16 Purines

OVERVIEW

In this chapter we describe the role of purine nucleosides and nucleotides as chemical mediators subserving a wide range of functions. The mechanisms responsible for their synthesis and release are considered, as well as the various receptors on which they act, and drugs that affect purinergic signalling.

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 produce a wide range of unrelated pharmacological effects. Interest in this field probably began with the observation in 1929 that adenosine injected into anaesthetised animals caused bradycardia, hypotension, vasodilatation and inhibition of intestinal movements. Since then, it has become clear that purines participate in many physiological control mechanisms, including the regulation of coronary 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 38).

There is, therefore, increasing interest in purine pharmacology and the potential role of purinergic agents in the treatment of pain and a variety of disorders, particularly of thrombotic and respiratory origin. 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.[1] There is no doubt that drugs affecting these systems will assume growing significance but, recognising that the overall picture is far from complete, we will focus our discussion on a few prominent areas.

Figure 16.1 summarises the mechanisms by which purines are 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 known about their signalling systems, their endogenous ligands and antagonists of pharmacological interest. It should be noted, however, that the family of purinergic receptors continues to grow and their pharmacology 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 it is possible to envisage a situation where ATP may produce effects at all three receptor subclasses depending upon the extent of its enzymatic hydrolysis.

The three main types of purine receptor are:

1. *Adenosine receptors* (A_1, A_{2A}, A_{2B} and A_3), formerly known as P_1 *receptors*, which are G-protein-coupled receptors that regulate cAMP.
2. *P2Y metabotropic receptors* ($P2Y_{1–14}$), which are G-protein-coupled receptors that utilise either cAMP or phospholipase C activation as their signalling system (see Ch. 3); they respond to various adenine nucleotides, generally preferring ATP over ADP or AMP.
3. *P2X ionotropic receptors* ($P2X_{1–7}$) which are multimeric ATP-gated cation channels.

The subtypes in each family may be distinguished on the basis of their molecular structure as well as their agonist and antagonist selectivity. The latter has usually been determined by the use of groups of experimental compounds with varying degrees of receptor selectivity and need not concern us here. 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, some members of this family also recognise pyrimidines such as UTP and UDP as well as purines, and as such are sometimes classed as *pyrimidinoceptors*. Little is known about the role of pyrimidines in cell signalling.

With the exception of platelet $P2Y_{12}$ antagonists such as **clopidogrel**, there are so far few therapeutic agents that act on these receptors, and we will confine this account to some prominent and interesting aspects; 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 and out mainly by a membrane transporter. Little is known about the way in which this is controlled but the extracellular concentrations are usually quite low 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).

Virtually all cells express one or more A-receptors and so adenosine produces many pharmacological effects, both in the periphery and in the CNS. Based on its ability to inhibit cell function and thus minimise the metabolic requirements of cells, one of its functions may be as an 'acute' protective agent that is released immediately when tissue integrity is threatened (e.g. by coronary or cerebral

[1]Indeed, a journal, *Purinergic Signalling*, devoted exclusively to these issues was launched recently.

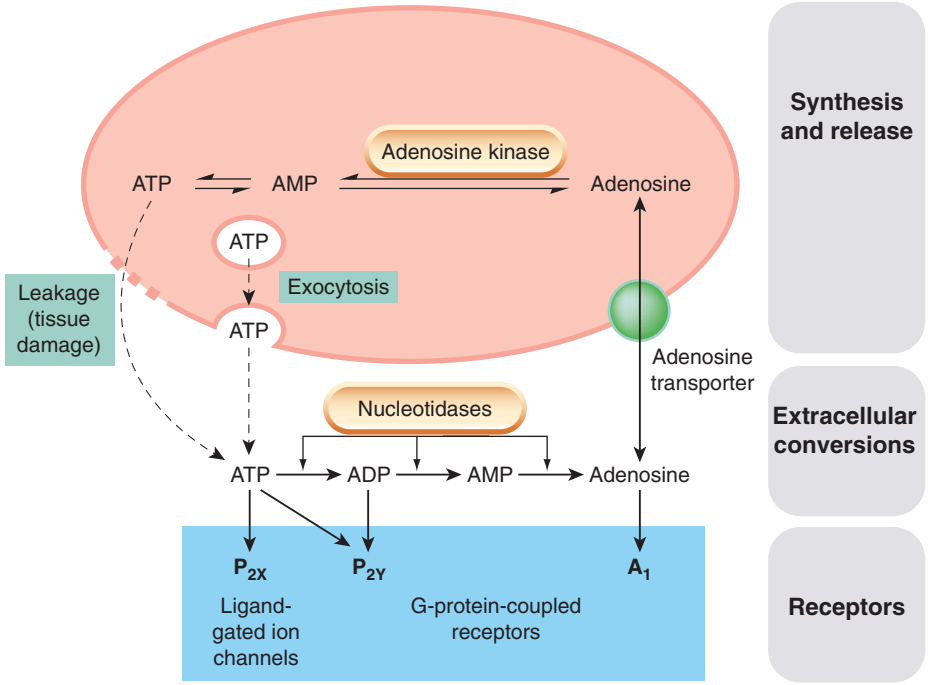

Fig. 16.1 Purines as mediators. ATP (and, in platelets, ADP) is stored in vesicles and released by exocytosis. It is also present in the cytosol of all cells, from which large quantities may be released by cellular damage. Adenosine is present in the cytosol of all cells, and is taken up and released via a specific membrane transporter. When released, ATP and ADP are rapidly converted to adenosine by the action of tissue nucleotidases.

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 **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 drug **clopidogrel**.
- *ATP* is stored in vesicles and released by exocytosis. Cytoplasmic ATP may be released when cells are damaged. It also functions as an intracellular mediator, inhibiting the opening of membrane potassium channels.
  - ATP may act on P2Y or on P2X receptors: these are ligand-gated ion channels.
  - Suramin blocks the ATP actions at most receptors.
  - Important sites of ATP action include the CNS, peripheral and central pathways and inflammatory cells.
  - Released ATP is rapidly converted to ADP and adenosine that may act on other purinergic receptors.

ischaemia; see Chs 21 and 39). 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 CARDIOVASULAR SYSTEM

Adenosine inhibits cardiac conduction and it is likely that all four of the adenosine receptors are involved in this effect. Because of this, adenosine itself may be used as a drug, being given as an intravenous bolus injection to terminate supraventricular tachycardia (Ch. 21). In this respect it is safer than alternatives such as β-adrenoceptor antagonists or **verapamil**, because of its short duration of action: it is destroyed or taken up within a few seconds of intravenous administration. 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).

Table 16.1 Purinergic receptors

Receptor	Subtype	Class	Principal endogenous ligands	Notes
Adenosine (also called P_1)	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	Adenosine (high affinity)	Caffeine, theophylline (antagonists)
	A_{2B}	G-protein coupled ($G_{s/q}$) Raises cAMP	Adenosine (low affinity)	Theophylline (antagonist)
	A_3	G-protein coupled ($G_{i/q}$) Lowers cAMP	Adenosine (low affinity)	Theophylline (antagonist)
P2Y 'metabotropic'[a]	$P2Y_1$	G-protein coupled (mainly G_q) Activates PLCβ mobilises Ca^{2+} Sometimes alters cAMP	ATP (antagonist or partial agonist)	Suramin (antagonist)
	$P2Y_2$		Adenine, UTP and ATP	Suramin (antagonist)
	$P2Y_4$		ATP (antagonist) and UTP	Pyrimidinoceptor
	$P2Y_6$		UDP	—
	$P2Y_{11}$		ATP > ADP	Suramin (antagonist)
	$P2Y_{12}$	G-protein coupled (mainly $G_{i/o}$) Reduces cAMP	ADP	Platelet ADP receptor Clopidigrel, prasugrel and ticlopidine (potent antagonists)
	$P2Y_{13}$		ADP	—
	$P2Y_{14}$		UDP-glucose	Pyrimidinoceptor
P2X 'ionotropic'	$P2X_1$	Receptor-gated cation-selective ion channels	ATP	Suramin (antagonist)
	$P2X_2$			
	$P2X_3$			
	$P2X_4$			
	$P2X_5$			
	$P2X_6$			
	$P2X_7$			

[a] Only functional human receptors are listed. The missing numbers in the sequence indicate that while these receptors have been cloned, 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.

ADENOSINE AND ASTHMA

Adenosine receptors are found on all the cell types involved in asthma and the overall pharmacology is complex. However, by 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. 27), 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 contributes to their pharmacological actions independently of adenosine receptor antagonism. Certain derivatives of theophylline are claimed to show greater selectivity for adenosine receptors over phosphodiesterase. In contrast to the A_1 receptor, activation of the A_{2A} subtype exerts a largely protective and anti-inflammatory effect.

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).

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. 47) occurs partly as a result of block of these receptors.

ADP AS A MEDIATOR

ADP is usually stored in vesicles in cells. When released, it exerts its biological effects predominantly through the P2Y family of receptors.

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 Chs 23 and 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 the earlier agent, **ticlopidine** (not available in the UK), are $P2Y_{12}$ antagonists and exert their antiaggregating effects through this mechanism (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. The 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 antagonise ATP and have broad-spectrum inhibitory activity at most P2X and P2Y receptors. ATP is present in all cells in millimolar concentrations and is released, independently of exocytosis, if the cells are damaged (e.g. by ischaemia). ATP released from cells is rapidly dephosphorylated by a range of tissue-specific nucleotidases, producing ADP and adenosine (Fig. 16.1), both of which produce a wide variety of receptor-mediated effects. The role of intracellular ATP in controlling membrane potassium channels, which is important in the control of vascular smooth muscle (Ch. 22) and of insulin secretion (Ch. 30), is quite distinct from its 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.

The nucleotide 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 functions as a conventional 'fast' transmitter in the CNS and in autonomic ganglia.

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, as a result of activation of $P2X_2$ and/or $P2X_3$ receptors in afferent neurons involved in the transduction of nociception (see Ch. 41). Oddly, perhaps, the same receptors seem to be involved in taste perception on the tongue. Elsewhere in the CNS, $P2X_4$ receptors on microglia may be important in the development of neuropathic pain.

ATP IN INFLAMMATION

The $P2X_7$ receptor is widely distributed on cells of the immune system, and ATP, apparently acting through this receptor, causes the release from macrophages and mast cells of cytokines and other mediators of the inflammatory response. Mice in which the receptor is deleted by genetic techniques show a reduced capacity to develop chronic inflammation.

FUTURE PROSPECTS

While it is true that few currently available drugs act through purinergic receptors when compared, for example, with 5-HT receptors discussed in Chapter 15, the area as a whole holds promise for future therapeutic exploitation, particularly in the treatment of asthma (Brown et al., 2008), pain (Liu et al., 2005; Burnstock, 2006) and gastrointestinal disorders (Burnstock, 2008), provided compounds with sufficient receptor selectivity can be found.

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 http://www.iuphar-db.org.)

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17 Local hormones: cytokines, biologically active lipids, amines and peptides

OVERVIEW

In Chapter 6 we discussed the cellular players in host defence and alluded to the crucial role played by soluble chemical messengers in the inflammatory response. Here, we take a close look at these mediators as well as others which, while having a normal physiological role, are pressed into service by the host defence mechanism when necessary. The exceptions are the *cytokines* and *chemokines* which, as a general rule, are mainly of importance in inflammation and immunity. Many of the mediators described here are important targets for anti-inflammatory and other drug action.

INTRODUCTION

A 'mediator' is operationally defined as a substance that fulfils a set of criteria generally modelled on the original suggestions of Sir Henry Dale in 1933. A modified version, more applicable to the field today, was considered by Dale & Foreman (1994). They defined a 'mediator' as a substance that fulfils certain criteria, including the following:

- Applying the substance should produce the effect in question, and receptors should be present locally.
- The synthetic pathway should be present, and the substance generated locally.
- There should be a mechanism for termination of its effects.
- Interfering with its synthesis, release or termination should modify the physiological reaction accordingly.

The principal mediators of inflammation will be described below beginning with the cytokines.

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 enormously important for the overall coordination of the inflammatory response. Cytokines act locally by *autocrine* or *paracrine* mechanisms. Their synthesis is massively upregulated during inflammatory episodes and they are usually active at very low (sub-nanomolar) concentrations.

On the target cell, they bind to and activate specific, high-affinity receptors that, in most cases, are also upregulated during inflammation. Except for *chemokines* (see below), 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 (Ch. 3).

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 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 the purposes of this chapter, however, Table 17.1 lists some significant species and their biological actions using a very simplified classification scheme. The cytokine aficionado can find further classification tables in Janeway et al. (2004), or by using the Web links listed at the end of the chapter.

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

INTERLEUKINS

Originally so named as they signalled between leukocytes, the distinction has become less useful with time. The primary proinflammatory interleukins (IL) are usually considered to be tumour necrosis factor (TNF)-α and IL-1. The latter cytokine actually comprises a family of three cytokines consisting of two agonists, IL-1α, IL-1β and, surprisingly, an endogenous IL-1-receptor *antagonist* (IL-1ra).[1] 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* (see below). Some interleukins are anti-inflammatory. These include transforming growth factor (TGF)-β, IL-4, IL-10 and IL-13. They inhibit chemokine production, and the anti-inflammatory interleukins can inhibit responses driven by T-helper (Th)1 cells, whose inappropriate activation is involved in the pathogenesis of several diseases. Both TNF-α and IL-1 are important targets for anti-inflammatory biopharmaceuticals (Ch. 26).

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

Table 17.1 Some examples of significant cytokines and their actions

Main function	Cytokine	Main cell source	Main target cells/action	Comments
Cytokines that stimulate immune cells to proliferate and differentiate	IL-1	Monocyte/macrophages and other cells	Stimulates proliferation, maturation and activation of Th, B and NK lymphocytes: causes inflammation, fever	Two subtypes and IL-1ra—a receptor antagonist
	IL-2	Th1 cells	Stimulates proliferation, maturation and activation of T, B and NK cells	
	IL-4	Th2 cells	Stimulates proliferation, maturation of T and B cells and IgG and E synthesis	
	IL-5	Th2 cells	Stimulates proliferation, maturation of B cells and IgA synthesis	
	IL-6	Monocyte/macrophages and Th2 cells	Stimulates differentiation of B cells, plasma cells and Ig secretion	Target for anti-inflammatory drugs (Ch. 26)
	IL-10	Th2 cells	Inhibits cytokine production by macrophages Activates B cells	
	IL-17	T cells, various	Stimulates Th17 cells	
	GM–CSF	Th cells	Stimulates growth of dendritic and other progenitor cells	
	IL-8	Macrophages, endothelial cells	Neutrophil chemotaxis	C-X-C chemokine
Cytokines that mainly signal other cellular functions	MIP-1	Macrophages/lymphocyes	Chemotaxis of monocytes/T cells	Two subtypes C-C chemokine
	TGF-α	T cells, monocytes	Chemotaxis of macrophages/ lymphocytes and IL-1 synthesis Stimulates B cell IgA synthesis	
	TNF-α	Macrophages, mast cells and NK cells	Stimulates macrophage cytokine expression Kills tumour cells	Target for anti-inflammatory drugs (Ch. 26)
	TNF-β	Th1 cells	Stimulates macrophage phagocytosis and NO production Kills tumour cells	
	Eotaxin	Several	Chemotaxis, activation of eosinophils	C-C chemokine
	MCP-1	Bone and other cells	Chemotaxis of T cells/dendritic cells	C-C chemokine
	RANTES	T cells	Chemotaxis of T cells Chemotaxis and activation of other leukocytes	
Interferons	IFN-α	Leukocytes	Inhibits viral replication in various cell types	Multiple molecular species
	IFN-γ	Th1, NK cells	Inhibits Th2 cell proliferation Stimulates macrophage pathogen killing	

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); NO, nitric oxide; RANTES, regulated on activation normal T cell expressed and secreted; TGF, transforming growth factor; Th, T-helper (cell); TNF, tumour necrosis factor.

CHEMOKINES

Chemokines are defined as *chemoattractant cytokines* 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, f-Met-Leu-Phe, etc; see Fig. 6.2). Furthermore, many chemokines have other actions, for example causing mast cell degranulation or promoting angiogenesis.

More than 40 chemokines have been identified, and for those of us who are not professional chemokinologists they can be conveniently distinguished by considering 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[2]) 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 coreceptor that allows it to penetrate the T cell (see Ch. 51).

INTERFERONS

There are three classes 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, and 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; see also Abbas et al., 1996).

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. A variety of dose-related side effects may occur. IFN-β is used in some patients with multiple sclerosis, whereas IFN-γ is used in chronic granulomatous disease in conjunction with antibacterial drugs (see clinical box for more details).

[2]MCP, monocyte chemoattractant protein; RANTES, regulated on activation normal T cell expressed and secreted.

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).
- **β:** 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.
- Interleukins 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.

Interfering with cytokine action using biopharmaceuticals has proved to be a particularly fertile area of drug development: several successful strategies have been adopted including direct antibody neutralisation 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.

HISTAMINE

In a classic study, Sir Henry Dale and his colleagues demonstrated that a local anaphylactic reaction (a type I or 'immediate hypersensitivity reaction'; see below) 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 described below) 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 the lungs and the skin, and in particularly high concentrations in the 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. 38). 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 C3a and C5a that 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 through a non-receptor action. 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

Histamine acts on G-protein-coupled receptors, of which four main types have been identified; all four are implicated in the inflammatory response (see Gutzmer et al., 2005, for a review). 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 of inflammation (notably rhinitis). Other clinical uses of subtype antagonists may be found in Chapters 27, 36 and 47. At the time of writing, 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. 27 and Fig. 27.3).

Cardiovascular effects. Histamine dilates human blood vessels 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 29.

Itching. Itching occurs if histamine is injected into the skin or applied to a blister base, because it stimulates sensory nerve endings by an H_1-dependent mechanism.

Central nervous system effects. Histamine is a transmitter in the CNS (Ch. 38).

The 'triple response'. When injected intradermally, histamine causes a reddening of the skin, accompanied by a weal with a surrounding flare. This is the *triple response* described by Sir Thomas Lewis over 80 years ago and is explained by the foregoing effects. 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 19 and 26).

Despite the fact that histamine release is evidently capable of reproducing many of the inflammatory signs and symptoms, histamine 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, significant in type I hypersensitivity reactions such as allergic rhinitis and urticaria. The use of H_1 antagonists in these and other conditions is dealt with in Chapter 26.

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 immunoglobulin E.
- Histamine produces effects by acting on H_1, H_2 or H_3 (and possibly 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), *weal* (direct action on blood vessels) 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. 36).

EICOSANOIDS

GENERAL REMARKS

Unlike histamine, eicosanoids are not preformed in cells but are generated from phospholipid precursors on demand. They are implicated in the control of many physiological processes, and are among the most important mediators and modulators of the inflammatory reaction (Fig. 17.1) and are a very significant target for drug action.

Interest in eicosanoids arose in the 1930s after reports that semen contained a lipid substance that 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 acids 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 double bonds (hence *eicosa*, referring to the 20 carbon atoms, and *tetraenoic*, referring to the four double bonds). 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 the *prostaglandins*, the *thromboxanes* and the *leukotrienes*, although other derivatives of arachidonate, for example the *lipoxins*, are also produced. (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 arachidonate, usually in a one-step process catalysed by the enzyme *phospholipase* A_2 (PLA_2; Fig. 17.2), although a multi-step process involving phospholipases C or D in conjunction with diacylglycerol lipase is sometimes utilised. Several species of PLA_2 exist, but the most important is probably the highly regulated *cytosolic* PLA_2. This enzyme generates not only arachidonic acid (and thus eicosanoids) but also *lysoglyceryl-phosphorylcholine* (*lyso-PAF*), the precursor of *platelet activating factor*, another inflammatory mediator (see Fig. 17.2).

Cytosolic PLA_2 is activated (and hence arachidonic acid liberated) by phosphorylation. This occurs in response to signal transduction events 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 the activation process. The free arachidonic acid is metabolised

[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, contains virtually none. Nevertheless the name stuck, outlasting the term *vesiglandin* which, while being suggested later, would have been more appropriate.

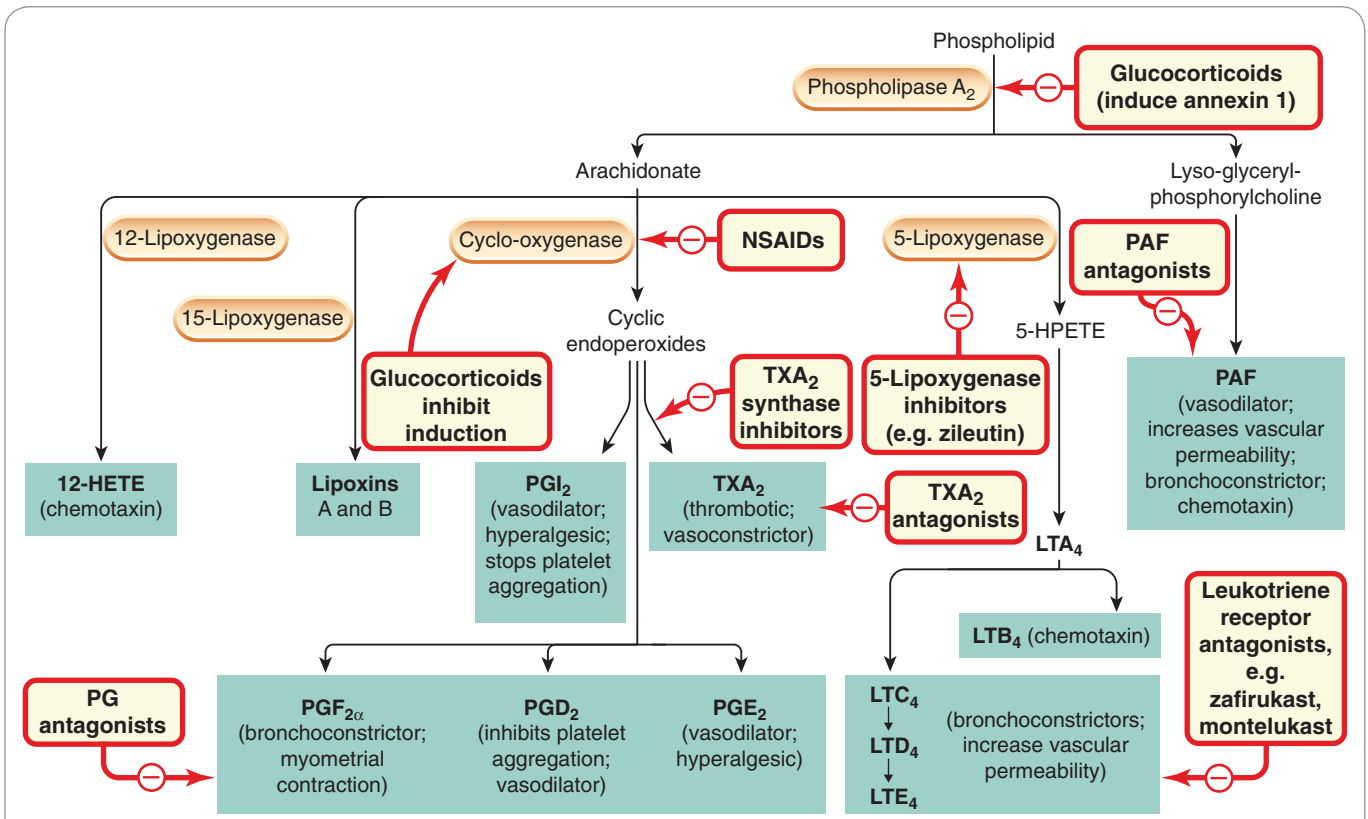


Fig. 17.1 Summary diagram of the inflammatory mediators derived from phospholipids, with an outline of their actions and the sites of action of anti-inflammatory drugs. The arachidonate metabolites are eicosanoids. The glucocorticoids inhibit transcription of the gene for cyclo-oxygenase-2, induced in inflammatory cells by inflammatory mediators. The effects of prostaglandin (PG)E_2 depend on which of the three receptors for this prostanoid are activated. HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LT, leukotriene; NSAID, non-steroidal anti-inflammatory drug; PAF, platelet-activating factor; PGI_2, prostacyclin; TX, thromboxane.

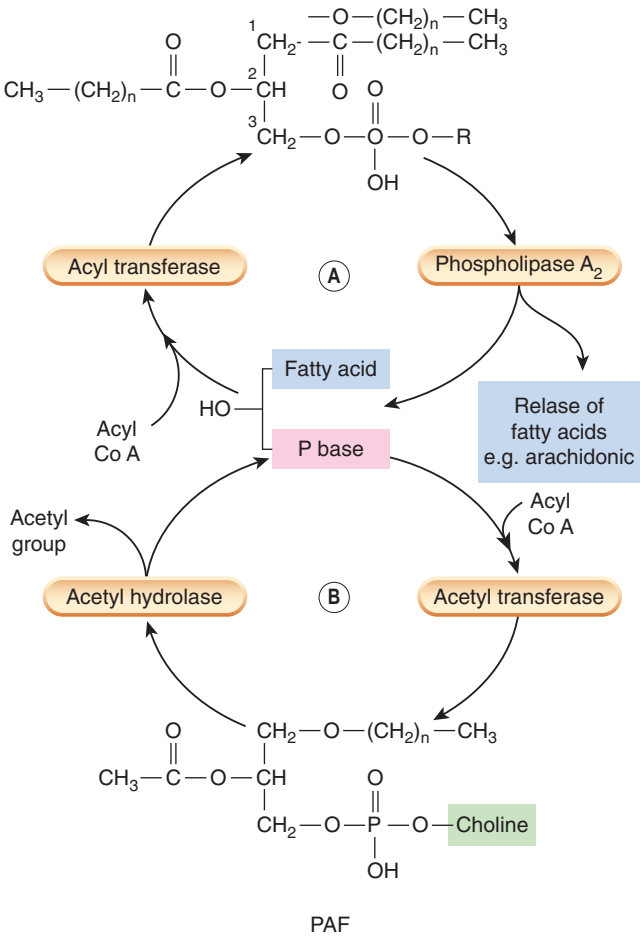


Fig. 17.2 The structure of phospholipids, the release of fatty acids and platelet-activating factor (PAF) precursors. The structure of a 'generic' phospholipid is shown. Different bases are found at C3 yielding phosphatidyl-choline, -ethanolamine, -serine or -inositol species. Generally speaking, unsaturated fatty acids such as arachidonic acid are esterified at the C2 position and saturated fatty acids are linked to C1. Two bonds are possible: an ether linkage or an ester linkage. Arachidonic acid can be removed by phospholipase A_2 and used for synthesis of eicosanoids. This yields a lyso-phospholipid that is normally rapidly reacylated and converted back to phospholipids (A). If the species is lysophosphatidyl choline and it contains an ether-linked hexadecyl or octadecyl fatty acid at C1, it can serve as a precursor for PAF. This is accomplished by a further acetylation step. PAF is inactivated by an acetylhydrolase that removes the acetyl group and converts it back to lyso-PAF, where it can be recycled (B).

separately (or sometimes jointly) by several pathways, including the following.

- *Fatty acid cyclo-oxygenase* (*COX*). Two main isoforms, COX-1 and COX-2, transform arachidonic acid to prostaglandins and thromboxanes.
- *Lipoxygenases*. Several subtypes synthesise leukotrienes, lipoxins or other compounds (Figs 17.1 and 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 that produces prostanoids that act as homeostatic regulators (e.g. modulating vascular responses), whereas COX-2 is not normally present (at least in most tissues) but it is strongly induced by inflammatory stimuli and therefore believed to be more relevant to inflammation therapy (see Ch. 26 for a full discussion of this point). Both enzymes catalyse the incorporation of two molecules of oxygen into each arachidonate molecule, forming the highly unstable *endoperoxides* PGG_2 and PGH_2. These are rapidly transformed by isomerase or synthase enzymes to PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and TXA_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 acid*, which contains five double bonds, yields PGE_3. The latter substrate is significant because it is present in abundance in some fish oils and may, if present in sufficient amounts in the diet, come to represent a significant fraction of cellular fatty acids. When this occurs, the production of the proinflammatory 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 *Resolvins* below).

CATABOLISM OF THE PROSTANOIDS

This is a multistep process. After carrier-mediated uptake, most prostaglandins are rapidly inactivated by 'prostaglandin-specific' enzymes, and the inactive products are further degraded by general fatty acid-oxidising enzymes. The prostaglandin-specific enzymes are present in high concentration in the lung, and 95% of infused PGE_2, PGE_1 or $PGF_{2\alpha}$ is inactivated on first passage. The half-life of most prostaglandins in the circulation is less than 1 min.

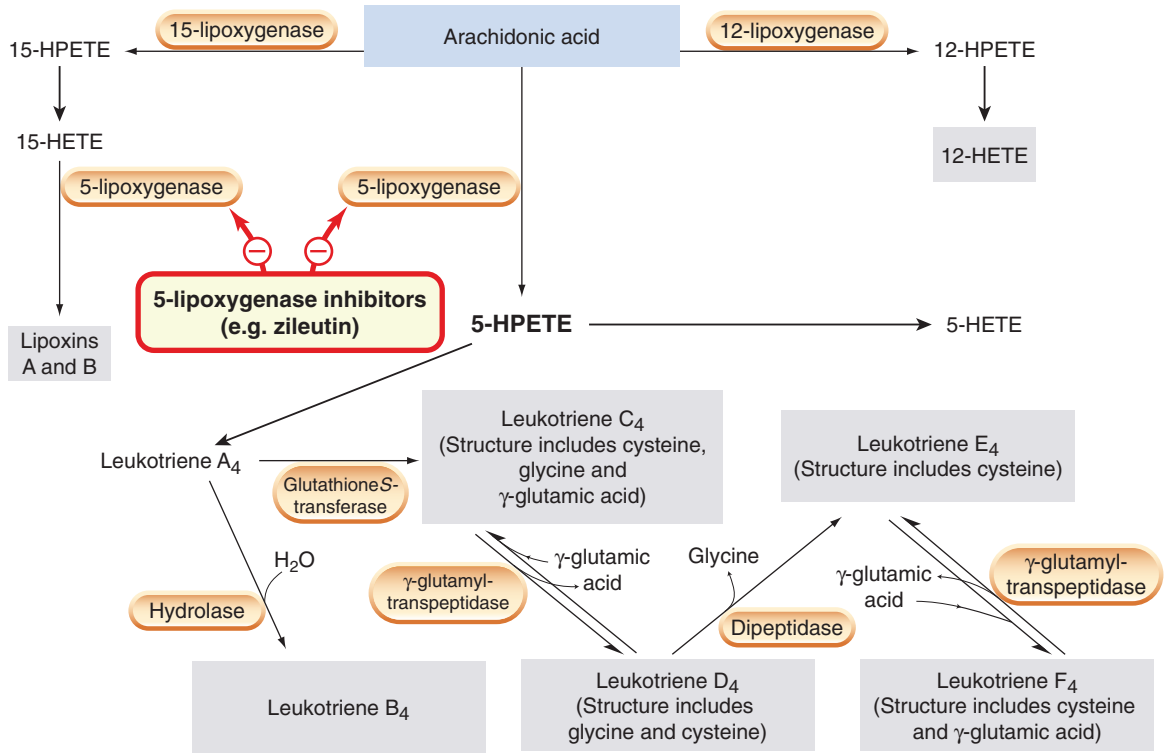


Fig. 17.3 The biosynthesis of leukotrienes from arachidonic acid. Compounds with biological action are shown in grey boxes. HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

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

PROSTANOID RECEPTORS

There are five main classes of prostanoid receptors (Coleman & Humphrey, 1993), all of which are typical G-protein-coupled receptors (Table 17.2). 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, the EP receptors are subdivided into four subgroups.

ACTIONS OF THE PROSTANOIDS

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

- *PGD_2* causes vasodilatation, inhibition of platelet aggregation, relaxation of gastrointestinal and uterine muscle, and modification of release of hypothalamic/pituitary hormones. It has a bronchoconstrictor effect through an action on TP receptors.
- *$PGF_{2\alpha}$* causes myometrial contraction in humans (see Ch. 34), 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).
- *PGE_2* 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. 29), increased gastric mucus secretion, inhibition of lipolysis, inhibition of autonomic neurotransmitter release and stimulation of contraction of the pregnant human uterus (Ch. 34).

THE ROLE OF THE 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

Table 17.2 Prostanoid receptors classified according to their general physiological effects

Receptor	Physiological ligands	Second messenger	General physiological effect	Distribution
IP	Iloprost[a] > $E_1 = E_2$	↑ cAMP	'Inhibitory': e.g. smooth muscle relaxation	Abundant in cardiovascular system, platelets and neurons
DP	D_2			Least abundant prostanoid receptor; restricted distribution (e.g. vascular smooth muscle and cutaneous blood vessels)
EP_2	$E_1 = E_2$			Least abundant EP receptor; induced in response to stimuli
EP_4	$E_1 = E_2$			Widespread distribution throughout body
TP	$TxA_2 > D_2$	↑ Ca^{2+}	'Excitatory': e.g. smooth muscle contraction	Abundant in cardiovascular system, platelets and immune cells
FP	$F_{2\alpha} > D_2$			Two subtypes known, opposing actions Very high expression in female reproductive organs
EP_1	$E_2 > E_1 > F_{2\alpha}$			Mainly kidney, lung and stomach
EP_3	$E_1 = E_2$ > iloprost	↓ cAMP	'Inhibitory': inhibits smooth muscle relaxation	Widespread distribution throughout body

[a] Iloprost is an analogue of PGI_2 and is used because I_2 is unstable.
(Adapted from Narumiya et al. 1999 Phys Rev 79: 1193–1226.)

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 this effect of histamine and bradykinin. Similarly, they do not themselves produce pain, but potentiate the effect of bradykinin by sensitising afferent C fibres (see Ch. 41) to the effects of other noxious stimuli. The anti-inflammatory effects of NSAIDs 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 there is evidence that 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 under some circumstances. 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. Several prostanoids are available for clinical use (see clinical box).

LEUKOTRIENES

Leukotrienes (*leuko-* because they are made by white cells, and *-trienes* because they contain a conjugated triene system of double bonds) 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 membrane, where it associates with a crucial accessory protein affectionately termed *FLAP* (five-lipoxygenase activating protein). 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 compound *leukotriene (LT)*A_4. This may be converted enzymically to LTB_4 and, utilising a separate pathway, is also the precursor of the cysteinyl-containing leukotrienes *LT*C_4, *LTD*$_4$, *LTE*$_4$ and *LTF*$_4$ (also referred to as the sulfidopeptide leukotrienes). Mixtures of these cysteinyl adducts constitute the *slow-reacting substance of anaphylaxis* (SRS-A), a substance shown many years ago to be generated in guinea pig lung during anaphylaxis, and believed to be important in asthma. LTB_4 is produced mainly by neutrophils, and the cysteinyl-leukotrienes mainly by eosinophils, mast cells, basophils and macrophages. *Lipoxins* and other active products, some of which have anti-inflammatory properties, are also produced from arachidonate by this pathway (Fig. 17.3).

LTB_4 is metabolised by a unique membrane-bound 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* if the ligand is LTB_4, and *CysLT* for the cysteinyl-leukotrienes. LTB_4 acts on specific LTB_4 receptors as defined by selective agonists and antagonists. The transduction mechanism utilises inositol trisphosphate and increased cytosolic Ca^{2+}.

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

Prostanoids

- The term *prostanoids* encompasses the prostaglandins and the thromboxanes.
- Cyclo-oxygenases (COX) oxidise arachidonate, producing the unstable intermediates prostaglandins 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.
- 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.

Clinical uses of prostanoids

- Gynaecological and obstetric (see Ch. 34):
  - 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. 29).
- 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.

selective antagonists. The CysLT-receptor antagonists **zafirlukast** and **montelukast** are now in use in the treatment of asthma (see Ch. 27). Cysteinyl-leukotrienes may mediate the cardiovascular changes of acute anaphylaxis. Agents that inhibit 5-lipoxygenase are under development as antiasthmatic agents (see Ch. 27) and anti-inflammatory agents. One such drug, **zileuton**, is available in some parts of the world but has not won a definite place in therapy yet (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). On neutrophils, it also upregulates membrane adhesion molecule expression, 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.

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

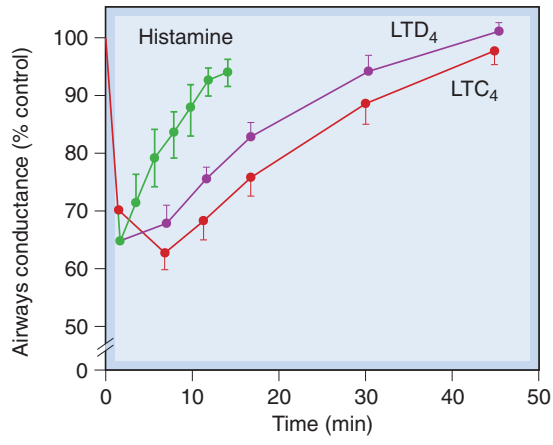


Fig. 17.4 The time course of action on specific airways conductance of the cysteinyl-leukotrienes and histamine, in six normal subjects. Specific airways conductance was measured in a constant volume whole-body plethysmograph, and the drugs were given by inhalation. (From Barnes P J, Piper P J, Costello J K 1984 Thorax 39: 500.)

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.

Platelet-activating factor

- 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 hyperresponsiveness and in the delayed phase of asthma.
- A PAF antagonist, **lexipafant**, is undergoing clinical trial in pancreatitis.

lavage fluid in subjects with allergic rhinitis. There is evidence that they contribute to the underlying bronchial hyperreactivity in asthmatics, and it is thought that they are among the main mediators of both the early and late phases of asthma (Fig. 27.2).

LIPOXINS AND RESOLVINS

A recently identified group of trihydroxy arachidonate metabolites termed *lipoxins* (Fig. 17.3) are formed by the concerted action of the 5- and the 12- or 15-lipoxygenase enzymes during inflammation. They act on polymorphonuclear leukocytes to oppose the action of proinflammatory stimuli, supplying what might be called 'stop signals' to inflammation. Lipoxins utilise the same formyl peptide G-protein-coupled receptor system that recognises other endogenous anti-inflammatory factors such as annexin-A1. Oddly, aspirin (a COX inhibitor, see Ch. 26) stimulates the synthesis of these substances 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 Ariel & Serhan, 2007, for a review of this promising area). The leukocyte receptor for resolvins is called *Chem 23*.

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). The name is somewhat misleading, 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. PAF is biosynthesised from acyl-PAF in a two-step process (Fig. 17.2). The action of PLA_2 on acyl-PAF produces lyso-PAF, which is then acetylated to give PAF. PAF, in turn, can be deacetylated to the inactive lyso-PAF. It is produced by platelets in response to thrombin, and by activated inflammatory cells.

ACTIONS AND ROLE IN INFLAMMATION

By acting on specific receptors, PAF is capable of producing 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 (Fig. 27.3). It can activate PLA_2 and initiates eicosanoid synthesis.

PAF stimulates arachidonate turnover and TXA_2 generation by platelets, producing shape change and the release of the granule contents. This is important in haemostasis and thrombosis (see Ch. 24). PAF has spasmogenic effects on both bronchial and ileal smooth muscle.

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.

BRADYKININ

Bradykinin and lysyl bradykinin (*kallidin*) are active peptides formed by proteolytic cleavage of circulating proteins termed kininogens through a protease cascade pathway (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 17.5. 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 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 (Fig. 17.6). 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.

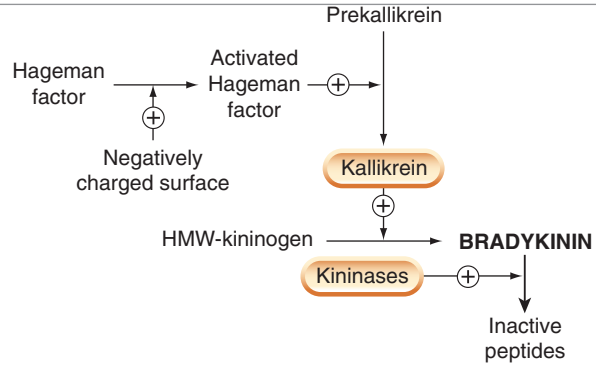


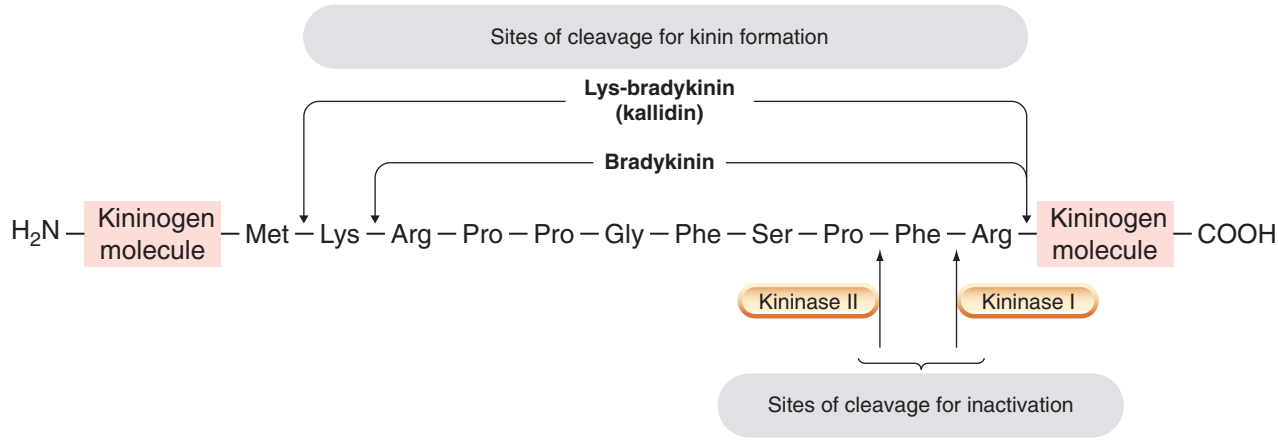
Fig. 17.5 The generation and breakdown of bradykinin. High-molecular-weight kininogen (HMW-kininogen) probably acts both as a substrate for kallikrein and as a co-factor in the activation of prekallikrein.

METABOLISM AND INACTIVATION OF BRADYKININ

Specific enzymes that inactivate bradykinin and related kinins are called *kininases* (Fig. 17.5). 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. 21), 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 (see below).

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



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. 17.6 Structure of bradykinin and some bradykinin antagonists. The sites of proteolytic cleavage of high-molecular-weight kininogen by kallikrein kallidin involved in the formation of bradykinin are shown in the upper half of the figure; the sites of cleavage associated with bradykinin 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, d-Tic and Oic, which are analogues of phenylalanine and proline.

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. 41), and antagonists could be developed for use in cough and neurological disorders (see Chung, 2005; 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**, which has recently been approved by the European Medicines Agency for treating 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 (Fig. 17.1) and release of nitric oxide (NO). It is a potent pain-producing agent at sensory neurons, and its action here is potentiated by prostaglandins (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 histamine (hence *brady*, which means 'slow').

Although bradykinin reproduces many inflammatory signs and symptoms, its role in inflammation and allergy has not been clearly defined, partly because its effects are often part 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. Physiologically, the release of bradykinin by tissue kallikrein may regulate blood flow to certain exocrine glands, and influence secretions. It also stimulates ion transport and fluid secretion by some epithelia, including intestine, airways and gall bladder.

NITRIC OXIDE

Chapter 20 discusses NO in detail, and here we will consider only its role in inflammation. *Inducible NO synthase* (iNOS) is the chief isoform relevant to inflammation, and virtually all inflammatory cells express the enzyme in response to cytokine stimulation. iNOS is also present in the bronchial epithelium of asthmatic subjects, in the mucosa of the colon in patients with ulcerative colitis, and in synoviocytes in inflammatory joint disease. NO probably has a net proinflammatory effect: it increases vascular permeability and prostaglandin production, and is a potent vasodilator. Some other properties may be seen as anti-inflammatory; for example, endothelial NO inhibits adhesion of neutrophils and platelets, and platelet aggregation. NO, or compounds derived from it, also has cytotoxic actions, killing bacteria, fungi, viruses and metazoan parasites, so in this respect NO enhances local defence mechanisms. However, produced in excess, it may also harm host cells.

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 *kininase II* (angiotensin-converting enzyme) in the lung.
- Pharmacological actions:
  - vasodilatation (largely dependent on endothelial cell nitric oxide and prostaglandin I_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.
- Des-Arg Hoe 140 is a selective competitive antagonist for B1 receptors (PA_2:8)
- Icatibant, a peptide analogue of BK, is a selective competitive antagonist for B_2 receptors (PA_2:9). It was recently approved in Europe for the treatment of 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.

Inhibitors of iNOS are under investigation for treatment of inflammatory conditions. Patients with septic shock have benefited from inhibitors of iNOS, and in experimental arthritis iNOS inhibitors reduce disease activity. Laboratory studies on compounds consisting of NSAIDs coupled with NO-releasing groups suggest that these have fewer side effects than conventional NSAIDs and greater anti-inflammatory efficacy (see Ch. 26).

NEUROPEPTIDES

Neuropeptides released from sensory neurons cause *neurogenic inflammation* (Maggi, 1996). The main peptides involved are substance P, neurokinin A and CGRP (see Ch. 19). Substance P and neurokinin A (members of the tachykinin family) act on mast cells, releasing histamine and other mediators, and producing smooth muscle contraction and mucus secretion, whereas CGRP is a potent vasodilator. 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.

CONCLUDING REMARKS

Even from the, albeit superficial, sketch of the host defence response that we have presented here and in Chapter 6, it must be evident to the reader that this is among the most complicated of all physiological responses. Perhaps that is not surprising, given the central importance of its mission to the very survival of the organism. Perhaps it is also

understandable that it can recruit so many different mediators that regulate and orchestrate the workings of the immune system that run into the hundreds.

What do come as a shock are experimental observations suggesting that the activity of many of these local hormones can apparently be blocked with little or no effect on the outcome of inflammation. This fact speaks to the redundancy of many of the component systems and goes at least some of the way to explaining why, until the advent of antibody-based therapies (see Ch. 26), our ability to curb the worst ravages of chronic inflammatory disease was very limited.

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Janeway, C.A., Travers, P., Nolan, A., et al., 2004. Immunobiology: the Immune System in Health and Disease, sixth ed. Churchill Livingstone, Edinburgh. (*Excellent textbook, good diagrams*)

Useful Web links

http://microvet.arizona.edu/Courses/MIC419/Tutorials/cytokines.html (*This is a useful Web site with a series of immunological tutorials. The cytokines module is worth looking at, and it has a good [although not complete] list of the most important members of the family, their targets and function. Also contains other material that is likely to be useful in understanding this chapter*)

http://www.copewithcytokines.de/ (*A very comprehensive site dealing with practically all known cytokines. Also contains a list of terms, links to reviews and short pieces on individual cytokines. Worth a look if you are stuck for some information*)

18 Cannabinoids

OVERVIEW

Modern pharmacological interest in cannabinoids dates from the discovery that Δ^9-tetrahydrocannabinol (THC) is the 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 36, 47 and 48.

PLANT-DERIVED CANNABINOIDS AND THEIR PHARMACOLOGICAL EFFECTS

Cannabis sativa, the hemp plant, has been used for its psychoactive properties for thousands of years (Ch. 47). Its medicinal use was advocated in antiquity, but serious interest resurfaced only in 1964 with the identification of *Δ^9-tetrahydrocannabinol* (THC, see Fig. 18.1), 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 peripheral 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—often a factor in road accidents. Cannabis users are less accident prone, 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. 47). Subjects report that time passes extremely slowly. The alarming sensations and paranoid delusions that often occur with LSD are seldom experienced after cannabis. Some studies support a connection between chronic use and subsequent schizophrenia and mood disorder (Henquet et al., 2005; Leweke & Koethe, 2008).

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

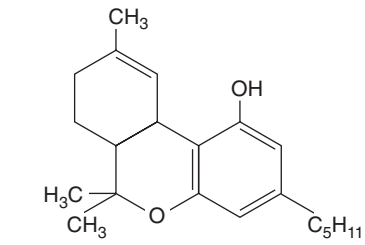
- impairment of short-term memory and simple learning tasks—subjective feelings of confidence and heightened creativity are not reflected in actual performance
- impairment of motor coordination (e.g. driving performance)
- catalepsy—the adoption of fixed unnatural postures
- hypothermia
- analgesia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

The main peripheral effects of cannabis are:

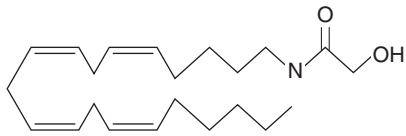
- tachycardia, which can be prevented by drugs that block sympathetic transmission
- vasodilatation, which is particularly marked on the scleral and conjunctival vessels, producing a bloodshot appearance characteristic of cannabis smokers
- reduction of intraocular pressure
- bronchodilatation.

Cannabis

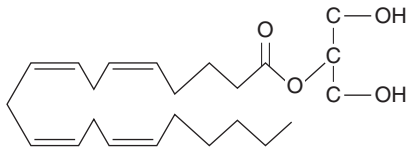
- Main active constituent is Δ^9-tetrahydrocannabinol (THC); a pharmacologically active 11-hydroxy metabolite is also important.
- Actions on the central nervous system include both depressant and psychotomimetic effects.
- Subjective experiences include euphoria and a feeling of relaxation, with sharpened 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.



Δ9-Tetrahydrocannabinol (THC)



Anandamide



2 Arachidonoyl glycerol (2-AG)

Fig. 18.1 Structures of Δ9-tetrahydrocannabinol and two endocannabinoids.

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 excretion continues for several days after a single dose. Radioimmunoassay of THC is bedevilled by cross-reactivity, and accurate identification and quantification of THC in biological fluids, important for medicolegal reasons, depends on mass spectrometry.

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** (see below) produce euphoria and drowsiness, sometimes accompanied by sensory distortion and hallucinations. These effects, together with legal restrictions on the use of cannabis, have precluded the widespread therapeutic use of cannabinoids.

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. The 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. 48), and it has been argued whether cannabis should be classified as addictive (see Fattore et al., 2008 and reviews by Maldonado & Rodríguez de Fonseca, 2002; Taber & Hurley 2009).

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 inward-rectifying potassium (GIRK) channels, causing hyperpolarisation (Fig. 18.2). These effects are similar to those mediated by opioid receptors (Ch. 41). 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 among the most abundant receptors in the brain, being comparable in this regard with receptors for glutamate and GABA—the main central excitatory and inhibitory neurotransmitters (Ch. 37). 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. 29 and below), substantia nigra, mesolimbic dopamine pathways that have been implicated in psychological 'reward' (Ch. 48), and in association areas of the cerebral cortex. There is a relative paucity of CB_1 receptors in the brain stem, perhaps explaining the lack of serious respiratory or cardiovascular toxicity of the cannabinoids. At a cellular level, CB_1 receptors are localised presynaptically, and inhibit transmitter release as explained above. 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.

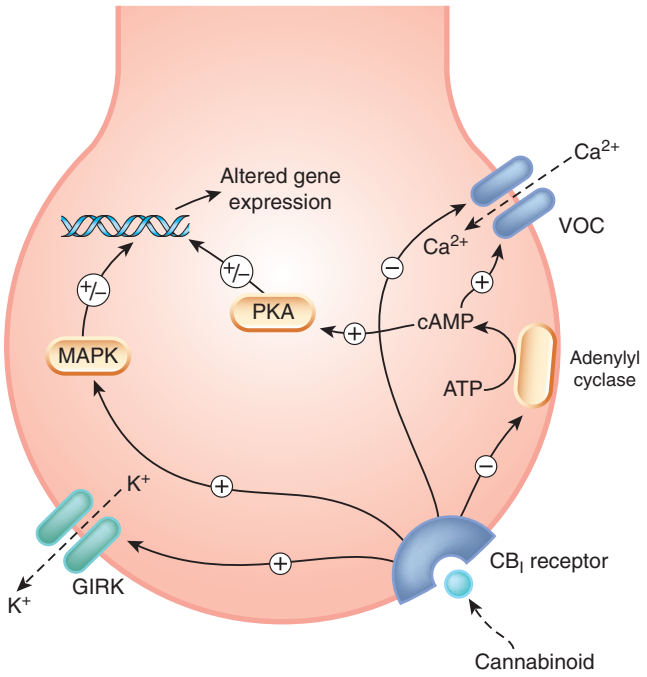


Fig. 18.2 Cellular actions of cannabinoids. CB_1 receptor activation inhibits neurotransmitter release via inhibition of Ca^{2+} entry and hyperpolarisation due to activation of potassium channels. It also alters gene expression. GIRK: G-protein-sensitive inward-rectifying potassium channel; MAPK: mitogen-activated protein kinase; PKA: protein kinase A; VOC, voltage-operated calcium channel. (Redrawn from Devane et al. 1992.)

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 through activation of CB_1 receptors, an action that could contribute to their effect on body weight (Cota et al., 2003).

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 (Ch. 36). 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 18.1). 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. 41). 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.

Table 18.1 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	
Virhodamine	$CB_2 > CB_1$
Noladin	$CB_1 >> CB_2$
N-Arachidonoyl dopamine	$CB_1 >> CB_2$

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. 17), the structure of which is shown in Figure 18.1. 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. 18.3). A few years later, a second endocannabinoid, *2-arachidonoyl glycerol* (2-AG, Fig 18.1), was identified, and more recently three further endocannabinoid candidates with distinct CB_1/CB_2 (see below) receptor selectivities have been added to the list (Table 18.1). Endocannabinoids are made 'on demand' like eicosanoids (see Ch. 17), rather than being presynthesised and stored for release when needed.

BIOSYNTHESIS OF ENDOCANNABINOIDS

Biosynthesis of anandamide and of 2-AG is summarised in Figure 18.4. 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 recently cloned *sn*-1-selective diacylglycerol lipases (DAGL-α and DAGL-β), which

[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!

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

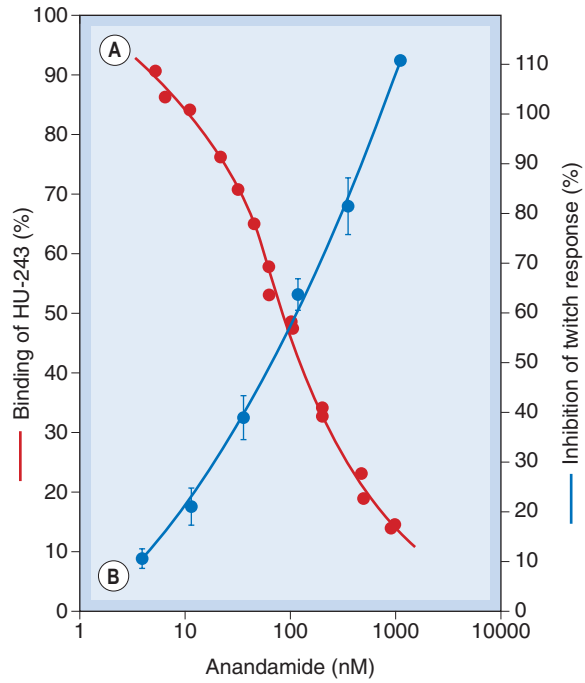


Fig. 18.3 Anandamide as an endocannabinoid. Anandamide is an endogenous cannabinoid. [**A**] Competitive inhibition of tritiated HU-243 (a cannabinoid receptor ligand) binding to synaptosomal membranes from rat brain by natural anandamide (red circles, left hand ordinate axis). [**B**] Inhibition of 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.)

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 below) in adult brain.

Little is known as yet about the biosynthesis of the more recent endocannabinoid candidates noladin, virhodamine and *N*-arachidonoyl dopamine. pH-dependent non-enzymatic interconversion of vihrodamine and anandamide is one possibility, and could result in a switch between CB_2- and CB_1-mediated responses (see Table 18.1).

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 18.4. 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. 43 for an

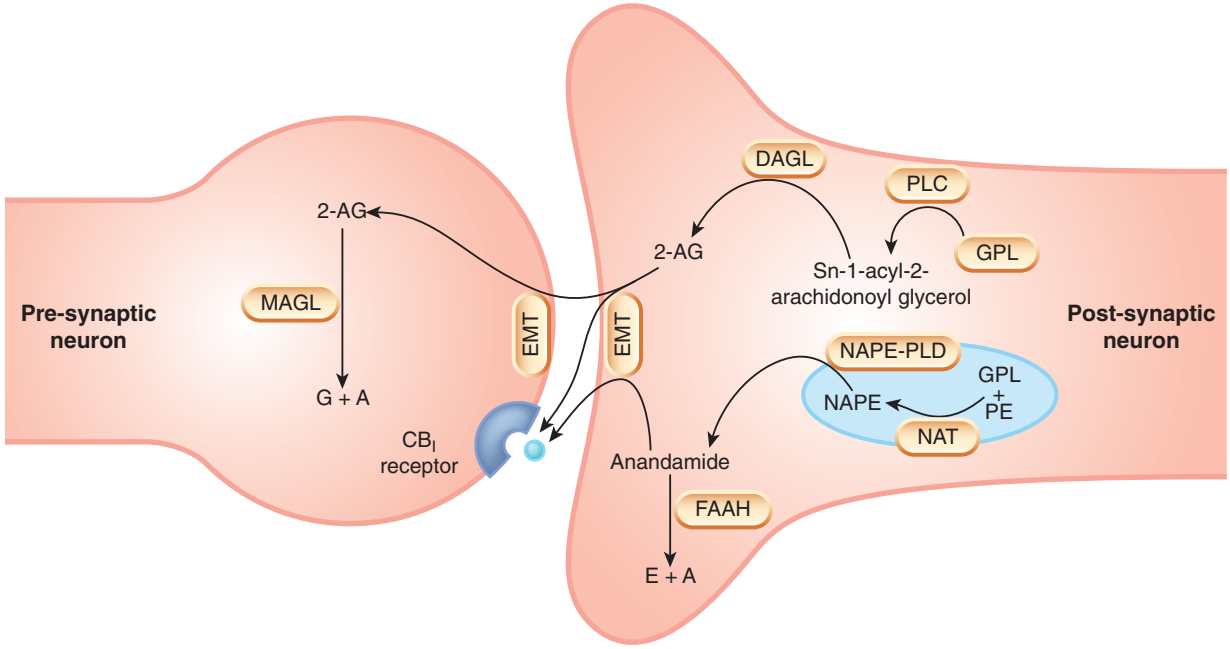


Fig. 18.4 Biosynthesis and inactivation of endocannabinoids. 2-AG, 2-arachidonoyl glycerol; A, arachidonic acid; DAGL, diacylglycerol lipase; E, ethanolamine; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; GPL, glycerophospholipid; MAGL, monoacyl glycerol lipase; NAPE, *N*-acyl-phosphatidylethanolamine; NAPE-PLD, *N*-acyl phosphatidylethanolamine-specific phopholipase D; NAT, *N*-acyl-transferase; PE, phosphatidylethanolamine; PLC, phospholipase C.

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. 17).

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 very incompletely defined. Increased intracellular Ca^{2+} concentration is probably an important cellular trigger because, as mentioned above, 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. 41.3) and long-term potentiation in the hippocampus (Fig. 37.7). 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. 18.4) fit nicely with the idea that the endocannabinoid 2-AG could be a 'retrograde' messenger in DSI (see Fig. 38.8).

Neuromodulatory actions of endocannabinoids could influence a wide range of physiological activities, including nociception, cardiovascular, respiratory and gastrointestinal function. Hypothalamic hormone interactions could 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 (reviewed by Wang et al., 2006). Effects of endocannabinoids on food intake are of particular interest, because of the importance of obesity (Ch. 29).

PATHOLOGICAL INVOLVEMENT

There is evidence, both from experimental animals and from human tissue, that endocannabinoid signalling is abnormal in various neurodegenerative diseases (see Ch. 39). 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 Wang et al., 2006) 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. 17).
- 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.
- **Rimonabant**, an antagonist at CB_1 receptors, causes sustained weight loss and may promote abstinence from tobacco.

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 41 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. 29). The cloning of CB_2 receptors, and their absence from healthy brain, 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, but was withdrawn

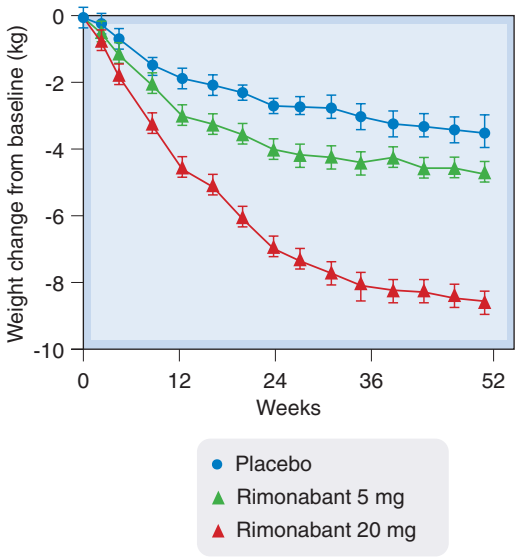


Fig. 18.5 Change from baseline in body weight in a double-blind, placebo-controlled trial of rimonabant versus placebo in 1507 overweight patients. (Redrawn from Van Gaal et al., 2005.)

because of 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. A substantial randomised controlled trial of THC in patients with multiple sclerosis found no objective evidence of benefit on spasticity but improved mobility (see also Ch. 39). 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 (see Pacher et al., 2006 for a review of emerging therapeutic targets) are given in the clinical box.

The CB_1 receptor antagonist **rimonabant**, combined with a reduced calorie diet, caused a dose-related weight loss (of approximately 6 kg at the higher dose) after 12 months' treatment in one placebo-controlled trial (see Fig. 18.5, and see also Ch. 29). Adverse effects included nausea and diarrhoea, and importantly depression and other psychological disturbances which led to its withdrawal from clinical use as an anorectic agent, but interest remains in the therapeutic potential of blocking the endocannabinoid system in order to reduce weight and improve cardiometabolic risk factors (reviewed by Samaha & Chou, 2009).

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 L-dopa; see Ch. 39).
- Antagonists:
  - obesity
  - tobacco dependence
  - drug addiction
  - alcoholism.

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19 Peptides and proteins as mediators

OVERVIEW

In this chapter we consider the special features of peptides and proteins, which are ubiquitous chemical mediators, and whose characteristics differ in some important respects from the small molecule mediators discussed in earlier chapters. The field is advancing rapidly through the application of molecular biological techniques, and many clinical applications are in prospect.

INTRODUCTION

Pharmacology has traditionally concerned itself with signalling molecules that are of low molecular weight and non-peptide in nature. Since the 1970s, however, it has emerged that peptides and proteins are at least as important—maybe even more so—as signalling molecules. Yet the pharmacological manipulation of peptide signalling is less advanced than that of, say, the cholinergic, adrenergic or 5-hydroxytryptamine systems (Chs 12–14). Pharmacology, one could say, has some catching up to do. In this chapter, we give an overview of the main characteristics of peptides and proteins as mediators and as drugs, bringing out the contrasts between these and non-peptides, and we evaluate the present and possible future use of peptides as therapeutic agents. For reviews, with more detail than can be provided here, see Buckel (1996), Cooper et al. (1996), Hökfelt et al. (2000) and Nestler et al. (2001).

HISTORICAL ASPECTS

▼ Despite the fact that some peptide mediators were discovered early in the history of our discipline (e.g. substance P was discovered in the 1930s), pharmacology has historically harboured a strong bias towards non-peptides. One reason for this apparently irrational dislike is that, at one time, most drugs were natural (mainly plant) products. Very few were peptides or acted through what we now recognise as peptide signalling systems. A second reason is that the methodology required to study peptides is of more recent origin. The development of high-performance liquid chromatography and solid-phase peptide synthesis, and the use of antibodies for radioimmunoassay and immunocytochemistry, as well as the use of molecular biology, have greatly accelerated the development of the area.

In 1953, du Vigneaud made history, and earned a Nobel Prize, by determining the structure of, and subsequently synthesising, oxytocin, the first peptide mediator to be characterised and the first to be produced commercially for clinical use. The structures of many other mediators, for example substance P, bradykinin and angiotensin, which had been identified as peptides in the 1930s, remained unsolved for many years. While all are small peptides of 11 residues or fewer, determination of their structure, and their total chemical synthesis, was a Herculean effort. The structure of bradykinin was not elucidated until 1960, while that of substance P was published in 1970.

By contrast, the use of contemporary techniques enabled endothelin (a much larger peptide) to be fully characterised, synthesised and cloned within about a year, the complete information being published in a single paper (Yanagisawa et al., 1988). Protein mediators, such as cytokines and growth factors (Ch. 17) containing 50 or more residues are still difficult to synthesise chemically, and major advances must rely largely on molecular biology. The use of recombinant proteins as therapeutic agents—a development driven mainly by the emergent biotechnology industry—is rapidly gaining ground (see Ch. 59). Whereas the discovery of new 'small molecule' mediators has virtually dried up, the discovery of new protein and peptide mediators continues apace.

GENERAL PRINCIPLES OF PEPTIDE PHARMACOLOGY

STRUCTURE OF PEPTIDES

Peptide and protein mediators generally vary from 3 to about 200 amino acid residues in size (Fig. 19.1), the arbitrary dividing line between peptides and proteins being about 50 residues. For convenience, in this chapter, we use the term peptide to cover both classes. Specific residues in peptides often undergo post-translational modifications, such as C-terminal *amidation*, *glycosylation*, *acetylation*, *carboxylation*, *sulfation* or *phosphorylation*. They also may contain *intramolecular* disulfide bonds, such that the molecule adopts a cyclic or partially cyclic conformation; or they may comprise two or more separate chains linked by *intermolecular* disulfide bonds.

It is difficult to determine the conformation of peptides in solution because they are so flexible, and peptides of fewer than about 40 residues have proved difficult to crystallise, precluding the use of X-ray diffraction methods to study their conformation (although some other techniques, such as nuclear magnetic resonance, have proved helpful). Larger proteins adopt more restricted conformations, but because of their size they generally interact with multiple sites on their receptors. To envisage peptides fitting into a receptor site in a precise 'lock and key' mode is to imagine that you can unlock your front door with a length of cooked spaghetti. Such problems have greatly impeded the rational design of non-peptide analogues (*peptidomimetics*) that mimic the action of peptides at their receptors. 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 (see below; Betancur et al., 1997—an exception is the opioid field; see Ch. 41).

TYPES OF PEPTIDE MEDIATOR

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:

1. *Neurotransmitters and neuroendocrine* mediators (discussed further in this chapter).
2. *Hormones from non-neural sources*: these comprise (a) plasma-derived peptides, notably angiotensin

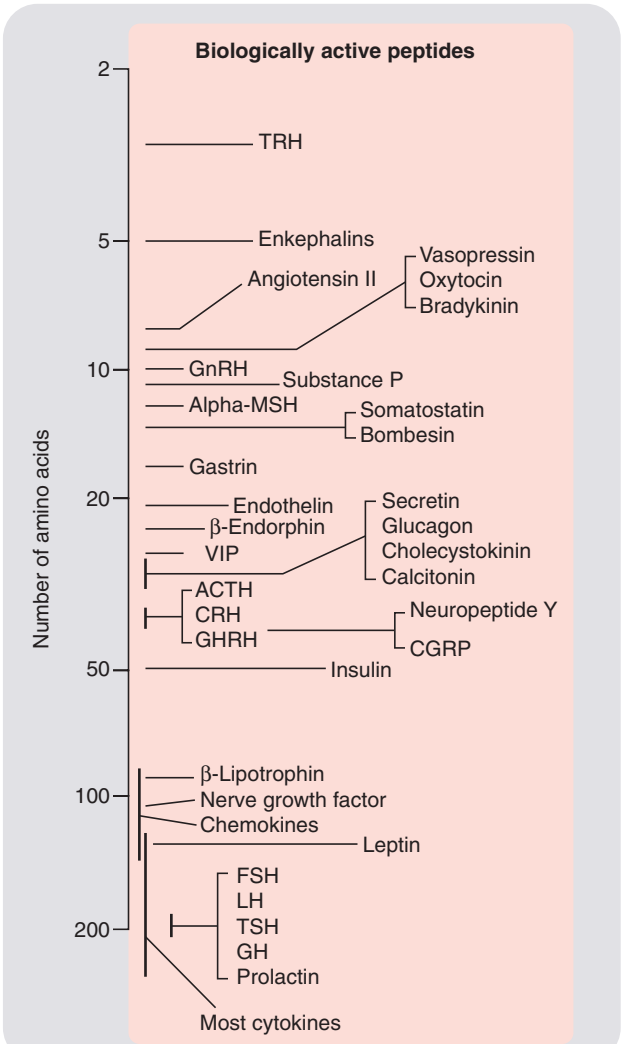

Fig. 19.1 Some typical peptide mediators. ACTH, adrenocorticotrophic hormone; Alpha-MSH, α-melanocyte-stimulating hormone; CGRP, calcitonin gene-related peptide; CRH, corticotrophin-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotrophin-releasing hormone; LH, luteinising hormone; TRH, thyrotrophin-releasing hormone; TSH, thyroid-stimulating hormone; VIP, vasoactive intestinal peptide.

(Ch. 22) and bradykinin (Ch. 17), and (b) substances such as insulin (Ch. 30) endothelin (Ch. 22), atrial natriuretic peptide (Ch. 21) and leptin (Ch. 31).

3. *Growth factors*: produced by many different cells and tissues that control cell growth and differentiation (see Ch. 5).
4. *Mediators of the immune system* (cytokines and chemokines; see Ch. 17).

Some important examples of peptide and protein mediators are shown in Figure 19.1.

ROLE OF MOLECULAR BIOLOGY

▼ Because peptide structures are represented directly in the genome, molecular biology has been the key to most of the recent advances in knowledge. It is used in many ways, as in the following examples.

- *Cloning of the genes encoding peptide precursors* has shown how several active peptides can arise from a single precursor protein. *Calcitonin gene-related peptide* (CGRP) was discovered in this way.
- *Cloning of the genes encoding peptide receptors* has revealed that most belong either to the class of G-protein-coupled receptors or the tyrosine kinase-linked receptors (see Ch. 3). Very few peptides act on ligand-gated channels.
- Several new peptide mediators have been discovered by *screening for ligands of 'orphan receptors'* (see Civelli et al., 2001). Searching in an extract of brain peptides for possible ligands for an opioid receptor-like orphan (called ORL1) led to the identification of the novel neuropeptide *nociceptin* (Meunier et al., 1995). When the gene encoding nociceptin was cloned, it was found also to encode another peptide, *nocistatin* that acted on yet another receptor (see Okuda-Ashitaka & Ito, 2000). The discovery of *orexins* (peptides involved in appetite and obesity; see Ch. 31) arose through similar molecular orienteering.
- 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.
- Transgenic animals with peptide or receptor genes deleted or overexpressed provide valuable clues to the functions of novel peptides. Antisense oligonucleotides and RNA interference techniques (see also Ch. 59) can also be used to silence such genes.

PEPTIDES IN THE NERVOUS SYSTEM: COMPARISON WITH CONVENTIONAL TRANSMITTERS

The abundance of neuropeptides in the brain and elsewhere became evident in the 1970–1980s, and new examples are still emerging. In most respects, neuropeptide-mediated transmission resembles transmission by 'conventional' non-peptide mediators; the mechanisms for peptide storage and release (summarised in Fig. 19.2) and the receptor mechanisms through which their effects are produced are essentially the same in both cases. One difference is that the vesicles are loaded with peptide precursors in the cell body, the active peptides being generated within the vesicles as they move to the nerve terminals. Following exocytosis, the vesicles cannot be reloaded in situ but must instead be replaced with new preloaded vesicles. Transmitter turnover is therefore less rapid than with conventional mediators, and recapture of the released transmitter does not occur.

As with other chemical mediators, the effects of peptides may be excitatory or inhibitory, pre- or postsynaptic, and exerted over short or long distances from the site of release. There are, however, certain monopolies of function between peptide and non-peptide mediators. For example, endogenous peptides rarely activate ligand-gated ion channels,[1]

[1]But there are non-physiological exceptions. Some spider venom peptides, for example, produce pain by activating the ion-channel linked capsaicin receptor TRPV1.

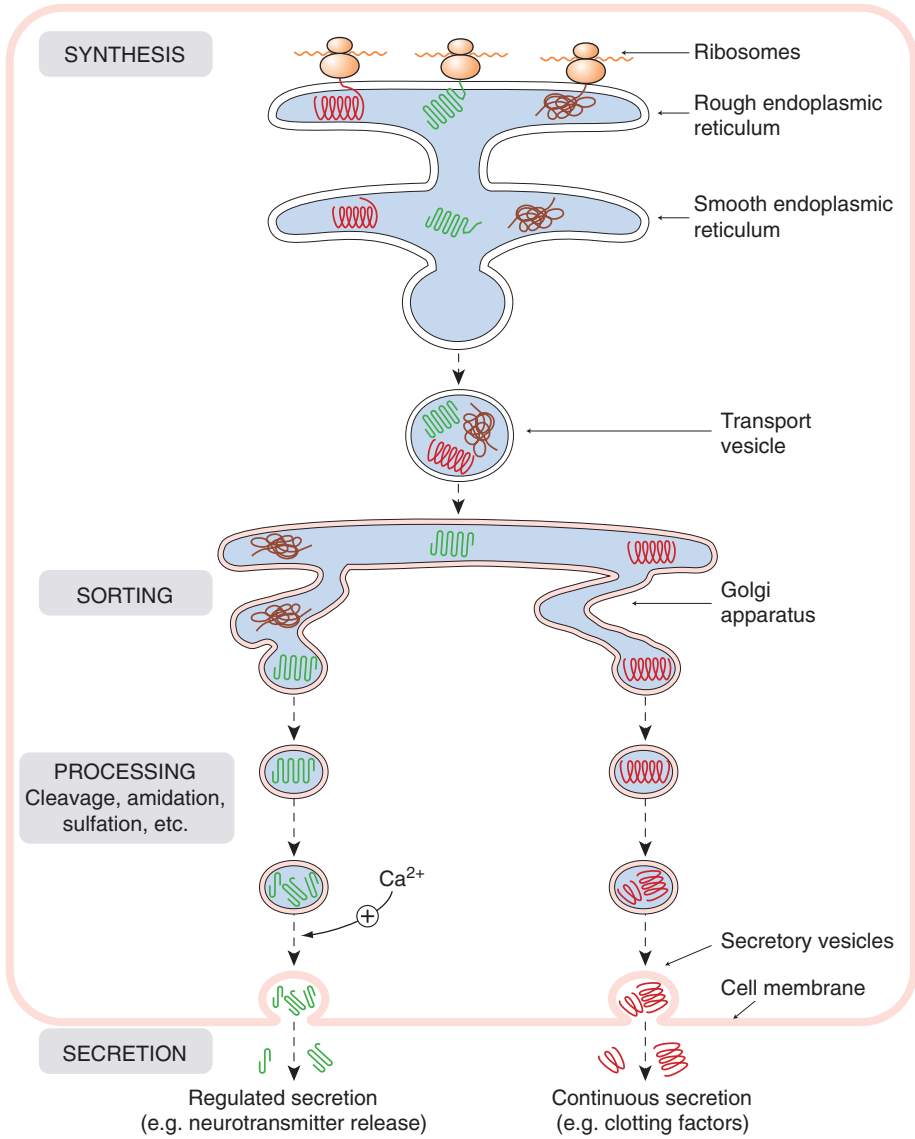

Fig. 19.2 Cellular mechanisms for peptide synthesis and release. Proteins synthesised by ribosomes are threaded through the membrane of the rough endoplasmic reticulum, from where they are conveyed in transport vesicles to the Golgi apparatus. Here, they are sorted and packaged into secretory vesicles. 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.

and therefore do not function as fast neurotransmitters in the manner of non-peptides such as acetylcholine, glutamate, glycine or GABA (see Chs 13 and 37). Instead, they serve (as do many non-peptides) mainly as neuromodulators, by activating G-protein-coupled receptors. In contrast, the ligands for tyrosine kinase-linked receptors are all peptides or proteins.

In summary, the similarities in function between peptide and non-peptide mediators are more striking than the differences. The main difference stems from the fact that peptides, being gene products, represent variations on a single theme—a linear string of amino acids. Such sequences are much more susceptible to evolutionary change than are the structures of non-peptide mediators, and the number of known peptide mediators now greatly exceeds that of non-peptides. As Iversen pointed out in 1983: 'almost overnight, the number of putative transmitters in the mammalian nervous system has jumped from the ten or so monoamine and amino acid candidates to more than 40'. Since then, no new monoamine transmitters have appeared, but there are at least another 60 peptides.

The role of peptides as co-transmitters is discussed in Chapter 12. Two well-documented examples (reviewed by Lundberg, 1996) are the parasympathetic nerves innervating the salivary glands (where the secretory response is produced by acetylcholine and the vasodilatation partly

by *vasoactive intestinal peptide*) and the sympathetic innervation to many tissues, which releases the vasoconstrictor *neuropeptide Y* in addition to noradrenaline (norepinephrine).

The distinction between neuropeptides and peripherally acting hormones is useful but not absolute. Thus the incretins and insulin (Ch. 30), angiotensin, atrial natriuretic peptide (Chs 21 and 22) and oxytocin (Ch. 34) are best known as hormones that are formed, released and act in the periphery. They are, however, also found in the brain, although their role there is uncertain. Similarly, endothelin (Ch. 22) was first discovered in blood vessels but is now known to occur extensively in the brain as well.

MULTIPLE PHYSIOLOGICAL ROLES OF PEPTIDES

▼ In common with many non-peptide mediators, such as noradrenaline, dopamine, 5-hydroxytryptamine or acetylcholine, the same peptides may function as mediators in several different organs, and intriguingly often appear to subserve some coordinated physiological function. For example, angiotensin acts on the cells of the hypothalamus to release antidiuretic hormone (vasopressin), which in turn causes water retention. Angiotensin also acts elsewhere in the brain to promote drinking behaviour and to increase blood pressure by activation of the sympathetic system; in addition, it releases aldosterone, which causes salt and water retention and acts directly to constrict blood vessels. Each of these effects plays a part in the overall response of the body to water deprivation and reduced circulating volume. There are other examples of what appears to be an orchestrated functional response produced by the various actions of a single mediator, but there are many more examples where the multiple effects seem just to be exactly that—multiple effects.

So far, the stream of new information about neuropeptides since the 1970s has led to few useful generalisations about their functional role, and surprisingly few new drugs—with the exception of antihypertensive drugs acting on the renin–angiotensin system (see Ch. 22), most of which are peptidomimetics. For whatever reason, peptide pharmacology has proved to be something of a graveyard for drug discovery projects. For example, substance P antagonists were confidently expected to be effective analgesic drugs based on copious data from animal studies, but proved to have no analgesic activity in humans, although one such drug, **aprepitant**, has been found to have a role in preventing vomiting caused by cisplatin-based cytotoxic chemotherapy (Ch. 55). They also have unexpected anxiolytic properties.

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 simpler. Peptide synthesis (Fig. 19.3) begins with the manufacture of a precursor protein in which the peptide sequence is embedded, along with specific proteolytic enzymes that 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. 19.2). 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 non-peptide mediators.

PEPTIDE PRECURSORS

The precursor protein, or *preprohormone*, 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 in which several copies of active peptide fragments may be contained. Often, several different peptides are found within one precursor, but sometimes there is only one in multiple copies. An extreme example occurs in the invertebrate *Aplysia*, in which the precursor contains 28 copies of the same short peptide. The signal peptide, 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*, of which two subtypes (PC1 and PC2) have been studied in detail (see Cullinan et al., 1991). Scrutiny of the prohormone sequence often reveals likely cleavage points that demarcate 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 preprohormones, which reflects the level of gene expression, is very sensitive to physiological conditions, and this type of transcriptional control is one of the main mechanisms by

Structure and function of peptide mediators

- Size varies from three to several hundred amino acid residues. Conventionally, molecules of fewer than 50 residues are called peptides, larger molecules being proteins.
- Neural and endocrine mediators range in size from 3 to over 200 residues. Cytokines, chemokines and growth factors are generally larger than 100 residues.
- Most known peptide mediators come from the nervous system and endocrine organs. However, some are found in the plasma, and many occur at other sites (e.g. vascular endothelium, heart, cells of the immune system). The same peptide may occur in several places and serve different functions.
- Small peptides and chemokines act mainly on G-protein-coupled receptors, and act through the same second messenger systems as those used by other mediators. Cytokines and growth factors generally act through tyrosine kinase-linked membrane receptors.
- Peptides frequently function in the nervous system as co-transmitters with other peptides or with non-peptide transmitters.
- The number of known peptide mediators now greatly exceeds that of non-peptides.

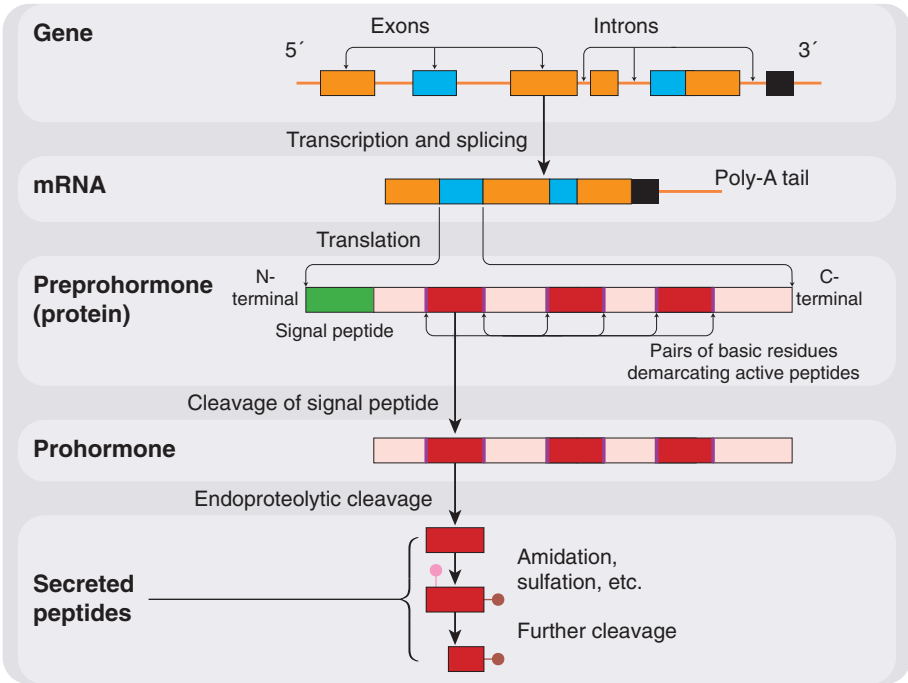

Fig. 19.3 Synthesis of a peptide mediator. The coding regions of the gene (exons) are transcribed and spliced to give rise to mRNA, segments of which (blue) are translated to produce preprohormones. Cleavage of the N-terminal signal peptide produces the prohormone, from which endopeptidases excise peptide fragments. These may be active as such, or they may undergo further post-translational processing (amidation etc.).

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, which is important in the genesis of inflammatory pain (see Ch. 41).

DIVERSITY WITHIN PEPTIDE FAMILIES

▼ Peptides commonly occur in families with similar or related sequences and actions. Opioid peptides (see Ch. 41) provide a good example of the representation of such a family at the genomic level. *Opioid peptides*, defined as peptides with opiate-like pharmacological effects, are coded by three distinct genes whose products are, respectively, *prepro-opiomelanocortin* (POMC), *preproenkephalin* and *prepro-dynorphin*. Each of these precursors contains the sequences of a number of opioid peptides (Fig. 19.4). Hughes and Kosterlitz, who discovered the enkephalins in 1975, noticed that the sequence of *met-enkephalin* is contained within that of a pituitary hormone, β-*lipotrophin*. About this time, three other peptides with morphine-like actions were discovered, *α-, β- and γ-endorphin*, which also were contained within the β-lipotrophin molecule. It was then found that the enkephalins actually come from the other gene products, *proenkephalin* and *prodynorphin*, POMC itself serving as a source of *adrenocorticotrophic hormone* (ACTH), *melanocyte-stimulating hormones* (MSH) and β-endorphin, but not of enkephalins.

The expression of the precursor proteins varies greatly in different tissues and brain areas. For example, POMC and its peptide products are found mainly in the pituitary and hypothalamus, whereas endorphin, met-enkephalin, leu-enkephalin and dynorphin are more widely distributed. In the spinal cord, dynorphin occurs mainly in interneurons, while the enkephalins are found mainly in long descending pathways from the midbrain to the dorsal horn. Opioid peptides are also produced by many non-neuronal cells, including endocrine and exocrine glands and cells of the immune system, as well as in brain areas distinct from those involved in nociception, and correspondingly they play a regulatory role in many different physiological systems, as reflected in the rather complex pharmacological properties of opiate drugs.

Diversity of members of a peptide family can also arise by *gene splicing* or during *post-translational processing* of the prohormone.

Gene splicing as a source of peptide diversity

▼ Genes contain coding regions (exons) interspersed with non-coding regions (introns), and when the gene is transcribed, RNA (*heterologous nuclear RNA*; hnRNA) is spliced to remove the introns and some of the exons, forming the final mRNA that is translated. Control of the splicing process allows a measure of cellular control over the peptides that are produced. Good examples of this are calcitonin/CGRP and substance P/neurokinin A.

The calcitonin gene codes for calcitonin itself (Ch. 35) and also for a completely dissimilar peptide, CGRP. Alternative splicing allows cells to produce either *procalcitonin* (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. The control of the splicing process is not well understood.

Post-translational modifications as a source of peptide diversity

▼ Many peptides, such as tachykinins and peptides related to ACTH (see Ch. 32), must undergo enzymatic amidation at the C-terminus to acquire full biological activity. Tissues may also generate peptides of

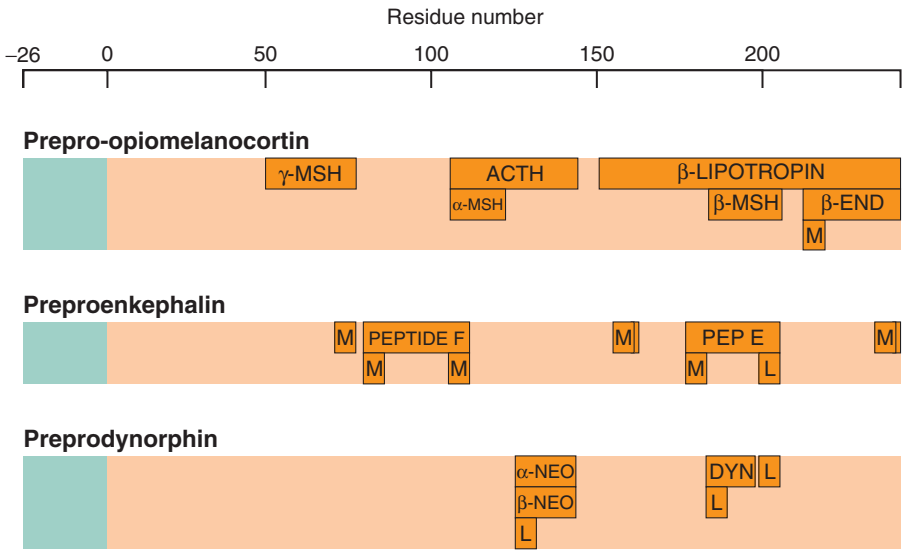

Fig. 19.4 **Opioid precursors.** Structures of the three opioid precursor proteins, showing the location of opioid and other peptides within the sequence. These embedded peptides are bounded by pairs of basic amino acids, which form points of attack for enzymatic cleavage. The signal peptide sequence is shown in green. β-END, β-endorphin; ACTH, adrenocorticotrophic hormone; DYN, dynorphin; L, leucine enkephalin; M, methionine enkephalin; MSH, melanocyte-stimulating hormone; NEO, neoendorphin.

varying length from the same primary sequence by the action of specific peptidases that cut the chain at different points. For example, procholecystokinin (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; Ch. 17), 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.

In some cases cyclic peptides may be produced. This is often seen in plant and fungal tissues and some of the products are pharmacologically important (e.g. ciclosporin; Ch. 26).

PEPTIDE TRAFFICKING AND SECRETION

The basic mechanisms by which peptides are synthesised, packaged into vesicles, processed and secreted are summarised in Figure 19.2 (see review by Perone et al., 1997). 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 mainly by 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 their selective release. Identification of the specific 'trafficking' proteins involved in particular secretory pathways may yield novel drug targets for the selective control of secretion, but the prospect is still some way off, and conventional receptor-based pharmacology will be the basis for shorter-term therapeutic developments.

Biosynthesis and release of peptides

- The genetically coded *preprohormone* is a large protein comprising a signal sequence (involved in transfer of the protein across the membrane) and the *prohormone*, containing the embedded sequences of one or more active peptides.
- The active peptides are produced intracellularly by selective enzymic cleavage, centred on pairs of adjacent Arg or Lys residues. In most cases, the active peptides are stored (often in vesicles) in a releasable form.
- A single precursor gene may give rise to several peptides by selective mRNA splicing before translation, by selective cleavage of the prohormone or by post-translational modification.
- Peptides and proteins are located in intracellular vesicles, which are budded off from the endoplasmic reticulum and Golgi apparatus.
- After sorting and post-translational processing of the peptide products, the vesicles differentiate into secretory vesicles, which discharge their contents by exocytosis.
- With constitutive release (e.g. plasma proteins, clotting factors), secretory vesicles are discharged as soon as they are formed, and secretion is continuous. With regulated release (neuropeptides and endocrine peptides), exocytosis is controlled by intracellular Ca^{2+}, as with release of conventional transmitters.
- There are many examples of closely related peptides, presumably produced by divergent evolution from a single gene, with different locations and physiological functions.

PEPTIDE ANTAGONISTS

Although selective antagonists are available for the great majority of non-peptide receptors, only a few peptide antagonists are so far in clinical use, although their therapeutic potential is considerable (see Betancur et al., 1997). Substitution into endogenous peptides of unnatural amino acids, such as D-amino acids, sometimes produces excellent antagonists. This strategy was successful in the case of substance P, angiotensin and bradykinin. However, for reasons discussed below, such peptide antagonists are of little use therapeutically, so effort has been channelled instead into discovering non-peptides that bind to peptide receptors. In a few cases, 'peptoids' have been produced by modifying the peptide backbone, while retaining as far as possible the disposition of the side-chain groups that are responsible for binding to the receptor. Such compounds have been developed as antagonists for several peptide receptors (e.g. CCK and neuropeptide Y). In other cases, random screening of large compound libraries has succeeded where rational approaches failed, resulting in highly potent and selective antagonists, some of which are in use, or under development, as therapeutic agents. The most important peptide receptor antagonists in clinical use, all of them non-peptides, include:

- **naloxone**, **naltrexone** (μ-opioid receptors): used to antagonise opiate effects (see Ch. 41)
- **losartan**, **valsartan**, etc. (angiotensin AT_1 receptors): used as antihypertensive drugs (see Ch. 22)
- **bosentan** (endothelin ET_1/ET_2 receptors): used in the treatment of pulmonary hypertension (see Ch. 22).

Antagonists for many other peptides, including bradykinin, substance P, CGRP, corticotrophin-releasing factor, neuropeptide Y, neurotensin, oxytocin, antidiuretic hormone and somatostatin, have been discovered but, with some notable exceptions (e.g. the oxytocin antagonist **atosiban**; see Ch. 34, and the substance P antagonist **aprepitant**; see Ch. 55), have not yet been developed for clinical use. Details can be found in Alexander et al. (2006) and in the review by Betancur et al. (1997).

▼ Few, if any, *agonists* at peptide receptors have been discovered by random screening, and morphine-like compounds are probably the most important clinical examples of non-peptide agonists at peptide receptors. It is becoming increasingly clear, however, that some peptide receptors are 'promiscuous', in that they can bind both peptide and non-peptide ligands. A recent example is that of the formyl peptide receptor (FPR) family of G-protein-coupled receptors, one member of which (FPRL1/ALX) recognises a whole range of molecular species including the bacterial tripeptide fMLP, several endogenous *anti-inflammatory* proteins and peptides including annexin A1 as well as the anti-inflammatory lipid lipoxin A_4 (Ch. 16). Binding of these ligands probably occurs at different receptor domains but, in an additional twist, this receptor can also recognise *proinflammatory* substances such as serum amyloid A and correctly transduce the appropriate signal to the cell (see Ye et al., 2009). How this occurs and what makes non-peptides chemically recognisable by peptide receptors is incompletely understood, much to the frustration of medicinal chemists who would dearly like to be able to design such compounds de novo. There remain many peptide mediators for which no antagonists are known, but strenuous efforts are being made to fill this gap in the hope of developing new therapeutic agents.

Not surprisingly, it has proved easier to find synthetic compounds that block receptors for small peptides (e.g. most neuropeptides), which have only a few points of attachment, than for large peptides and proteins (e.g. cytokines and growth factors), which can interact with the receptor at many points. These receptors are not easily fooled by small molecules, and efforts to target them therapeutically rely on protein-based approaches (see below).

PROTEINS AND PEPTIDES AS DRUGS

Many proteins, including hormones, antibodies, decoy receptors, cytokines, enzymes and clotting factors, are registered for use as therapeutic agents in specific conditions; they are mainly given by injection but occasionally by other routes (see Table 19.1). Many of the proteins currently in therapeutic use are functional human proteins prepared by recombinant technology, which are used to supplement the action of endogenous mediators. Although their preparation requires advanced technology, such proteins are relatively straightforward to develop as drugs, because they rarely cause toxicity (although some may be immunogenic) and have a more predictable therapeutic effect than synthetic drugs.

While clearly different from conventional drugs in many ways, the same principles of *pharmacodynamics* apply to proteins and peptides although their *pharmacokinetic* properties are usually radically different from those of their small-molecule cousins, largely because of the way that they are metabolised (see Lin, 2009, for a good discussion of this point).

'Designer proteins'—genetically engineered variants of natural proteins—for specific purposes are already a reality (see Ch. 59). Examples include 'humanised antibodies' and fusion proteins consisting of an antibody (targeted, for

Peptides and proteins as drugs

- Despite the large number of known peptide mediators, only a few peptides, mostly close analogues of endogenous mediators, are currently useful as drugs.
- In most cases, peptides make poor drugs, because:
  - they are poorly absorbed when given orally
  - they have a short duration of action because of rapid degradation in vivo
  - they do not predictably cross the blood–brain barrier
  - they are expensive and difficult to manufacture
  - they may be immunogenic.
- Peptide antagonists were slow to be discovered, but many are now available for experimental purposes and in development as therapeutic agents.
- Important peptide antagonists used clinically include *naloxone*, *losartan* and *bosentan*.
- Protein-based therapeutic agents are of growing importance and include hormones (e.g. *insulin*, *growth hormone*), clotting factors, cytokines, antibodies and enzymes. In many cases, these are produced using recombinant technology.
- 'Designer proteins' prepared by recombinant techniques are expected to play an increasing therapeutic role in the future.

Table 19.1 Some peptide and protein drugs

Drug	Use	Route
Peptides		
Captopril/enalapril (peptide related)	Hypertension, heart failure (Ch. 22)	Oral
ADH, desmopressin and lypressin	Diabetes insipidus (Ch. 30)	Intranasal, injection
Oxytocin	Induction of labour (Ch. 34)	Injection
GnRH analogues (e.g. buserelin)	Infertility, suppression of ovulation (Ch. 34), prostate and breast tumours	Intranasal, injection
ACTH	Diagnosis of adrenal insufficiency (Ch. 32)	Injection
TSH/TRH	Diagnosis of thyroid disease (Ch. 33)	Injection
Calcitonin	Paget's disease of bone (Ch. 35)	Intranasal, injection
Insulin	Diabetes (Ch. 30)	Injection
Somatostatin, octreotide	Acromegaly, gastrointestinal tract tumours (Ch. 32)	Intranasal, injection
Growth hormone	Dwarfism (Ch. 32)	Injection
Ciclosporin	Immunosuppression (Ch. 26)	Oral
F(ab) fragment	Digoxin overdose	Injection
Proteins		
Streptokinase, TPA	Thromboembolism (Ch. 24)	Injection
Asparaginase	Tumour chemotherapy (Ch. 55)	Injection
DNAase	Cystic fibrosis (Ch. 27)	Inhalation
Glucocerebrosidase	Gaucher's disease	Injection
Interferons	Tumour chemotherapy (Chs 17 and 55), multiple sclerosis (Ch. 39)	Injection
Erythropoietin, GMCF, etc.	Anaemia (Ch. 25)	Injection
Clotting factors	Clotting disorders (Ch. 24)	Injection
Monoclonal antibodies (e.g. TNF-α)	Inflammatory diseases (Chs 6, 26)	Injection, infusion
Antibodies, vaccines, etc.	Infectious diseases	Injection, oral
Enfurvitide	HIV infection (Ch. 51)	Injection

ACTH, adrenocorticotrophic hormone; ADH, antidiuretic hormone; GMCF, granulocyte colony-stimulating factor; GnRH, gonadotrophin-releasing hormone; TNF-α, tumour necrosis factor-α; TPA, tissue plasminogen activator; TRH, thyrotrophin-releasing hormone; TSH, thyroid-stimulating hormone.

example, at a tumour antigen) or a peptide (e.g. bombesin or somatostatin, which bind to receptors on tumour cells) linked to a toxin (such as ricin or diphtheria toxin) to kill the target cells (see Ch. 55). Many ingenious ideas are being explored, and some prophets anticipate the dawn of a new era of therapeutics, as the hegemony of small-molecule therapeutics begins to fade. Pharmacologists, needless to say, are somewhat sceptical, but nobody can afford to ignore the potential of biotechnology-based therapeutics in the future. A full discussion of this exciting area is provided in Ch. 59.

Smaller peptides are used therapeutically mainly when there is simply no viable alternative (e.g. insulin and its designer variants, Ch. 30) but, in general, peptides make bad drugs. There are several reasons for this:

- Most must be administered by injection or nasal spray, because they are poorly absorbed or metabolised in the gut. (An important exception is **ciclosporin**, discussed in Ch. 26, which contains so many unnatural amino acids that no peptidase will touch it.)
- They are expensive to manufacture.
- They usually have a short biological half-life because of hydrolysis by plasma and tissue peptidases, although there are exceptions to this.
- Penetration of the blood–brain barrier is unpredictable.
- They may be immunogenic

A list of some important therapeutic proteins and peptides is given in Table 19.1.

CONCLUDING REMARKS

The physiology and pharmacology of peptides—particularly neuropeptides—has stimulated a formidable corpus of research since the early 1980s, and the flow of data continues unabated. With more than a dozen major families of peptides, and a host of minor players, it is beyond the scope of this book to cover them individually

or in detail. Instead, we will introduce information on peptide pharmacology wherever it has relevance to the physiology and pharmacology under discussion. Examples are bradykinin (Ch. 17) and monoclonal antibodies (Chs 26 and 59) in inflammation; endothelins and angiotensin in cardiovascular regulation (Ch. 22); tachykinins in asthma (Ch. 27); tachykinins and opioid peptides in nociception (Ch. 41); and leptin, neuropeptide Y and orexins in obesity (Ch. 31). Useful general accounts of peptide pharmacology include Sherman et al. (1989), Cooper et al. (1996), Hökfelt (1991), Hökfelt et al. (2000) and Nestler et al. (2001).

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20 Nitric oxide

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.

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 was discovered in the vasculature when it was shown that the *endothelium-derived relaxing factor* described by Furchgott & Zawadzki (1980) is NO (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 nerves, 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, and for the inactivation of NO by haemoglobin (see below).

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 36–38, 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 known isoforms: an *inducible* form (iNOS or NOS-II; 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 so-called *constitutive* forms, which are present under physiological conditions in endothelium (eNOS or NOS-III) and in neurons (nNOS or NOS-I). eNOS 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. The constitutive enzymes generate small amounts of NO, whereas iNOS produces much greater amounts both because of its high activity and because of its abundance, at least in pathological states associated with cytokine release.[1]

▼ 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. Calcium–calmodulin regulates electron transfer within the molecule.

Both nNOS and iNOS are soluble cytosolic enzymes, and eNOS is dually 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, eNOS is associated with *caveolin*, a membrane protein involved in signal transduction. Association of eNOS with caveolin is reversible, dissociation from caveolin activating the enzyme. Oxidised low-density lipoprotein (oxLDL) displaces eNOS from caveolae by binding to endothelial cell CD36 receptors. This depletes the caveolae of cholesterol, disturbing eNOS function.

The nitrogen atom in NO is derived from the terminal guanidino group of L-arginine. NOS enzymes are functionally 'bimodal', in that they combine oxygenase and reductase activities associated with distinct structural domains. 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. 240 below).

L-Arginine is usually present in excess in endothelial cell cytoplasm, so the rate of production of NO is determined by the activity of the enzyme rather than by substrate availability. Nevertheless, very high doses of L-arginine can restore endothelial NO biosynthesis in some pathological states (e.g. hypercholesterolaemia; see below) in which

[1]It is possible that some of the NO made in healthy animals under basal conditions is derived from the action of iNOS, just as the inducible form of cyclo-oxygenase is active under basal conditions (Ch. 17)—whether this is because there is some iNOS 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.

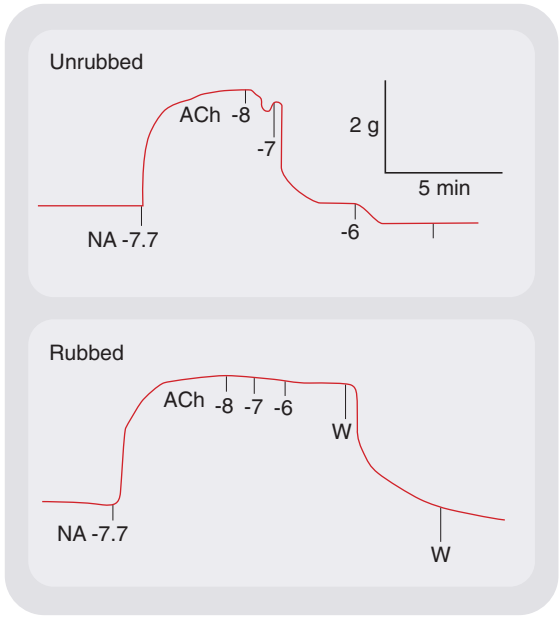

Fig. 20.1 Endothelium-derived relaxing factor. Acetylcholine (ACh) relaxes a strip of rabbit aorta precontracted with noradrenaline (NA) if the endothelium is intact ('unrubbed': upper panel), but not if it has been removed by gentle rubbing ('rubbed': lower panel). The numbers are logarithms of molar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

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 the synthase enzyme, which can become depleted despite apparently plentiful total cytoplasmic arginine concentrations
- competition with endogenous inhibitors of NOS such as *asymmetric dimethylarginine* (ADMA; see below), 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 eNOS or nNOS.
2. Phosphorylation of specific residues on eNOS controls its sensitivity to calcium–calmodulin; this can alter NO synthesis in the absence of any change in $[Ca^{2+}]_i$.

One important physiological stimulus controlling endothelial NO synthesis in resistance vessels is believed to be shear stress. This is sensed by endothelial mechanorecep-

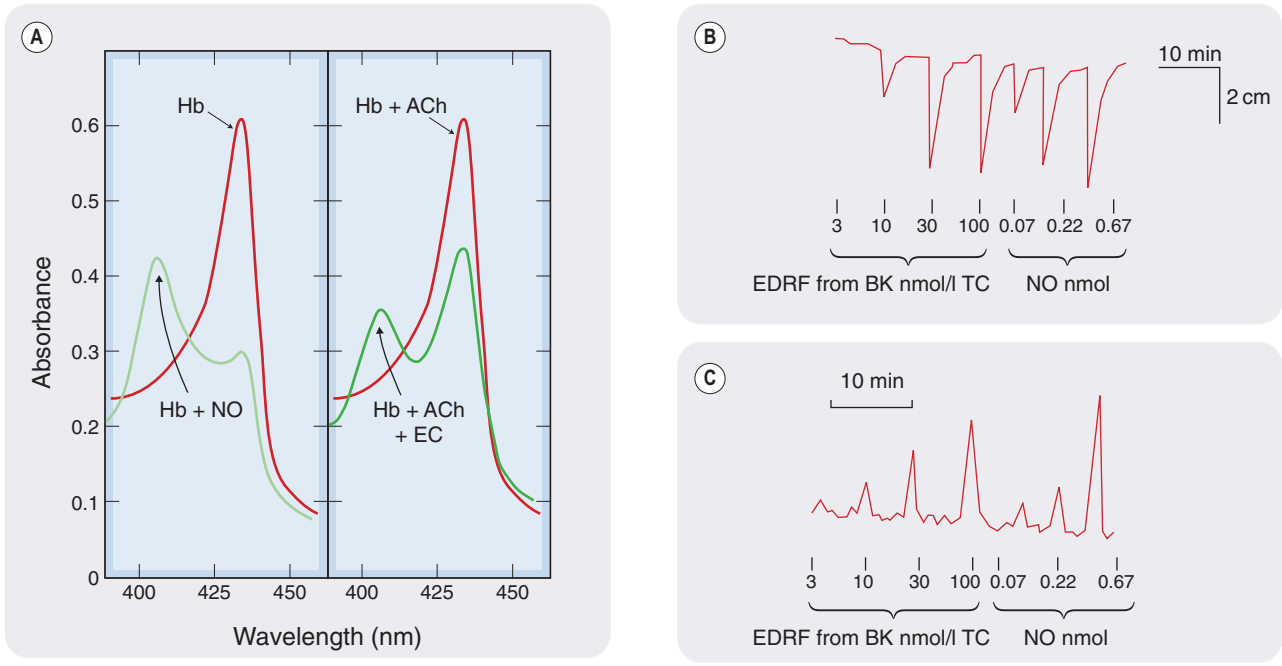

Fig. 20.2 Endothelium-derived relaxing factor (EDRF) is closely related to nitric oxide (NO). [**A**] EDRF released from aortic endothelial cells (EC) by acetylcholine (ACh) (right-hand panel) has the same effect on the absorption spectrum of deoxyhaemoglobin (Hb) as does authentic NO (left panel). [**B**] 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 et al. 1987 Circ Res 61: 866–879; and Palmer et al. 1987 Nature 327: 524–526.)

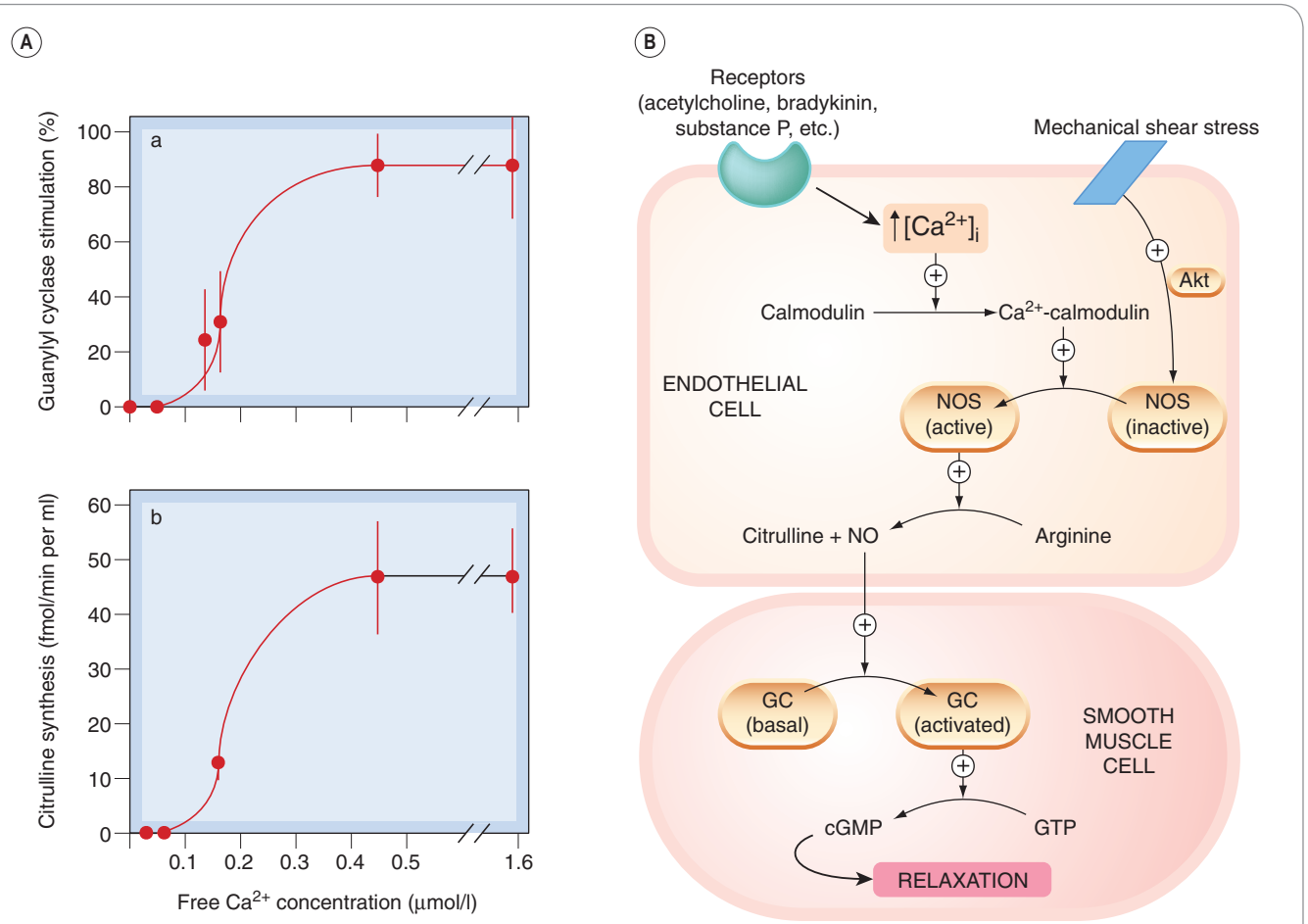

Fig. 20.3 Control of constitutive nitric oxide synthase (NOS) by calcium–calmodulin. [**A**] Dependence on Ca^{2+} of nitric oxide (NO) and citrulline synthesis from L-arginine by rat brain synaptosomal cytosol. Rates of synthesis of NO from L-arginine were determined by stimulation of guanylyl cyclase (GC) (upper) or by synthesis of [^{3}H]-citrulline from L-[^{3}H]-arginine (lower). [**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. (From: [**A**] Knowles R G et al. 1989 Proc Natl Acad Sci USA 86: 5159–5162.)

tors and transduced via a serine–threonine protein kinase called Akt. Agonists that increase cAMP in endothelial cells (e.g. β_2-adrenoceptor agonists) also increase eNOS activity, but via protein kinase A-mediated phosphorylation,[2] whereas protein kinase C reduces eNOS activity by phosphorylating residues in the calmodulin-binding domain, thereby reducing the binding of calmodulin. Insulin increases eNOS activity via tyrosine kinase activation (and also increases the expression of nNOS in diabetic mice).

In contrast to constitutive NOS isoforms, the activity of iNOS 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 cytokines synthesised in response to lipopolysaccharide, 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 iNOS, but they each synergise with interferon-γ in this regard (see Ch. 17). Induction of iNOS is inhibited by glucocorticoids and by several cytokines, including transforming growth factor-β. There are important species differences in the inducibility of iNOS, which is less readily induced in human than in mouse cells.

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 follow:

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

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

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

Low concentrations of NO are relatively stable in air, so small amounts of NO produced in the lung escape degradation and can be detected in exhaled air. In contrast, NO reacts very rapidly with even low concentrations of

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

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, and constitutive endothelial and neuronal forms (respectively, iNOS, eNOS and nNOS). 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.
- iNOS is induced in macrophages and other cells by interferon-γ.
- nNOS is present in the central nervous system (see Chs 36–38) and in autonomic nerves (see Ch. 12).
- eNOS is present in platelets and other cells in addition to endothelium.
- NO is inactivated by combination with the haem of haemoglobin or by oxidation to nitrite and nitrate, which are excreted in urine.
- NO is unstable but 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.

superoxide anion (O_2^-) to produce peroxynitrite anion ($ONOO^-$), which is responsible for some of its toxic effects. Haem has an affinity for NO > 10 000 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 oxidised to methaemoglobin.

Endothelium-derived NO acts locally on underlying vascular smooth muscle or on adherent monocytes or platelets. A strong, but still controversial, case has been made that NO can also act at a distance in the mammalian circulation via reversible interactions with haemoglobin.[3]

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. 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 below. Readers who require a more detailed account of the case that NO acts at a distance within the mammalian circulation are directed to reviews by Singel & Stamler (2005) and, for a sceptical view, Schechter & Gladwyn (2003).

[3]The potential for action at a distance elswhere in the animal kingdom is neatly demonstrated by *Rhodnius prolixus*, a blood-sucking insect that produces a salivary vasodilator/platelet inhibitor with the properties of a nitrovasodilator. This consists of a mixture of nitrosylated haemoproteins, which bind NO in the salivary glands of the insect but release it in the tissues of its prey. The consequent vasodilatation and inhibition of platelet activation presumably facilitates extraction of the bug's meal in liquid form.

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 several investigational drugs.

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. 34). 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. 37). 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 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.[4]

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 that occur in response to contractile or proaggregatory 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

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

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

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 peroxynitrite anions). These contribute to host defence, but also to the neuronal destruction that occurs when there is overstimulation of NMDA receptors by glutamate (see Chs 37 and 39). Paradoxically, NO is also cytoprotective under some circumstances (see Ch. 39).

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 for eNOS are hypertensive, consistent with a role for NO biosynthesis in the physiological control of blood pressure. In addition, NO derived from nNOS has recently been implicated in the control of basal resistance vessel tone in human forearm and cardiac muscle vascular beds (Seddon et al., 2008, 2009). Increased NO generation 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 (Ch. 12), and is important in the upper airways, gastrointestinal tract and control of penile erection (Chs 27, 29 and 34). It is implicated in the control of neuronal development and of synaptic plasticity in the CNS (Chs 36 and 38). Mice carrying a mutation disrupting the gene coding nNOS 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). nNOS 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. 17)

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 to *Leishmania major* (to which wild-type mice are highly

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.

resistant) of mice lacking iNOS. 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.

THERAPEUTIC APPROACHES

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 inhaled NO 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. Ethyl nitrite gas has been investigated in newborns (who are at much increased risk of respiratory distress syndrome because of their immature lungs) as a potentially less toxic alternative.

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 above) selectively inhibits platelet function. 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 (Webb et al., 2008).[5]

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 (see above), 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 functionally essential 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.

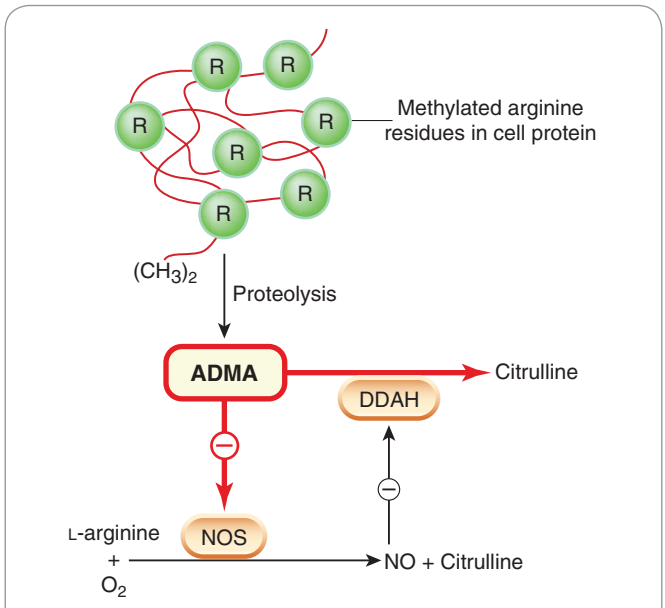

Fig. 20.4 Effect of asymmetric dimethylarginine (ADMA). DDAH, dimethylarginine dimethylamino hydrolase; NO, nitric oxide; NOS, nitric oxide synthase.

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 nNOS (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 iNOS 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 iNOS has been implicated (e.g. asthma). 7-Nitroindazole selectively inhibits nNOS, the mechanism of selectivity being uncertain. *S*-methyl-L-thiocitrulline is a potent and selective inhibitor of human nNOS (Furfine et al., 1994), and has recently provided new understanding of the importance of nNOS in control of human resistance vessel tone in vivo (Seddon et al., 2008, 2009).

[5]Perhaps dietary nitrate contributes to the beneficial effects of a vegetable-rich diet, highlighting the potential of a 'natural' low-cost approach for the prevention of cardiovascular disease.

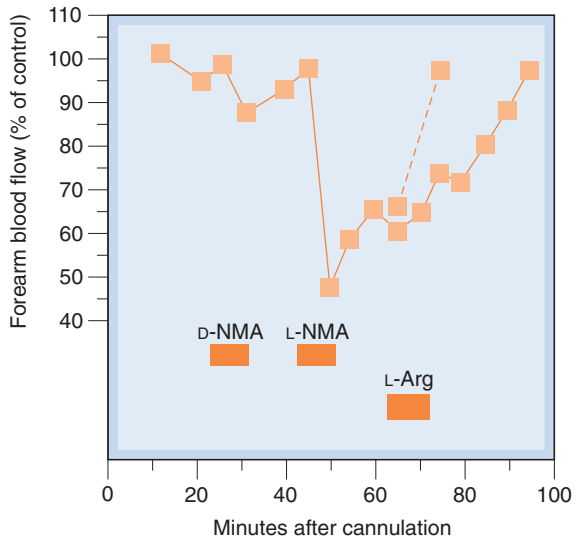

Fig. 20.5 Basal blood flow in the human forearm is influenced by nitric oxide (NO) biosynthesis. Forearm blood flow is expressed as a percentage of the flow in the non-cannulated control arm (which does not change). Brachial artery infusion of the D-isomer of the arginine analogue N^G-monomethyl-L-arginine (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 et al. 1989 Lancet ii: 997–1000.)

Inhibition of the L-arginine/nitric oxide pathway

- Glucocorticoids inhibit biosynthesis of inducible (but not constitutive) nitric oxide synthase (NOS).
- Synthetic arginine analogues (e.g. L-NMMA, L-NAME; see text) compete with arginine and are useful experimental tools.
- Endogenous NOS inhibitors include ADMA (see text) and PIN (a protein that inhibits NOS dimerisation).
- Isoform-selective inhibitors include *S*-methyl-L-thiocitrulline (selective for nNOS).

An endogenous protein inhibitor of nNOS (termed *PIN*) works by an entirely different mechanism, namely destabilising the NOS dimer (Jaffrey & Snyder, 1996).

POTENTIATION OF NITRIC OXIDE

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 above)
- dietary supplementation with L-arginine (see above)
- 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, 30 and 34)
- 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 above and Ch. 34).

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, 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. 27).

Nitric oxide biosynthesis is reduced in patients with *hypercholesterolaemia* and some other disorders that

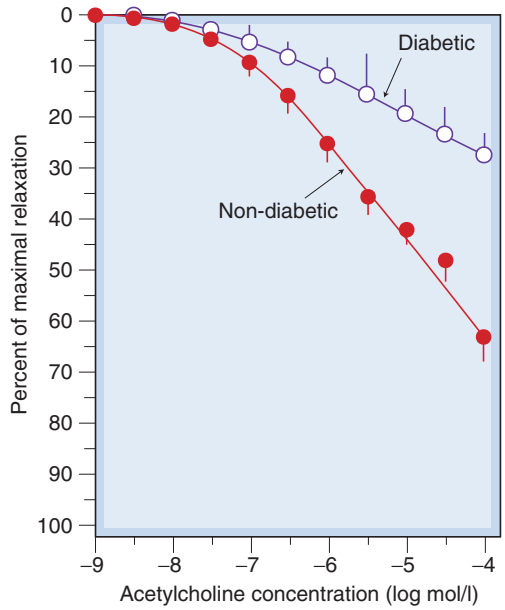

Fig. 20.6 Impaired endothelium-mediated relaxation of penile smooth muscle from diabetic men with erectile dysfunction. Mean (± SE) relaxation responses to acetylcholine in corpora cavernosa tissue (obtained at the time of performing surgical implants to treat impotence) from 16 diabetic men and 22 non-diabetic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

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. 39) and in septic shock (Ch. 22).

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), suggesting that basal NO synthesis may be reduced throughout their circulation.

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. 39). nNOS 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.

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. 34).
- 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).

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