clinical uses of drugs that influence the L-arginine/NO system are summarised in the clinical box.

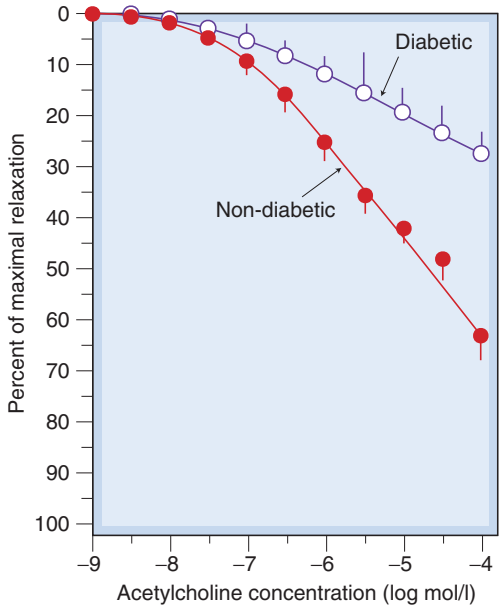

Fig. 20.6 Impaired endothelium-mediated relaxation of penile smooth muscle from diabetic men with erectile dysfunction. Mean (± SE) relaxation responses to acetylcholine in corpora cavernosa tissue (obtained at the time of performing surgical implants to treat impotence) from 16 diabetic men and 22 non-diabetic subjects. *(Data from Saenz de Tejada I, Carson MP, de las Morenas A et al. 1989 N Engl J Med 320, 1025–1030.)*

Nitric oxide in therapeutics

- Nitric oxide (NO) donors (e.g. **nitroprusside** and organic nitrovasodilators) are well established (see Chs 21 and 22).
- Type V phosphodiesterase inhibitors (e.g. **sildenafil**, **tadalafil**) potentiate the action of NO. They are used to treat erectile dysfunction (Ch. 35).
- Other possible uses (e.g. pulmonary hypertension, gastric stasis) are being investigated.
- Inhaled NO is used in adult and neonatal respiratory distress syndrome.
- Inhibition of NO biosynthesis is being investigated in disorders where there is overproduction of NO (e.g. inflammation and neurodegenerative disease). Disappointingly, **L-NMMA** increases mortality in one such condition (sepsis).

RELATED MEDIATORS

Nitric oxide (NO), promoted from pollutant to 'molecule of the year',[6] was joined, similarly implausibly, by carbon monoxide (CO) – a potentially lethal exhaust gas – and by hydrogen sulfide (H_2S), which are also formed in mammalian tissues. There are striking similarities between these three gases, as well as some contrasts. All three are highly diffusible labile molecules that are rapidly eliminated from the body: NO as nitrite and nitrate in urine as well as NO in exhaled air (see p. 240); CO in exhaled air; H_2S as thiosulfate, sulfite and sulfate in urine (Figure 20.7) as well as in exhaled breath. All three react with haemoglobin, and all three affect cellular energetics via

[6]By the American Association for the Advancement of Science in 1992.

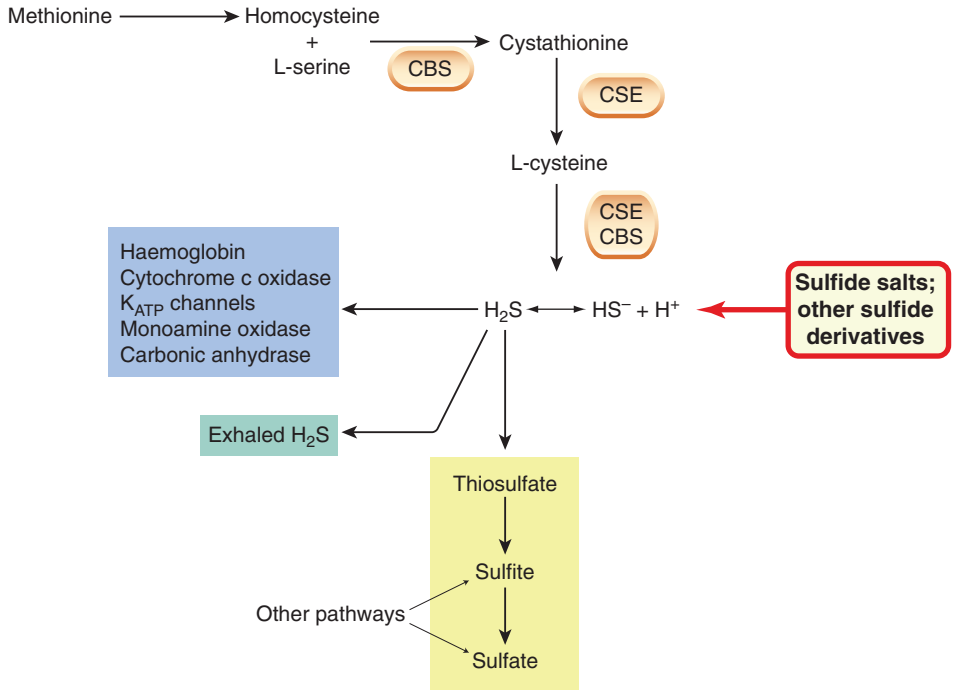

Fig. 20.7 Synthesis, sites of action and disposition of H_2S. Endogenous biosynthesis from sulfur-containing amino acids (methionine, cycteine) via actions of the regulated enzymes methionine cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS) is shown; pharmacological H_2S donors (red-rimmed box) may be administered exogenously. Most H_2S is probably renally excreted as sulfate (yellow box). Some is eliminated in exhaled air (green box). Some molecular targets of H_2S are indicated in the blue box. *(Adapted with permission from Ritter JM 2010 Human pharmacology of hydrogen sulfide: putative gaseous mediator. Br J Clin Pharmacol 69, 573–575.)*

actions on cytochrome c oxidase. All have vasodilator effects (although chronic exposure to CO can cause vasoconstriction), and all have anti-inflammatory and cytoprotective effects at low concentrations but cause cellular injury at higher concentrations.

CARBON MONOXIDE (CO)

▼ CO is synthesised, together with biliverdin, by inducible and/or constitutive forms of haem oxygenase, and has been implicated as a signalling molecule in the cardiovascular and central nervous systems (especially olfactory pathways) and in controlling respiratory, gastrointestinal, endocrine and reproductive functions (see Wu & Wang, 2005). There is evidence that prostanoid-induced cerebral vasodilatation is mediated by CO, and that CO also interacts with NO in modulating cerebral vascular tone (Leffler et al., 2011). There are as yet no therapeutic drugs acting via this pathway, but it remains worth watching.

HYDROGEN SULFIDE (H_2S)

▼ All H_2S has been known to generations of schoolboys as the source of the odour of rotten eggs and the proposal that it too is a gaseous mediator was met with some scepticism. Its toxicology includes actions on enzymes including monoamine oxidase and carbonic anhydrase, but more recent work has demonstrated a diverse pharmacology consistent with functions as a signalling molecule under physiological conditions.

Endogenous H_2S is produced from L-cysteine by cystathionine γ-lyase (also known as cystathionase or CSE) and cystathionine β-synthase (CBS). Large amounts of CBS occur in mammalian brain (especially hippocampus and cerebellar Purkinje cells), whereas CSE activity is greatest in liver, kidney and media of blood vessels. These enzymes are under regulatory control (e.g. by lipopolysaccharide and by TNF-α) and their expression is altered in experimental diseases (including pancreatitis and diabetes mellitus). Pharmacological inhibitors of H_2S synthesis are so far only of modest potency and specificity and have been of limited use in elucidating its physiological role. Several assays of H_2S in biological fluids grossly overestimate the true concentrations. Thiosulfate excretion (Fig. 20.7) may represent a better analytical approach than plasma sulfide to estimating overall turnover of H_2S; sulfite and sulfate (to which thiosulfate is converted) are not satisfactory, as their production from other sources of sulfur swamps the contribution of H_2S.

Pharmacological effects and therapeutic potential. H_2S has potent pharmacological effects in the cardiovascular system, including vasorelaxation secondary to activation of vascular smooth muscle K_{ATP} channels (see Ch. 4), in models of inflammation and in the central nervous system. Endocrine effects include inhibition of glucose-stimulated insulin secretion; actions on K_{ATP} channels may be important here also (see Ch. 31). One of the most striking effects of H_2S is to induce a state of suspended animation, described first in nematode worms, but then also in rodents, together with hypothermia. Subsequently, a whole range of cytotoxic (high concentration) and cytoprotective (low concentration) effects of H_2S and H_2S donors have been described in a wide variety of cell types in many different tissues (reviewed by Szabo, 2007). These findings provided a rationale for studies of effects of H_2S donors in animal models of diseases as diverse as pulmonary vasoconstriction, ischaemic heart disease, pulmonary fibrosis and stroke. The results have been sufficiently encouraging to provide a rationale for studying H_2S donors in man. Several sulfide-releasing derivatives based on **diclofenac** (Ch. 26) and on **mesalazine** (Ch. 30), as well as inorganic sodium sulfide, are under investigation as potential therapeutic agents. Again, a case of 'watch this space'.

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