21 The heart

OVERVIEW

In this chapter, we review briefly the physiology of cardiac function in terms of electrophysiology, of contraction, of oxygen consumption and coronary blood flow, of autonomic control and as a source of peptide hormones. This provides a basis for understanding effects of drugs on the heart and their place in treating cardiac disease. The main drugs considered are drugs that act directly on the heart, namely antidysrhythmic drugs and drugs that increase the force of contraction of the heart (especially digoxin); antianginal drugs are also covered in this chapter. The commonest forms of heart disease are caused by atheroma in the coronary arteries, and thrombosis on ruptured atheromatous plaques; drugs to treat and prevent these are considered in Chapters 23 and 24. Heart failure is mainly treated by drugs that work indirectly on the heart via actions on vascular smooth muscle, discussed in Chapter 22, by diuretics (Ch. 28) and β-adrenoceptor antagonists (Ch. 14).

INTRODUCTION

In this chapter, we consider effects of drugs on the heart under three main headings:

1. Rate and rhythm.
2. Myocardial contraction.
3. Metabolism and blood flow.

The effects of drugs on these aspects of cardiac function are not, of course, independent of each other. For example, if a drug affects the electrical properties of the myocardial cell membrane, it is likely to influence both cardiac rhythm and myocardial contraction. Similarly, a drug that affects contraction will inevitably alter metabolism and blood flow as well. Nevertheless, from a therapeutic point of view, these three classes of effect represent distinct clinical objectives in relation to the treatment, respectively, of cardiac dysrhythmias, cardiac failure and coronary insufficiency (as occurs during angina pectoris or myocardial infarction).

PHYSIOLOGY OF CARDIAC FUNCTION

CARDIAC RATE AND RHYTHM

The chambers of the heart normally contract in a coordinated manner, pumping blood efficiently by a route determined by the valves. Coordination of contraction is achieved by a specialised conducting system. Physiological sinus rhythm is characterised by impulses arising in the sinoatrial (SA) node and conducted in sequence through the atria, the atrioventricular (AV) node, bundle of His, Purkinje fibres and ventricles. Cardiac cells owe their electrical excitability to voltage-sensitive plasma membrane channels selective for various ions, including Na^+, K^+ and Ca^{2+}, the structure and function of which are described in Chapter 4. Electrophysiological features of cardiac muscle that distinguish it from other excitable tissues include:

- pacemaker activity
- absence of fast Na^+ current in SA and AV nodes, where slow inward Ca^{2+} current initiates action potentials
- long action potential ('plateau') and refractory period
- influx of Ca^{2+} during the plateau.

Thus several of the special features of cardiac rhythm relate to Ca^{2+} currents. The heart contains *intracellular* calcium channels (i.e. ryanodine receptors and inositol trisphosphate-activated calcium channels described in Ch. 4, which are important in myocardial contraction) and voltage-dependent calcium channels in the plasma membrane, which are important in controlling cardiac rate and rhythm. The main type of voltage-dependent calcium channel in adult working myocardium is the L-type channel, which is also important in vascular smooth muscle; L-type channels are important in specialised conducting regions as well as in working myocardium.

The action potential of an idealised cardiac muscle cell is shown in Figure 21.1A and is divided into five phases: 0 (fast depolarisation), 1 (partial repolarisation), 2 (plateau), 3 (repolarisation) and 4 (pacemaker).

▼ Ionic mechanisms underlying these phases can be summarised as follows.

Phase 0, rapid depolarisation, occurs when the membrane potential reaches a critical firing threshold (about –60 mV), at which the inward current of Na^+ flowing through the voltage-dependent sodium channels becomes large enough to produce a regenerative ('all-or-nothing') depolarisation. This mechanism is the same as that responsible for action potential generation in neurons (see Ch. 4). Activation of sodium channels by membrane depolarisation is transient, and if the membrane remains depolarised for more than a few milliseconds, they close again (inactivation). They are therefore closed during the plateau of the action potential and remain unavailable for the initiation of another action potential until the membrane repolarises.

Phase 1, partial repolarisation, occurs as the Na^+ current is inactivated. There may also be a transient voltage-sensitive outward current.

Phase 2, the *plateau*, results from an inward Ca^{2+} current. Calcium channels show a pattern of voltage-sensitive activation and inactivation qualitatively similar to sodium channels, but with a much slower time course. The plateau is assisted by a special property of the cardiac muscle membrane known as inward-going rectification, which means that the K^+ conductance falls to a low level when the membrane is depolarised. Because of this, there is little tendency for outward K^+ current to restore the resting membrane potential during the plateau, so a relatively small inward Ca^{2+} current suffices to maintain the plateau.

Phase 3, repolarisation, occurs as the Ca^{2+} current inactivates and a delayed outwardly rectifying K^+ current (analogous to, but much slower than, the K^+ current that causes repolarisation in nerve fibres; Ch. 4) activates, causing outward K^+ current. This is augmented by another K^+ current, which is activated by high intracellular Ca^{2+} concentrations, $[Ca^{2+}]_i$ during the plateau, and sometimes also by other K^+ currents, including one through channels activated by

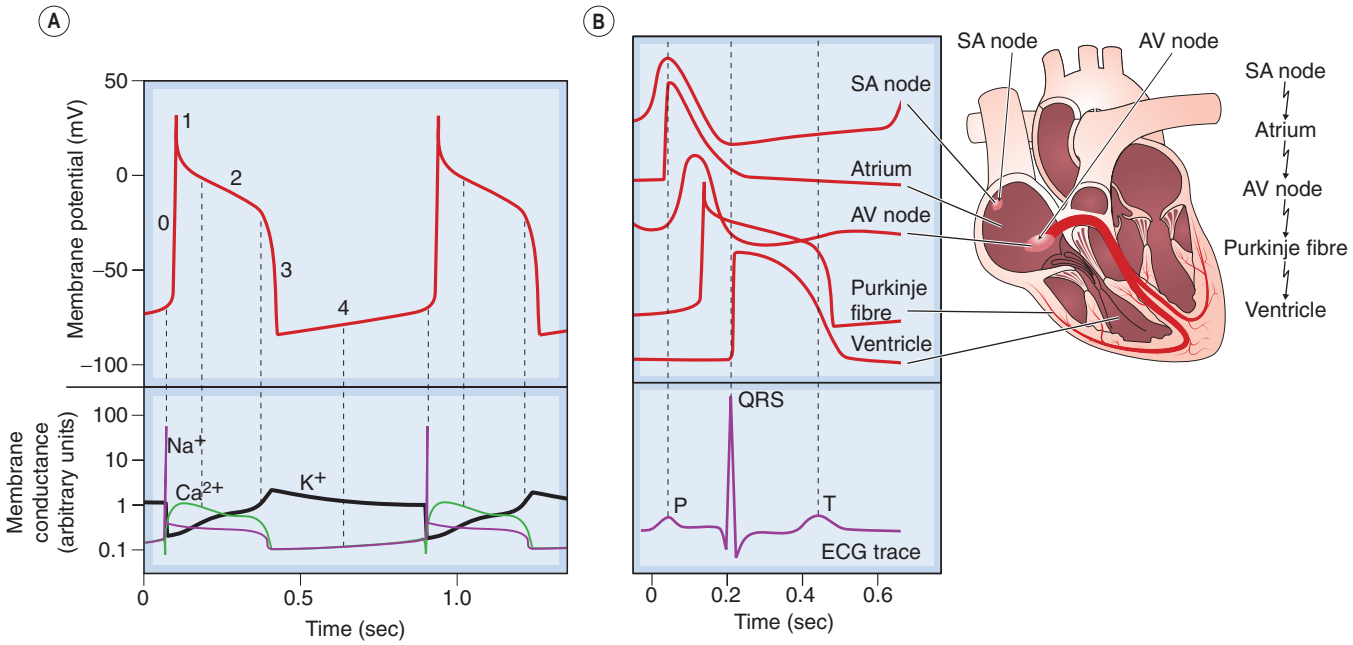


Fig. 21.1 The cardiac action potential. [**A**] Phases of the action potential: 0, rapid depolarisation; 1, partial repolarisation; 2, plateau; 3, repolarisation; 4, pacemaker depolarisation. The lower panel shows the accompanying changes in membrane conductance for Na^+, K^+ and Ca^{2+}. [**B**] Conduction of the impulse through the heart, with the corresponding electrocardiogram (ECG) trace. Note that the longest delay occurs at the atrioventricular (AV) node, where the action potential has a characteristically slow waveform. SA, sinoatrial. (Adapted from: [**A**] Noble D 1975 The initiation of the heartbeat. Oxford University Press, Oxford.)

acetylcholine (see below) and another that is activated by arachidonic acid, which is liberated under pathological conditions such as myocardial infarction.

Phase 4, the *pacemaker potential*, is a gradual depolarisation during diastole. Pacemaker activity is normally found only in nodal and conducting tissue. The pacemaker potential is caused by a combination of increasing inward currents and declining outward currents during diastole. It is usually most rapid in cells of the SA node, which therefore acts as pacemaker for the whole heart. Cells in the SA node have a greater background conductance to Na^+ than do atrial or ventricular myocytes, leading to a greater background inward current. In addition, inactivation of voltage-dependent calcium channels wears off during diastole, resulting in increasing inward Ca^{2+} current during late diastole. Activation of T-type calcium channels during late diastole contributes to pacemaker activity in the SA node. The negative membrane potential early in diastole activates a cation channel that is permeable to Na^+ and K^+, giving rise to another inward current, called I_f.[1] An inhibitor of this current, **ivabradine**, slows the heart and is used therapeutically (see below).

Several voltage- and time-dependent outward currents play a part as well: delayed rectifier K^+ current (I_K), which is activated during the action potential, is turned off by the negative membrane potential early in diastole. Current from the electrogenic Na^+/K^+ pump also contributes to the outward current during the pacemaker potential.

Figure 21.1B shows the action potential configuration in different parts of the heart. Phase 0 is absent in the nodal regions, where the conduction velocity is correspondingly slow (~5 cm/s) compared with other regions such as the Purkinje fibres (conduction velocity ~200 cm/s), which propagate the action potential rapidly to the ventricles. Regions that lack a fast inward current have a much longer refractory period than fast-conducting regions. This is because recovery of the slow inward current following its inactivation during the action potential takes a considerable time (a few hundred milliseconds), and the refractory period outlasts the action potential. With fast-conducting fibres, inactivation of the Na^+ current recovers rapidly, and the cell becomes excitable again almost as soon as it is repolarised.

The orderly pattern of sinus rhythm can be disrupted either by heart disease or by the action of drugs or circulating hormones, and an important therapeutic use of drugs is to restore a normal cardiac rhythm where it has become disturbed. The commonest cause of cardiac dysrhythmia is ischaemic heart disease, and many deaths following myocardial infarction result from *ventricular fibrillation* ('fibrillation' is a state where heart chambers stop contracting in a coordinated way because of chaotic electrical activity; instead, the affected heart chambers 'fibrillate'—rapid uncoordinated contractions within ventricles or atria that are visible to the naked eye but do not support output from the affected chambers) rather than directly from contractile failure.

DISTURBANCES OF CARDIAC RHYTHM

Clinically, dysrhythmias are classified according to:

- the site of origin of the abnormality—atrial, junctional or ventricular
- whether the rate is increased (*tachycardia*) or decreased (*bradycardia*).

They may cause palpitations (awareness of the heartbeat) or symptoms from cerebral hypoperfusion (faintness or loss of consciousness). Their diagnosis depends on the

[1] 'f' for 'funny', because it is unusual for cation channels to be activated by hyperpolarisation; electrophysiologists have a peculiar sense of humour!

surface electrocardiogram (ECG), and details are beyond the scope of this book—see Braunwald & Opie (2001). The commonest types of tachyarrhythmia are *atrial fibrillation*, where the heartbeat is completely irregular, and *supraventricular tachycardia* (SVT), where the heartbeat is rapid but regular. Occasional ectopic beats (ventricular as well as supraventricular) are common. Sustained ventricular tachyarrhythmias are much less common but much more serious; they include *ventricular tachycardia*, and *ventricular fibrillation* where the electrical activity in the ventricles is completely chaotic and cardiac output ceases. Bradyarrhythmias include various kinds of *heart block* (e.g. at the AV or SA node) and complete cessation of electrical activity (‘*asystolic arrest*’). It is often unclear which of the various mechanisms discussed below are responsible. These cellular mechanisms nevertheless provide a useful starting point for understanding how antidysrhythmic drugs work. Four basic phenomena underlie disturbances of cardiac rhythm:

1. Delayed after-depolarisation.
2. Re-entry.
3. Ectopic pacemaker activity.
4. Heart block.

The main cause of delayed after-depolarisation is abnormally raised $[Ca^{2+}]_i$, which triggers inward current and hence a train of abnormal action potentials (Fig. 21.2). After-depolarisation is the result of a net inward current, known as the transient inward current. A rise in $[Ca^{2+}]_i$ activates Na^+/Ca^{2+} exchange. This transfers one Ca^{2+} ion out of the cell in exchange for entry of three Na^+ ions, resulting in a net influx of one positive charge and hence membrane depolarisation. Additionally, raised $[Ca^{2+}]_i$ opens non-selective cation channels in the plasma membrane, causing depolarisation analogous to the endplate potential at the neuromuscular junction (Ch. 13). Consequently, hypercalcaemia (which increases the entry of Ca^{2+}) promotes after-depolarisation. Hypokalaemia also influences repolarisation,via an effect on the gating of cardiac delayed rectifier potassium channels. Many drugs, including ones whose principal effects are on other organs, delay cardiac repolarisation by binding to potassium or other cardiac channels or by influencing electrolyte concentrations (see Roden, 2004). Delayed repolarisation increases Ca^{2+} entry during the prolonged action potential, leading to after-depolarisation. Prolongation of the QT interval, which carries a risk of causing dangerous ventricular dysrhythmias, is a concern in drug development (see section below, Class III drugs, and see Ch. 57).

Normally, a cardiac action potential dies out after it has activated the ventricles because it is surrounded by refractory tissue, which it has just traversed. Re-entry (Fig. 21.3) describes a situation in which the impulse re-excites regions of the myocardium after the refractory period has subsided, causing continuous circulation of action potentials. It can result from anatomical anomalies or, more commonly, from myocardial damage. Re-entry underlies many types of dysrhythmia, the pattern depending on the site of the re-entrant circuit, which may be in the atria, ventricles or nodal tissue. A simple ring of tissue can give rise to a re-entrant rhythm if a transient or unidirectional conduction block is present. Normally, an impulse originating at any point in the ring will propagate in both directions and die out when the two impulses meet, but if a damaged area causes either a transient block (so that one impulse is blocked but the second can get through; Fig. 21.3) or a unidirectional block, continuous circulation of the impulse can occur. This is known as *circus movement* and was demonstrated experimentally on rings of jellyfish tissue many years ago.

Although the physiological pacemaker resides in the SA node, other cardiac tissues can take on pacemaker activity. This provides a safety mechanism in the event of failure of the SA node but can also trigger tachyarrhythmias. Ectopic pacemaker activity is encouraged by sympathetic activity and by partial depolarisation, which may occur during ischaemia. Catecholamines, acting on β_1 adrenoceptors (see below), increase the rate of depolarisation during phase 4

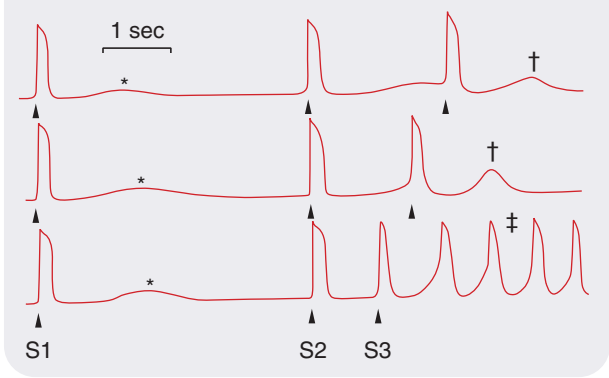


Fig. 21.2 After-depolarisation in cardiac muscle recorded from a dog coronary sinus in the presence of noradrenaline (norepinephrine). The first stimulus (S1) causes an action potential followed by a small after-depolarisation. As the interval S2–S3 is decreased, the after-depolarisation gets larger (†) until it triggers an indefinite train of action potentials (‡). (Adapted from Wit A L, Cranefield P F 1977 Circ Res 41: 435.)

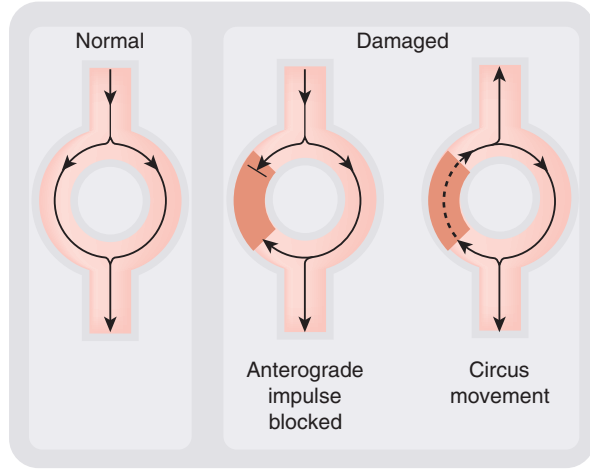


Fig. 21.3 Generation of a re-entrant rhythm by a damaged area of myocardium. The damaged area (brown) conducts in one direction only. This disturbs the normal pattern of conduction and permits continuous circulation of the impulse to occur.

Cardiac dysrhythmias

- Dysrhythmias arise because of:
  - delayed after-depolarisation, which triggers ectopic beats
  - re-entry, resulting from partial conduction block
  - ectopic pacemaker activity
  - heart block.
- Delayed after-depolarisation is caused by an inward current associated with abnormally raised intracellular Ca^{2+}.
- Re-entry is facilitated when parts of the myocardium are depolarised as a result of disease.
- Ectopic pacemaker activity is encouraged by sympathetic activity.
- Heart block results from disease in the conducting system, especially the atrioventricular node.
- Clinically, dysrhythmias are divided:
  - according to their site of origin (supraventricular and ventricular)
  - according to whether the heart rate is increased or decreased (tachycardia or bradycardia).

and can cause normally quiescent parts of the heart to take on a spontaneous rhythm. Several tachyarrhythmias (e.g. paroxysmal atrial fibrillation) can be triggered by circumstances associated with increased sympathetic activity. Pain (e.g. during myocardial infarction) increases sympathetic discharge and releases adrenaline (epinephrine) from the adrenal gland. Partial depolarisation resulting from ischaemic damage also causes abnormal pacemaker activity.

Heart block results from fibrosis of, or ischaemic damage to, the conducting system (often in the AV node). In complete heart block, the atria and ventricles beat independently of one another, the ventricles beating at a slow rate determined by whatever pacemaker picks up distal to the block. Sporadic complete failure of AV conduction causes sudden periods of unconsciousness (Stokes–Adams attacks) and is treated by implanting an artificial pacemaker.

CARDIAC CONTRACTION

Cardiac output is the product of heart rate and mean left ventricular stroke volume (i.e. the volume of blood ejected from the ventricle with each heartbeat). Heart rate is controlled by the autonomic nervous system (Chs 13 and 14, and see below). Stroke volume is determined by a combination of factors, including some intrinsic to the heart itself and other haemodynamic factors extrinsic to the heart. Intrinsic factors regulate myocardial contractility via $[Ca^{2+}]_i$ and ATP, and are sensitive to various drugs and pathological processes. Extrinsic circulatory factors include the elasticity and contractile state of arteries and veins, and the volume and viscosity of the blood, which together determine cardiac load (preload and afterload). Drugs that influence these circulatory factors are of paramount importance in treating patients with heart failure. They are covered in Chapter 22.

MYOCARDIAL CONTRACTILITY AND VIABILITY

The contractile machinery of myocardial striated muscle is basically the same as that of voluntary striated muscle (Ch. 4). It involves binding of Ca^{2+} to troponin C; this changes the conformation of the troponin complex, permitting cross-bridging of myosin to actin and initiating contraction. **Levosimendan** (a drug used to treat acute decompensated heart failure; Ch. 22), increases the force of contraction of the heart by binding troponin C and sensitising it to the action of Ca^{2+}.

Many effects of drugs on cardiac contractility can be explained in terms of actions on $[Ca^{2+}]_i$, via effects on calcium channels in plasma membrane or sarcoplasmic reticulum, or on the Na^+/K^+ pump, which indirectly influences the Na^+/Ca^{2+} pump (see below). Other factors that affect the force of contraction are the availability of oxygen and a source of metabolic energy such as free fatty acids. Myocardial *stunning*—contractile dysfunction that persists after ischaemia and reperfusion despite restoration of blood flow and absence of cardiac necrosis—is incompletely understood but can be clinically important. Its converse is known as *ischaemic preconditioning*; this refers to an improved ability to withstand ischaemia following previous ischaemic episodes. This potentially beneficial state could be clinically important. There is some evidence that it is mediated by *adenosine* (see Ch. 2), which accumulates as ATP is depleted. Exogenous adenosine affords protection similar to that caused by ischaemic preconditioning, and blockade of adenosine receptors prevents the protective effect of preconditioning (see Gross & Auchampach, 2007). There is considerable interest in developing strategies to minimise harmful effects of ischaemia while maximising preconditioning.

VENTRICULAR FUNCTION CURVES AND HEART FAILURE

The force of contraction of the heart is determined partly by its intrinsic contractility (which, as described above, depends on $[Ca^{2+}]_i$ and availability of ATP), and partly by extrinsic haemodynamic factors that affect end-diastolic volume and hence the resting length of the muscle fibres. The end-diastolic volume is determined by the end-diastolic pressure, and its effect on stroke work is expressed in the Frank–Starling law of the heart, which reflects an inherent property of the contractile system. The Frank–Starling law can be represented as a ventricular function curve (Fig. 21.4). The area enclosed by the pressure–volume curve during the cardiac cycle provides a measure of ventricular stroke work. It is approximated by the product of stroke volume and mean arterial pressure. As Starling showed, factors extrinsic to the heart affect its performance in various ways, two patterns of response to increased load being particularly important.

1. Increased cardiac filling pressure (*preload*), whether caused by increased blood volume or by venoconstriction, increases ventricular end-diastolic volume. This increases stroke volume and hence cardiac output and mean arterial pressure. Cardiac work and cardiac oxygen consumption both increase.
2. Resistance vessel vasoconstriction increases *afterload*. End-diastolic volume and hence stroke work are initially unchanged, but constant stroke work in the face of increased vascular resistance causes reduced

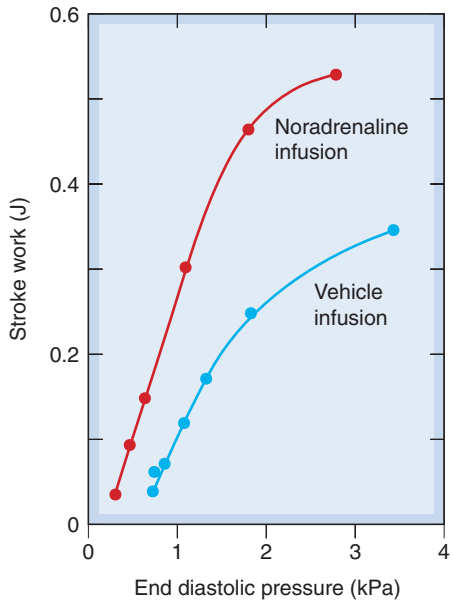


Fig. 21.4 Ventricular function curves in the dog. Infusion of physiological saline increases blood volume and hence end-diastolic pressure. This increases stroke work ('extrinsic' control) by increasing the force of contraction of the heart. This relationship is called the Starling curve. Noradrenaline has a direct action on the heart ('intrinsic' control), increasing the slope of the Starling curve. (Redrawn from Sarnoff S J et al. 1960 Circ Res 8: 1108.)

stroke volume and hence increased end-diastolic volume. This in turn increases stroke work, until a steady state is re-established with increased end-diastolic volume and the same cardiac output as before. As with increased preload, cardiac work and cardiac oxygen consumption both increase.

Normal ventricular filling pressure is only a few centimetres of water, on the steep part of the ventricular function curve, so a large increase in stroke work can be achieved with only a small increase in filling pressure. The Starling mechanism plays little part in controlling cardiac output in healthy subjects (e.g. during exercise), because changes in contractility, mainly as a result of changes in sympathetic nervous activity, achieve the necessary regulation without any increase in ventricular filling pressure (Fig. 21.4). In contrast, the denervated heart in patients who have received a heart transplant relies on the Starling mechanism to increase cardiac output during exercise.

In heart failure, the cardiac output is insufficient to meet the needs of the body, initially only when these are increased during exercise but ultimately, as disease progresses, also at rest. It has many causes, most commonly ischaemic heart disease. In patients with heart failure (see Ch. 22), the heart may be unable to deliver as much blood as the tissues require, even when its contractility is increased by sympathetic activity. Under these conditions, the basal (i.e. at rest) ventricular function curve is greatly depressed, and there is insufficient reserve, in the sense of extra contractility that can be achieved by sympathetic activity, to enable cardiac output to be maintained during exercise without a large increase in central venous pressure (Fig. 21.4). Oedema of peripheral tissues (causing swelling of the legs) and the lungs (causing breathlessness) is an important consequence of cardiac failure. It is caused by the increased venous pressure, and retention of Na^+ (see Ch. 22).

Myocardial contraction

- Controlling factors are:
  - intrinsic myocardial contractility
  - extrinsic circulatory factors.
- Myocardial contractility depends critically on intracellular Ca^{2+}, and hence on:
  - Ca^{2+} entry across the cell membrane
  - Ca^{2+} storage in the sarcoplasmic reticulum.
- The main factors controlling Ca^{2+} entry are:
  - activity of voltage-gated calcium channels
  - intracellular Na^+, which affects Ca^{2+}/Na^+ exchange.
- Catecholamines, cardiac glycosides and other mediators and drugs influence these factors.
- Extrinsic control of cardiac contraction is through the dependence of stroke work on the end-diastolic volume, expressed in the Frank–Starling law.
- Cardiac work is affected independently by afterload (i.e. peripheral resistance and arterial compliance) and preload (i.e. central venous pressure).

MYOCARDIAL OXYGEN CONSUMPTION AND CORONARY BLOOD FLOW

Relative to its large metabolic needs, the heart is one of the most poorly perfused tissues in the body. Coronary flow is, under normal circumstances, closely related to myocardial oxygen consumption, and both change over a nearly 10-fold range between conditions of rest and maximal exercise.

PHYSIOLOGICAL FACTORS

The main physiological factors that regulate coronary flow are:

- physical factors
- vascular control by metabolites
- neural and humoral control.

Physical factors

During systole, the pressure exerted by the myocardium on vessels that pass through it equals or exceeds the perfusion pressure, so coronary flow occurs only during diastole. Diastole is shortened more than systole during tachycardia, reducing the period available for myocardial perfusion. During diastole, the effective perfusion pressure is equal to the difference between the aortic and ventricular pressures (Fig. 21.5). If diastolic aortic pressure falls or diastolic ventricular pressure increases, perfusion pressure falls and so (unless other control mechanisms can compensate) does coronary blood flow. Stenosis of the aortic valve reduces aortic pressure but increases left ventricular pressure upstream of the narrowed valve, and often causes ischaemic chest pain (angina) even in the absence of coronary artery disease.

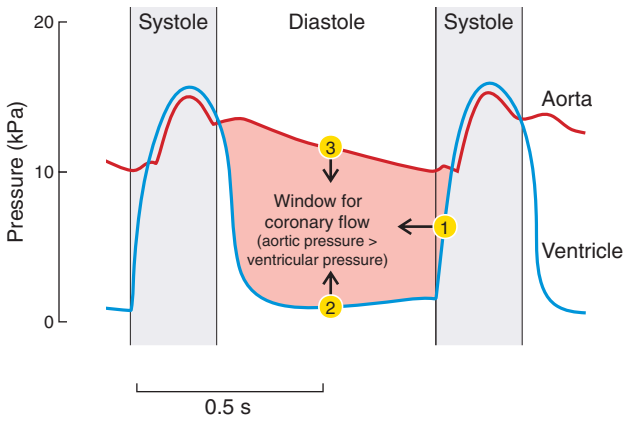


Fig. 21.5 Mechanical factors affecting coronary blood flow. The 'window' for coronary flow may be encroached on by: (1) shortening diastole, when heart rate increases; (2) increased ventricular end-diastolic pressure; and (3) reduced diastolic arterial pressure.

Vascular control by metabolites/mediators

Vascular control by metabolites is the most important mechanism by which coronary flow is regulated. A reduction in arterial partial pressure of oxygen (P_{O_2}) causes marked vasodilatation of coronary vessels in situ but has little effect on isolated strips of coronary artery. This suggests that it is a change in the pattern of metabolites produced by the myocardial cells, rather than the change in P_{O_2} per se, that controls the state of the coronary vessels, a popular candidate for the dilator metabolite being *adenosine* (see Ch. 16).

Neural and humoral control

Coronary vessels have a dense sympathetic innervation, but sympathetic nerves (like circulating catecholamines) exert only a small direct effect on the coronary circulation. Large coronary vessels possess α adrenoceptors that mediate vasoconstriction, whereas smaller vessels have β_2 adrenoceptors that have a dilator effect. Coronary vessels are also innervated by purinergic, peptidergic and nitrergic nerves, and basal coronary blood flow in patients with angiographically normal coronary arteries is reduced by about one-third by selective inhibition of nNOS (Seddon et al., 2009). Coronary vascular responses to altered mechanical and metabolic activity during exercise or pathological events overshadow neural and endocrine effects.

AUTONOMIC CONTROL OF THE HEART

The sympathetic and parasympathetic systems (see Chs 12–14) each exert a tonic effect on the heart at rest. They influence each of the aspects of cardiac function that have been discussed above, namely rate and rhythm, myocardial contraction, and myocardial metabolism and blood flow.

SYMPATHETIC SYSTEM

The main effects of sympathetic activity on the heart are:

- increased force of contraction (positive *inotropic* effect; Fig. 21.6)

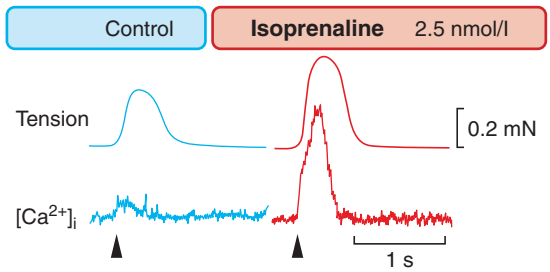


Fig. 21.6 The calcium transient in frog cardiac muscle. A group of cells was injected with the phosphorescent Ca^{2+} indicator aequorin, which allows $[Ca^{2+}]_i$ to be monitored optically. Isoprenaline causes a large increase in the tension and in the $[Ca^{2+}]_i$ transient caused by an electrical stimulus (▲). (From Allen D G, Blinks J R 1978 Nature 273: 509.)

Coronary flow, ischaemia and infarction

- The heart has a smaller blood supply in relation to its oxygen consumption than most organs.
- Coronary flow is controlled mainly by:
  - physical factors, including transmural pressure during systole
  - vasodilator metabolites.
- Autonomic innervation is less important.
- Coronary ischaemia is usually the result of atherosclerosis and causes angina. Sudden ischaemia is usually caused by thrombosis and may result in cardiac infarction.
- Coronary spasm sometimes causes angina (variant angina).
- Cellular Ca^{2+} overload results from ischaemia and may be responsible for:
  - cell death
  - dysrhythmias.

- increased heart rate (positive *chronotropic* effect; Fig. 21.7)
- increased *automaticity*
- repolarisation and *restoration of function* following generalised cardiac depolarisation
- reduced cardiac *efficiency* (i.e. oxygen consumption is increased more than cardiac work).

These effects all result from activation of β_1-adrenoceptors. The β_1 effects of catecholamines on the heart, although complex, probably all occur through increased intracellular cAMP (see Ch. 3). cAMP activates protein kinase A, which phosphorylates sites on the α1 subunits of calcium channels. This increases the probability that the channels will open, increasing inward Ca^{2+} current and hence force of cardiac contraction (Fig. 21.6). Activation of β_1-adrenoceptors also increases the Ca^{2+} sensitivity of the contractile machinery, possibly by phosphorylating troponin C; furthermore, it facilitates Ca^{2+} capture by the sarcoplasmic reticulum, thereby increasing the amount of Ca^{2+} available for release by the action potential. The net result of

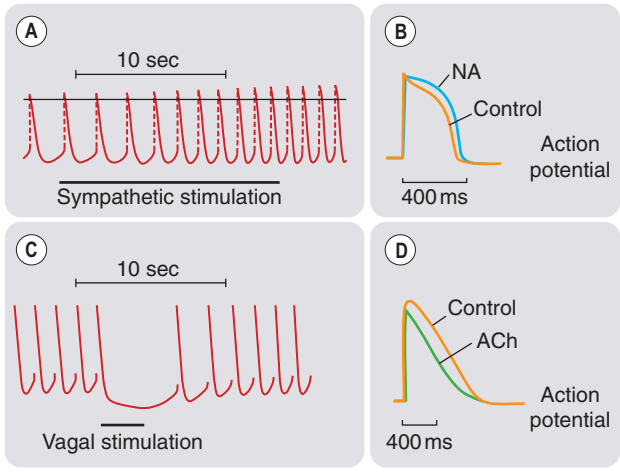


Fig. 21.7 Autonomic regulation of the heartbeat. [**A**] and [**B**] Effects of sympathetic stimulation and noradrenaline (NA). [**C**] and [**D**] Effects of parasympathetic stimulation and acetylcholine (ACh). Sympathetic stimulation [**A**] increases the slope of the pacemaker potential and increases heart rate, whereas parasympathetic stimulation [**C**] abolishes the pacemaker potential, hyperpolarises the membrane and temporarily stops the heart (frog sinus venosus). NA [**B**] prolongs the action potential, while ACh [**D**] shortens it (frog atrium). (From: [**A**] and [**C**] Hutter O F, Trautwein W 1956 J Gen Physiol 39: 715; [**B**] Reuter H 1974 J Physiol 242: 429; [**D**] Giles W R, Noble S J 1976 J Physiol 261: 103.)

catecholamine action is to elevate and steepen the ventricular function curve (Fig. 21.4). The increase in heart rate results from an increased slope of the pacemaker potential (Figs 21.1 and 21.7A). Increased Ca^{2+} entry also increases automaticity because of the effect of $[Ca^{2+}]_i$ on the transient inward current, which can result in a train of action potentials following a single stimulus (Fig. 21.2).

Activation of β_1-adrenoceptors repolarises damaged or hypoxic myocardium by stimulating the Na^+/K^+ pump. This can restore function if asystole has occurred following myocardial infarction, and **adrenaline** is one of the most important drugs used during cardiac arrest.

The reduction of cardiac efficiency by catecholamines is important because it means that the oxygen requirement of the myocardium increases. This limits the use of β agonists such as adrenaline and **dobutamine** for circulatory shock (Ch. 22). Myocardial infarction activates the sympathetic nervous system (see Fig. 21.8), which has the undesirable effect of increasing the oxygen needs of the damaged myocardium.

PARASYMPATHETIC SYSTEM

Parasympathetic activity produces effects that are, in general, opposite to those of sympathetic activation. However, in contrast to sympathetic activity, the parasympathetic nervous system has little effect on contractility, its main effects being on rate and rhythm, namely:

- cardiac slowing and reduced automaticity
- inhibition of AV conduction.

These effects result from activation of muscarinic (M_2) acetylcholine receptors, which are abundant in nodal and atrial tissue but sparse in the ventricles. These receptors are negatively coupled to adenylyl cyclase and thus reduce cAMP formation, acting to inhibit the opening of L-type Ca^{2+} channels and reduce the slow Ca^{2+} current, in opposition to β_1-adrenoceptors. M_2 receptors also open a potassium channel (called K_{ACh}). The resulting increase in K^+ permeability produces a hyperpolarising current that opposes the inward pacemaker current, slowing the heart and reducing automaticity (see Fig. 21.7C). Vagal activity is often increased during myocardial infarction, both in association with vagal afferent stimulation and as a side effect of opioids used to control the pain, and parasympathetic effects are important in predisposing to acute dysrhythmias.

Vagal stimulation decreases the force of contraction of the atria associated with marked shortening of the action potential (Fig. 21.7D). Increased K^+ permeability and reduced Ca^{2+} current both contribute to conduction block at the AV node, where propagation depends on the Ca^{2+} current. Shortening the atrial action potential reduces the refractory period, which can lead to re-entrant arrhythmias. Coronary vessels lack cholinergic innervation; consequently, the parasympathetic nervous system has little effect on coronary artery tone (see Ch. 13).[2]

Autonomic control of the heart

- Sympathetic activity, acting through β_1-adrenoceptors, increases heart rate, contractility and automaticity, but reduces cardiac efficiency (in relation to oxygen consumption).
- The β_1-adrenoceptors act by increasing cAMP formation, which increases Ca^{2+} currents.
- Parasympathetic activity, acting through muscarinic M_2 receptors, causes cardiac slowing, decreased force of contraction (atria only) and inhibition of atrioventricular conduction.
- M_2 receptors inhibit cAMP formation and also open potassium channels, causing hyperpolarisation.

CARDIAC NATRIURETIC PEPTIDES

Cardiac natriuretic peptides are an important family of mediators (see Potter et al., 2009, for a review). Atrial cells contain secretory granules, and store and release *atrial natriuretic peptide* (ANP). This has powerful effects on the kidney and vascular system. Release of ANP occurs during volume overload in response to stretching of the atria, and intravenous saline infusion is sufficient to stimulate its release. B-natriuretic peptide (BNP) is released from ventricular muscle and opposes ventricular fibrosis; its plasma concentration is increased in patients with heart failure and is used as an aid to diagnosis. C-natriuretic peptide (CNP) is stored in endothelium and in addition to vascular actions influences development of long bones.

The main effects of natriuretic peptides are to increase Na^+ and water excretion by the kidney; relax vascular

[2]The Creator has, however, thoughtfully provided coronary endothelium with muscarinic receptors linked to nitric oxide synthesis (see Ch. 20), presumably for the delectation of vascular pharmacologists.

smooth muscle (except efferent arterioles of renal glomeruli; see below); increase vascular permeability; and inhibit the release and/or actions of several hormones and mediators, including aldosterone, angiotensin II, endothelin and antidiuretic hormone. They exert their effects by combining with membrane receptors (natriuretic peptide receptors, NPRs, which exist in at least two subtypes, designated A and B).[3]

Both NPR-A and NPR-B incorporate a catalytic guanylyl cyclase moiety (see Ch. 3), and, when activated, increase intracellular cGMP. Organic nitrates (see later) and endothelium-derived nitric oxide (Ch. 20) act similarly, though they interact with soluble rather than membrane-bound guanylyl cyclase. Renal glomerular afferent arterioles are dilated by ANP but efferent arterioles are constricted, so filtration pressure is increased, leading to increased glomerular filtration and enhanced Na^+ excretion. Elsewhere, natriuretic peptides cause vasorelaxation and reduce blood pressure. Their therapeutic potential, which remains controversial (see Richards, 2009, for a recent editorial commentary), is considered in Chapter 22.

ISCHAEMIC HEART DISEASE

Atheromatous deposits are ubiquitous in the coronary arteries of adults living in developed countries. They are asymptomatic for most of the natural history of the disease (see Ch. 23), but can progress insidiously, culminating in acute myocardial infarction and its complications, including dysrhythmia and heart failure. Details of ischemic heart disease are beyond the scope of this book, and excellent accounts (e.g. Braunwald, 2005) are available for those seeking pathological and clinical information. Here, we merely set the scene for understanding the place of drugs that affect cardiac function in treating this most common form of heart disease.

Important consequences of coronary atherosclerosis include:

- angina (chest pain caused by cardiac ischaemia)
- myocardial infarction.

ANGINA

Angina occurs when the oxygen supply to the myocardium is insufficient for its needs. The pain has a characteristic distribution in the chest, arm and neck, and is brought on by exertion, cold or excitement. A similar type of pain occurs in skeletal muscle when it is made to contract while its blood supply is interrupted, and Lewis showed many years ago that chemical factors released by ischaemic muscle are responsible. Possible candidates include K^+, H^+ and adenosine (Ch. 16), all of which sensitise or stimulate nociceptors (see Ch. 41). It is possible that the same mediator that causes coronary vasodilatation is responsible, at higher concentration, for initiating pain.

Three kinds of angina are recognised clinically: stable, unstable and variant.

Stable angina. This is predictable chest pain on exertion. It is produced by an increased demand on the heart and is caused by a fixed narrowing of the coronary vessels, almost always by atheroma. Symptomatic therapy is directed at reducing cardiac work with organic nitrates, β-adrenoceptor antagonists and/or calcium antagonists (as described below), together with treatment of the underlying atheromatous disease, usually including a statin (Ch. 23), and prophylaxis against thrombosis with an antiplatelet drug, usually **aspirin** (Ch. 24).

Unstable angina. This is characterised by pain that occurs with less and less exertion, culminating in pain at rest. The pathology is similar to that involved in myocardial infarction, namely platelet–fibrin thrombus associated with a ruptured atheromatous plaque, but without complete occlusion of the vessel. Treatment is as for myocardial infarction without ST-segment elevation on the cardiogram (NSTEMI). Antiplatelet drugs (aspirin and/or an ADP antagonist such as **clopidogrel** or **prasugrel**) reduce the risk of myocardial infarction in this setting, and **heparin** and platelet glycoprotein receptor antagonists add to this benefit (Ch. 24) at the cost of increased risk of haemorrhage, and organic nitrates relieve ischaemic pain.

Variant angina. This is uncommon. It occurs at rest and is caused by coronary artery spasm, again usually in association with atheromatous disease. Therapy is with coronary artery vasodilators (e.g. organic nitrates, calcium antagonists).

MYOCARDIAL INFARCTION

Myocardial infarction occurs when a coronary artery has been blocked by thrombus. This may be fatal and is a common cause of death, usually as a result of mechanical failure of the ventricle or from dysrhythmia. Cardiac myocytes rely on aerobic metabolism. If the supply of oxygen remains below a critical value, a sequence of events leading to cell death (by necrosis or apoptosis) ensues (see Ch. 5 for a fuller account of apoptosis), detected clinically by an elevation of circulating *troponin* (the gold-standard biochemical marker of myocardial injury). The sequences leading from vascular occlusion to cell death via the two pathways are illustrated in Figure 21.8. The relative importance of necrosis and apoptosis in myocardial cell death in clinically distinct settings is unknown, but it has been suggested that apoptosis may be an adaptive process in hypoperfused regions, sacrificing some jeopardised myocytes but thereby avoiding the disturbance of membrane function and risk of dysrhythmia inherent in necrosis. Consequently, it is currently unknown if pharmacological approaches to promote or inhibit this pathway could be clinically beneficial.

Prevention of irreversible ischaemic damage following an episode of coronary thrombosis is an important therapeutic aim. Opening the occluded artery is key, and it is important that this is achieved promptly, irrespective of the means by which it is done. If logistically possible, *angioplasty* (performed using a catheter with an inflatable balloon near its tip, with a glycoprotein IIb/IIIa antagonist—see Chapter 24—to prevent reocclusion) is somewhat more effective than thrombolytic drugs. The main therapeutic drugs (see Fig. 21.8) include drugs to improve cardiac function by maintaining oxygenation and reducing cardiac

[3]The nomenclature of natriuretic peptides and their receptors is peculiarly obtuse. The peptides are named 'A' for atrial, 'B' for brain—despite being present mainly in cardiac ventricle—and 'C' for A, B, C …; NPRs are named NPR-A, which preferentially binds ANP; NPR-B, which binds C natriuretic peptide preferentially; and NPR-C for 'clearance' receptor, because until recently clearance via cellular uptake and degradation by lysosomal enzymes was the only definite known function of this binding site.

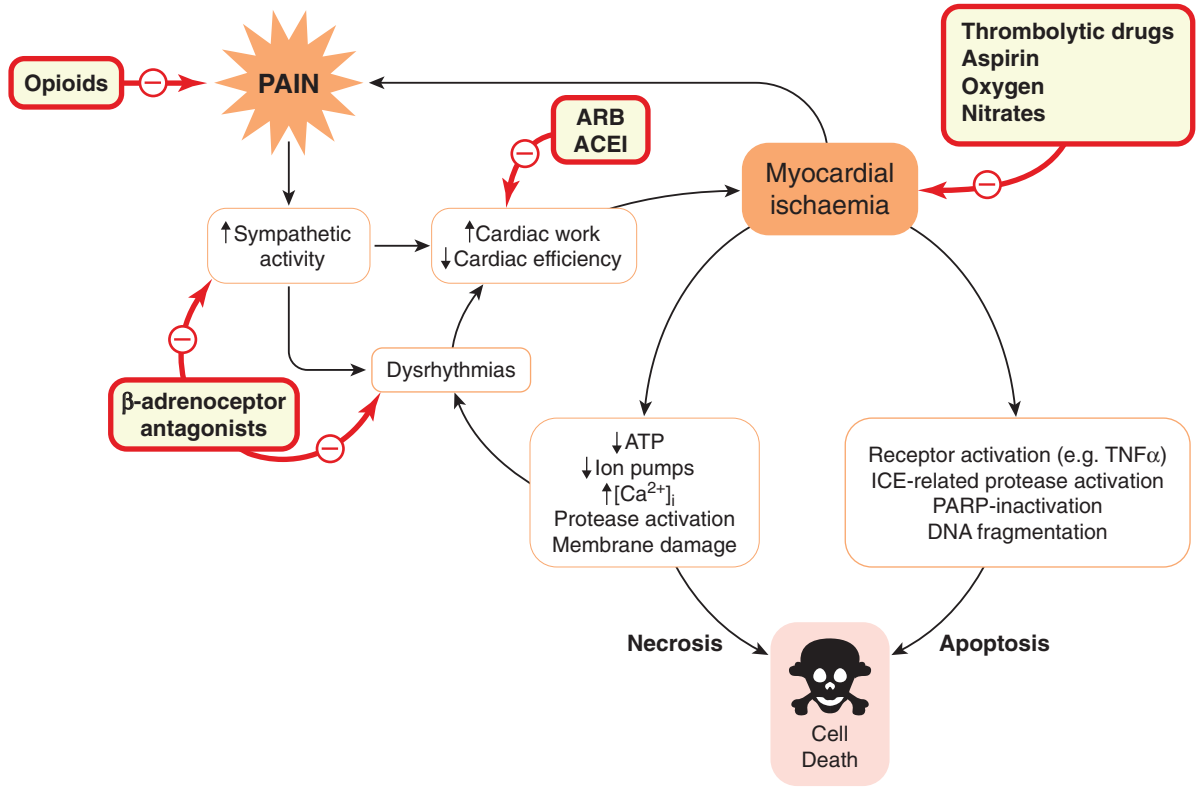


Fig. 21.8 Effects of myocardial ischaemia. This leads to cell death by one of two pathways: necrosis or apoptosis. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin AT_1 receptor antagonist; ICE, interleukin-1-converting enzyme; PARP, poly-[ADP-ribose]-polymerase; TNF-α, tumour necrosis factor-α.

work as well as treating pain and preventing further thrombosis. They are used in combination, and include:

- combinations of thrombolytic, antiplatelet (aspirin and clopidogrel) and antithrombotic (a heparin preparation) drugs to open the blocked artery and prevent reocclusion (see Ch. 24)
- oxygen if there is arterial hypoxia
- opioids (given with an antiemetic) to prevent pain and reduce excessive sympathetic activity
- organic nitrate
- β-adrenoceptor antagonists
- angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin AT_1 receptor antagonists (ARBs; see Ch. 22).

β-Adrenoceptor antagonists reduce cardiac work and thereby the metabolic needs of the heart, and are used as soon as the patient is stable. ACEIs and ARBs also reduce cardiac work and improve survival as does opening the coronary artery (with angioplasty or thrombolytic drug) and antiplatelet treatment.

DRUGS THAT AFFECT CARDIAC FUNCTION

Drugs that have a major action on the heart can be divided into three groups.

1. *Drugs that affect myocardial cells directly*. These include:
   - autonomic neurotransmitters and related drugs
   - antidysrhythmic drugs
   - cardiac glycosides and other inotropic drugs
   - miscellaneous drugs and hormones; these are dealt with elsewhere (e.g. **doxorubicin**, Ch. 55; thyroxine, Ch. 33; glucagon, Ch. 30).
2. *Drugs that affect cardiac function indirectly*. These have actions elsewhere in the vascular system. Some antianginal drugs (e.g. nitrates) fall into this category, as do most drugs that are used to treat heart failure (e.g. diuretics and ACEIs).
3. *Calcium antagonists*. These affect cardiac function by a direct action on myocardial cells and also indirectly by relaxing vascular smooth muscle.

ANTIDYSRHYTHMIC DRUGS

A classification of antidysrhythmic drugs based on their electrophysiological effects was proposed by Vaughan Williams in 1970. It provides a good starting point for discussing mechanisms, although many useful drugs do not fit neatly into this classification (Table 21.1). Furthermore, emergency treatment of serious dysrhythmias is usually by physical means (e.g. pacing or electrical cardioversion by applying a direct current shock to the chest or via an implanted device) rather than drugs.

There are four classes (see Table 21.2).

- Class I: drugs that block voltage-sensitive sodium channels. They are subdivided: Ia, Ib and Ic (see below).
- Class II: β-adrenoceptor antagonists.

Table 21.1 Antidysrhythmic drugs unclassified in the Vaughan Williams system

Drug	Use
Atropine	Sinus bradycardia
Adrenaline (epinephrine)	Cardiac arrest
Isoprenaline	Heart block
Digoxin	Rapid atrial fibrillation
Adenosine	Supraventricular tachycardia
Calcium chloride	Ventricular tachycardia due to hyperkalaemia
Magnesium chloride	Ventricular fibrillation, digoxin toxicity

Table 21.2 Summary of antidysrhythmic drugs (Vaughan Williams classification)

Class	Example(s)	Mechanism
Ia	Disopyramide	Sodium channel block (intermediate dissociation)
Ib	Lidocaine	Sodium channel block (fast dissociation)
Ic	Flecainide	Sodium channel block (slow dissociation)
II	Propranolol	β-Adrenoceptor antagonism
III	Amiodarone, sotalol	Potassium channel block
IV	Verapamil	Calcium channel block

- Class III: drugs that substantially prolong the cardiac action potential.
- Class IV: calcium antagonists.

The phase of the action potential on which each of these classes of drug have their main effect is shown in Figure 21.9.

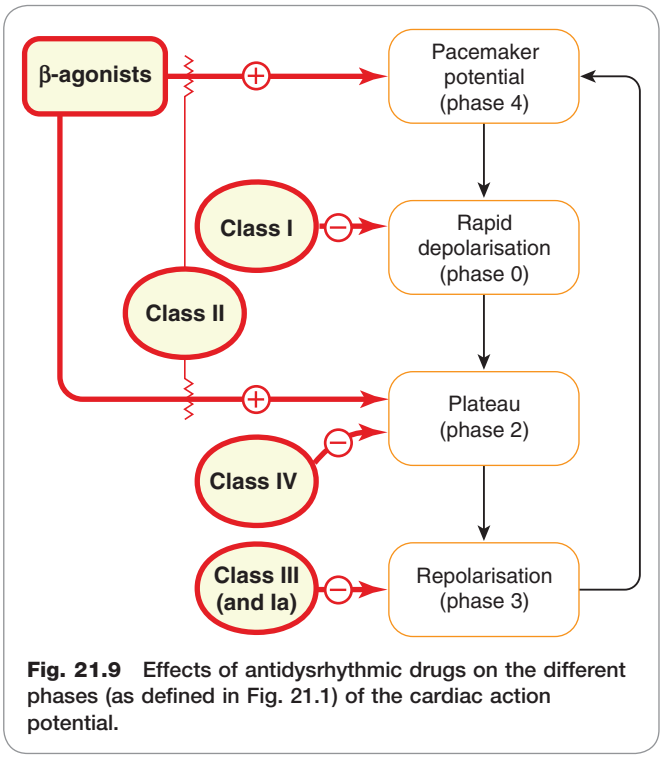


Fig. 21.9 Effects of antidysrhythmic drugs on the different phases (as defined in Fig. 21.1) of the cardiac action potential.

MECHANISMS OF ACTION

Class I drugs

Class I drugs block sodium channels, just as local anaesthetics do, by binding to sites on the α subunit (see Chs 4 and 42). Because this inhibits action potential propagation in many excitable cells, it has been referred to as 'membrane-stabilising' activity, a phrase best avoided now that the ionic mechanism is understood. The characteristic effect on the action potential is to reduce the maximum rate of depolarisation during phase 0.

The reason for further subdivision of these drugs into classes Ia, Ib and Ic is that the earliest examples, **quinidine** and **procainamide** (class Ia), have different effects from many of the more recently developed drugs, even though all share the same basic mechanism of action. A partial explanation for these functional differences comes from electrophysiological studies of the characteristics of the sodium channel block produced by different class I drugs.

The central concept is of *use-dependent channel block*. It is this characteristic that enables all class I drugs to block the high-frequency excitation of the myocardium that occurs in tachyarrhythmias, without preventing the heart from beating at normal frequencies. Sodium channels exist in three distinct functional states: resting, open and refractory (see Ch. 4). Channels switch rapidly from resting to open in response to depolarisation; this is known as *activation*. Maintained depolarisation, as in ischaemic muscle, causes channels to change more slowly from open to refractory (*inactivation*), and the membrane must then be repolarised for a time to restore the channel to the resting state before it can be activated again. Class I drugs bind to channels most strongly when they are in either the open or the refractory state, less strongly to channels in the resting state. Their action therefore shows the property of 'use dependence' (i.e. the more frequently the channels are activated, the greater the degree of block produced).

Class Ib drugs, for example **lidocaine**, associate and dissociate rapidly within the timeframe of the normal heartbeat. The drug binds to open channels during phase 0 of the action potential (affecting the rate of rise very little, but leaving many of the channels blocked by the time the action potential reaches its peak). Dissociation occurs in time for the next action potential, provided the cardiac rhythm is normal. A premature beat, however, will be aborted because the channels are still blocked. Furthermore, class Ib drugs bind selectively to refractory channels and thus block preferentially when the cells are depolarised, for example in ischaemia.

Class Ic drugs, such as **flecainide** and **encainide**, associate and dissociate much more slowly, thus reaching a steady-state level of block that does not vary appreciably during the cardiac cycle. They markedly inhibit conduction through the His–Purkinje system.

Class Ia, the oldest group (e.g. **quinidine**, **procainamide**, **disopyramide**), lies midway in its properties between Ib and Ic but, in addition, prolongs repolarisation, albeit less markedly than class III drugs (see below).

Class II drugs

Class II drugs comprise the β-adrenoceptor antagonists (e.g. **metoprolol**).

Adrenaline can cause dysrhythmias by its effects on the pacemaker potential and on the slow inward Ca^{2+} current (see above). Ventricular dysrhythmias following myocardial infarction are partly the result of increased sympathetic activity (see Fig. 21.8), providing a rationale for using β-adrenoceptor antagonists in this setting. AV conduction depends critically on sympathetic activity; β-adrenoceptor antagonists increase the refractory period of the AV node and can therefore prevent recurrent attacks of supraventricular tachycardia (SVT). The β-adrenoceptor antagonists are also used to prevent paroxysmal attacks of atrial fibrillation when these occur in the setting of sympathetic activation.

Class III drugs

The class III category was originally based on the unusual behaviour of a single drug, **amiodarone** (see below), although others with similar properties (e.g. **sotalol**) have since been described. Both amiodarone and sotalol have more than one mechanism of antidysrhythmic action. The special feature that defines them as class III drugs is that they substantially prolong the cardiac action potential. The mechanism of this effect is not fully understood, but it involves blocking some of the potassium channels involved in cardiac repolarisation, including the outward (delayed) rectifier. Action potential prolongation increases the refractory period, accounting for powerful and varied antidysrhythmic activity, for example by interrupting re-entrant tachycardias and suppressing ectopic activity. However, all drugs that prolong the cardiac action potential (detected clinically as prolonged QT interval on the ECG; see above) can paradoxically also have *proarrhythmic* effects, notably a polymorphic form of ventricular tachycardia called (somewhat whimsically) *torsade de pointes* (because the appearance of the ECG trace is said to be reminiscent of this ballet sequence). This occurs particularly in patients taking other drugs that can prolong QT, including several antipsychotic drugs; those with disturbances of electrolytes involved in repolarisation (e.g. hypokalaemia, hypercalcaemia); or individuals with hereditary prolonged QT (Ward–Romano syndrome).[4] The mechanism of the dysrhythmia is not fully understood; possibilities include increased dispersion of repolarisation (i.e. lack of spatial homogeneity) and increased Ca^{2+} entry during the prolonged action potential, leading to increased after-depolarisation.

Class IV drugs

Class IV agents act by blocking voltage-sensitive calcium channels. Class IV drugs in therapeutic use as antidysrhythmic drugs (e.g. **verapamil**) act on L-type channels. Class IV drugs slow conduction in the SA and AV nodes where action potential propagation depends on slow inward Ca^{2+} current, slowing the heart and terminating SVT by causing partial AV block. They shorten the plateau of the action potential and reduce the force of contraction. Reduced Ca^{2+} entry reduces after-depolarisation and thus suppresses premature ectopic beats. In contrast, L-type calcium channel blockers that act mainly on vascular smooth muscle (e.g. **nifedipine**) indirectly increase sympathetic tone via their hypotensive effect and so may actually provoke tachyarrhythmias.

DETAILS OF INDIVIDUAL DRUGS

Quinidine, procainamide and disopyramide (class Ia)

Quinidine and **procainamide** are pharmacologically similar. They are now mainly of historical interest. **Disopyramide** resembles quinidine, including in its marked atropine-like effects, which result in blurred vision, dry mouth, constipation and urinary retention. It has more negative inotropic action than quinidine but is less likely to cause hypersensitivity reactions.

Lidocaine (class Ib)

Lidocaine, also well known as a local anaesthetic (see Ch. 42), is given by intravenous infusion to treat and prevent ventricular dysrhythmias in the immediate aftermath of myocardial infarction. It is almost completely extracted from the portal circulation by hepatic first-pass metabolism (Ch. 9), and so cannot usefully be administered orally. Its plasma half-life is normally about 2 h, but its elimination is slowed if hepatic blood flow is reduced, for example by reduced cardiac output following myocardial infarction or by drugs that reduce cardiac contractility (e.g. β-adrenoceptor antagonists). Dosage must be reduced accordingly to prevent accumulation and toxicity. Indeed, its clearance has been used to estimate hepatic blood flow, analogous to the use of *para*-aminohippurate clearance to measure renal blood flow.

The adverse effects of lidocaine are mainly due to its actions on the central nervous system and include drowsiness, disorientation and convulsions. Because of its relatively short half-life, the plasma concentration can be adjusted fairly rapidly by varying the infusion rate.

Phenytoin (an antiepileptic drug, see Ch. 44), acts similarly, but is no longer used in treating dysrhythmias.

Flecainide and encainide (class Ic)

Flecainide and **encainide** suppress ventricular ectopic beats. They are long acting and reduce the frequency of ventricular ectopic beats when administered orally. However, in clinical trials, they increase the incidence of sudden death associated with ventricular fibrillation after myocardial infarction, so they are no longer used in this setting. This counterintuitive result had a profound impact on the way clinicians and drug regulators view the use of seemingly reasonable intermediate end points (in this case, reduction of frequency of ventricular ectopic beats) as evidence of efficacy in clinical trials. Currently, the main use of flecainide is in prophylaxis against paroxysmal atrial fibrillation.

[4] A 3-year-old girl began to have blackouts, which decreased in frequency with age. Her ECG showed a prolonged QT interval. When 18 years of age, she lost consciousness running for a bus. When she was 19, she became quite emotional as a participant in a live television audience and died suddenly. The molecular basis of this rare inherited disorder is now known. It is caused by a mutation in either the gene coding for a particular potassium channel—called *HERG*—or another gene, *SCN5A*, which codes for the sodium channel and disruption of which results in a loss of inactivation of the Na^+ current (see Welsh & Hoshi, 1995, for a commentary).

Clinical uses of class I antidysrhythmic drugs

- **Class Ia** (e.g. **disopyramide**)
  - ventricular dysrhythmias
  - prevention of recurrent paroxysmal atrial fibrillation triggered by vagal overactivity.
- **Class Ib** (e.g. intravenous **lidocaine**)
  - treatment and prevention of ventricular tachycardia and fibrillation during and immediately after myocardial infarction.
- **Class Ic**
  - to prevent paroxysmal atrial fibrillation (flecainide)
  - recurrent tachyarrhythmias associated with abnormal conducting pathways (e.g. Wolff–Parkinson–White syndrome).

Clinical uses of class II antidysrhythmic drugs (e.g. propranolol, timolol)

- To reduce mortality following myocardial infarction.
- To prevent recurrence of tachyarrhythmias (e.g. paroxysmal atrial fibrillation) provoked by increased sympathetic activity.

Clinical uses of class III antidysrhythmic drugs

- Amiodarone: tachycardia associated with the Wolff–Parkinson–White syndrome. It is also effective in many other supraventricular and ventricular tachyarrhythmias but has serious adverse effects.
- (Racemic) sotalol combines class III with class II actions. It is used in paroxysmal supraventricular dysrhythmias and suppresses ventricular ectopic beats and short runs of ventricular tachycardia.

β-Adrenoceptor antagonists (class II)

The most important β-adrenoceptor antagonists are described in Chapter 14. Their clinical use for rhythm disorders is shown in the clinical box. **Propranolol**, like several other drugs of this type, has some class I action in addition to blocking β-adrenoceptors. This may contribute to its antidysrhythmic effects, although probably not very much, because an isomer with little β antagonist activity has little antidysrhythmic activity, despite similar activity as a class I agent.

Adverse effects are described in Chapter 14, the most important being worsening bronchospasm in patients with asthma, a negative inotropic effect, bradycardia and fatigue. It was hoped that the use of β_1-selective drugs (e.g. **metoprolol**, **atenolol**) would reduce the risk of bronchospasm, but their selectivity is insufficient to achieve this goal in clinical practice, although the once-a-day convenience of several such drugs has led to their widespread use in patients without lung disease.

Class III

Amiodarone is highly effective at suppressing dysrhythmias (see the clinical box). Like other drugs that interfere with cardiac repolarisation, it is important to monitor plasma electrolyte concentrations (especially of K^+) during its use to avoid precipitating torsades de pointes. Unfortunately it has several peculiarities that complicate its use. It is extensively bound in tissues, has a long elimination half-life (10–100 days) and accumulates in the body during repeated dosing. For this reason, a loading dose is used, and for life-threatening dysrhythmias this is given intravenously via a central vein (it causes phlebitis if given into a peripheral vessel). Adverse effects are numerous and important; they include photosensitive skin rashes and a slate-grey/bluish discoloration of the skin; thyroid abnormalities (hypo- and hyper-, connected with its high iodine content); pulmonary fibrosis, which is late in onset but may be irreversible; corneal deposits; and neurological and gastrointestinal disturbances, including hepatitis. **Dronedarone** is a related benzofuran with somewhat different effects on individual ion channels. It lacks iodine and was designed to be less lipophilic than amiodarone in hopes of reducing thyroid and pulmonary toxicities. Its elimination $t_{1/2}$ is shorter than that of amiodarone and while it increased mortality in patients with severe heart failure (Køber et al., 2008), it improved survival in high-risk patients with atrial fibrillation (Hohnloser et al., 2009) and has recently been approved by the Food and Drug Administration for this indication.

Sotalol is a non-selective β-adrenoceptor antagonist, this activity residing in the L isomer. Unlike other β antagonists, it prolongs the cardiac action potential and the QT interval by delaying the slow outward K^+ current. This class III activity is present in both L and D isomers. Racemic sotalol (the form prescribed) appears to be somewhat less effective than amiodarone in preventing chronic life-threatening ventricular tachyarrhythmias. It shares the ability of amiodarone to cause torsades de pointes but lacks its other adverse effects; it is valuable in patients in whom β-adrenoceptor antagonists are not contraindicated. As with amiodarone, close monitoring of plasma K^+ is important during its use because of the risk of proarryhthmia.

Verapamil and diltiazem (class IV)

Verapamil is given by mouth. (Intravenous preparations are available but are dangerous and almost never needed.) It has a plasma half-life of 6–8 h and is subject to quite extensive first-pass metabolism, which is more marked for the isomer that is responsible for its cardiac effects. A slow-release preparation is available for once-daily use, but it is less effective when used for prevention of dysrhythmia than the regular preparation because the bioavailability of the cardioactive isomer is reduced through the presentation of a steady low concentration to the drug-metabolising enzymes in the liver. If verapamil is added to **digoxin** in patients with poorly controlled atrial fibrillation, the dose of digoxin should be reduced and plasma digoxin concentration checked after a few days, because verapamil both

displaces digoxin from tissue-binding sites and reduces its renal elimination, hence predisposing to digoxin accumulation and toxicity (see Ch. 56).

Verapamil is contraindicated in patients with Wolff–Parkinson–White syndrome (a pre-excitation syndrome caused by a rapidly conducting pathway between atria and ventricles anatomically distinct from the physiological conducting pathway that predisposes to re-entrant tachycardia), and is ineffective and dangerous in ventricular dysrhythmias. Adverse effects of verapamil and diltiazem are described below in the section on calcium channel antagonists.

Diltiazem is similar to verapamil but has relatively more effect on smooth muscle while producing less bradycardia (said to be 'rate neutral').

Adenosine (unclassified in the Vaughan Williams classification)

Adenosine is produced endogenously and is an important chemical mediator (Ch. 16) with effects on breathing, cardiac and smooth muscle, vagal afferent nerves and on platelets, in addition to the effects on cardiac conducting tissue that underlie its therapeutic use. The A_1 receptor is responsible for its effect on the AV node. These receptors are linked to the same cardiac potassium channel (K_{ACh}) that is activated by acetylcholine, and adenosine hyperpolarises cardiac conducting tissue and slows the rate of rise of the pacemaker potential accordingly. It is administered intravenously to terminate SVT if this rhythm persists despite manoeuvres such as carotid artery massage designed to increase vagal tone. It has largely replaced verapamil for this purpose, because it is safer owing to its effect being short lived. This is a consequence of its pharmacokinetics: it is taken up via a specific nucleoside transporter by red blood cells and is metabolised by enzymes on the lumenal surface of vascular endothelium. Consequently, the effects of a bolus dose of adenosine last only 20–30 s. Once SVT has terminated, the patient usually remains in sinus rhythm, even though adenosine is no longer present in plasma. Its short-lived unwanted effects include chest pain, shortness of breath, dizziness and nausea. **Theophylline** and other xanthine alkaloids block adenosine receptors and inhibit the actions of intravenous adenosine, whereas **dipyridamole** (a vasodilator and antiplatelet drug; see below and Ch. 24) blocks the nucleoside uptake mechanism, potentiating adenosine and prolonging its adverse effects. Both these interactions are clinically important.

Clinical uses of class IV antidysrhythmic drugs

- Verapamil is the main drug. It is used:
  - to prevent recurrence of paroxysmal supraventricular tachycardia (SVT)
  - to reduce the ventricular rate in patients with atrial fibrillation, provided they do not have Wolff–Parkinson–White or a related disorder.
- Verapamil was previously given intravenously to terminate SVT; it is now seldom used for this because adenosine is safer.

DRUGS THAT INCREASE MYOCARDIAL CONTRACTION

CARDIAC GLYCOSIDES

Cardiac glycosides come from foxgloves (*Digitalis* spp.) and related plants. Withering (1775) wrote on the use of the foxglove: 'it has a power over the motion of the heart to a degree yet unobserved in any other medicine …' Foxgloves contain several cardiac glycosides with similar actions. Their basic chemical structure consists of three components: a sugar moiety, a steroid and a lactone ring, The lactone is essential for activity, the other parts of the molecule mainly determining potency and pharmacokinetic properties. Therapeutically the most important cardiac glycoside is **digoxin**.

Endogenous cardiotonic steroids (CTSs), also called digitalis-like factors, have been mooted for nearly half a century. There is evidence in mammals of an endogenous digitalis-like factor closely similar to **ouabain**, a short-acting cardiac glycoside (see Schoner & Scheiner-Bobis, 2007). CTSs were first considered important in the regulation of renal sodium transport and arterial pressure, but they have now been implicated in the regulation of cell growth, differentiation, apoptosis, fibrosis, the modulation of immunity and of carbohydrate metabolism, and the control of various central nervous functions (Bagrov et al., 2009).

Actions and adverse effects

The main actions of glycosides are on the heart, but some of their adverse effects are extracardiac, including nausea, vomiting, diarrhoea and confusion. The cardiac effects are:

- cardiac slowing and reduced rate of conduction through the AV node
- increased force of contraction
- disturbances of rhythm, especially:
  - block of AV conduction
  - increased ectopic pacemaker activity.

Adverse effects are common and can be severe. One of the main drawbacks of glycosides in clinical use is the narrow margin between effectiveness and toxicity.

Mechanism

The mechanism whereby cardiac glycosides increase the force of cardiac contraction (positive inotropic effect) is inhibition of the Na^+/K^+ pump in the cardiac myocytes. Cardiac glycosides bind to a site on the extracellular aspect of the α subunit of the Na^+-K^+-ATPase (which is an αβ heterodimer), and are useful experimental tools for studying this important transport system. The molecular mechanism underlying increased vagal tone (negative chronotropic effect) is unknown, but could also be due to inhibition of the Na^+/K^+ pump.

Rate and rhythm

Cardiac glycosides slow AV conduction by increasing vagal outflow. Their beneficial effect in established rapid atrial fibrillation results partly from this. If ventricular rate is excessively rapid, the time available for diastolic filling is inadequate. Increasing the refractory period of the AV node reduces ventricular rate. The atrial dysrhythmia persists, but the pumping efficiency of the heart improves owing to improved ventricular filling. SVT can be terminated by cardiac glycosides, which slow AV conduction, although other drugs are usually employed for this indication (see below).

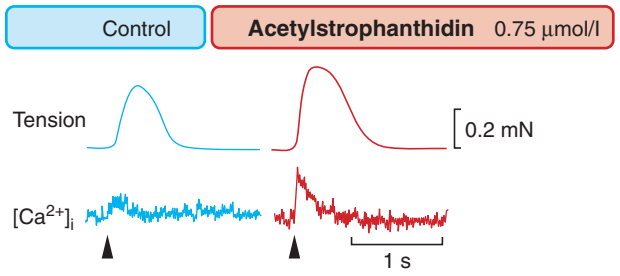


Fig. 21.10 Effect of a cardiac glycoside (acetylstrophanthidin) on the Ca^{2+} transient and tension produced by frog cardiac muscle. The effect was recorded as in Figure 21.6 (From Allen D G, Blinks J R 1978 Nature 273: 509.)

Larger doses of glycosides disturb sinus rhythm. This can occur at plasma concentrations of digoxin within, or only slightly above, the therapeutic range. Slowing of AV conduction can progress to AV block. Glycosides can also cause ectopic beats. Because Na^+/K^+ exchange is electrogenic, inhibition of the pump by glycosides causes depolarisation, predisposing to disturbances of cardiac rhythm. Furthermore, the increased $[Ca^{2+}]_i$ causes increased afterdepolarisation, leading first to coupled beats (bigeminy), in which a normal ventricular beat is followed by an ectopic beat; this is followed by ventricular tachycardia and eventually by ventricular fibrillation.

Force of contraction

Glycosides cause a large increase in twitch tension in isolated preparations of cardiac muscle. Unlike catecholamines, they do not accelerate relaxation (compare Fig. 21.6 with Fig. 21.10). Increased tension is caused by an increased $[Ca^{2+}]_i$ transient (Fig. 21.10). The action potential is only slightly affected and the slow inward current little changed, so the increased $[Ca^{2+}]_i$ transient probably reflects a greater release of Ca^{2+} from intracellular stores. The most likely mechanism is as follows (see also Ch. 4):

1. Glycosides inhibit the Na^+/K^+ pump.
2. Increased $[Na^+]_i$ slows extrusion of Ca^{2+} via the Na^+/Ca^{2+} exchange transporter. Increasing $[Na^+]_i$ reduces the inwardly directed gradient for Na^+; the smaller this gradient, the slower is extrusion of Ca^{2+} by Na^+/Ca^{2+} exchange.
3. Increased $[Ca^{2+}]_i$ is stored in the sarcoplasmic reticulum, and thus increases the amount of Ca^{2+} released by each action potential.

The effect of extracellular potassium

Effects of cardiac glycosides are increased if plasma $[K^+]$ decreases, because of reduced competition at the K^+-binding site on the Na^+-K^+-ATPase. This is clinically important, because diuretics (Ch. 28) are often used to treat heart failure, and most such drugs decrease plasma $[K^+]$, thereby increasing the risk of glycoside-induced dysrhythmia.

Pharmacokinetic aspects

Digoxin is administered by mouth or, in urgent situations, intravenously. It is a polar molecule; elimination is mainly by renal excretion and involves P-glycoprotein (Ch. 8), leading to clinically significant interactions with other drugs used to treat heart failure, such as **spironolactone**, and with antidysrhythmic drugs such as **verapamil** and **amiodarone**. Elimination half-time is approximately 36 h in patients with normal renal function, but considerably longer in elderly patients and those with overt renal failure, in whom reduced doses are indicated. A loading dose is used in urgent situations. The therapeutic range of plasma concentrations, below which digoxin is unlikely to be effective and above which the risk of toxicity increases substantially, is fairly well defined (1–2.6 nmol/l). Determination of plasma digoxin concentration is useful when lack of efficacy or toxicity is suspected.

Clinical uses of cardiac glycosides (e.g. digoxin)

- To slow ventricular rate in rapid persistent atrial fibrillation.
- Treatment of heart failure in patients who remain symptomatic despite optimal use of diuretics and angiotensin-converting enzyme inhibitors (Ch. 22).

OTHER DRUGS THAT INCREASE MYOCARDIAL CONTRACTION

Certain β_1-adrenoceptor agonists, for example **dobutamine**, are used to treat acute but potentially reversible heart failure (e.g. following cardiac surgery or in some cases of cardiogenic or septic shock) on the basis of their positive inotropic action. Dobutamine, for reasons that are not well understood, produces less tachycardia than other β_1 agonists. It is administered intravenously. **Glucagon** also increases myocardial contractility by increasing synthesis of cAMP, and has been used in patients with acute cardiac dysfunction owing to overdosage of β-adrenoceptor antagonists.

Inhibitors of the heart-specific subtype (type III) of phosphodiesterase, the enzyme responsible for the intracellular degradation of cAMP, increase myocardial contractility. Consequently, like β-adrenoceptor agonists, they increase intracellular cAMP but cause dysrhythmias for the same reason. Compounds in this group include **amrinone** and **milrinone**. They improve haemodynamic indices in patients with heart failure but paradoxically worsen survival, presumably because of dysrhythmias. This dichotomy has had a sobering effect on clinicians and drug regulatory authorities.

ANTIANGINAL DRUGS

The mechanism of anginal pain is discussed above. Angina is managed by using drugs that improve perfusion of the myocardium or reduce its metabolic demand, or both. Two of the main groups of drugs, organic nitrates and calcium antagonists, are vasodilators and produce both these effects. The third group, β-adrenoceptor antagonists, slow the heart and hence reduce metabolic demand. Organic nitrates and calcium antagonists are described below. The β-adrenoceptor antagonists are covered in Ch. 14, and their antidysrhythmic actions are described above. **Ivabradine** slows the heart by inhibiting the sinus node I_f current (see above), and is an alternative to β-adrenoceptor antagonists in patients in whom these are not tolerated or are contraindicated.

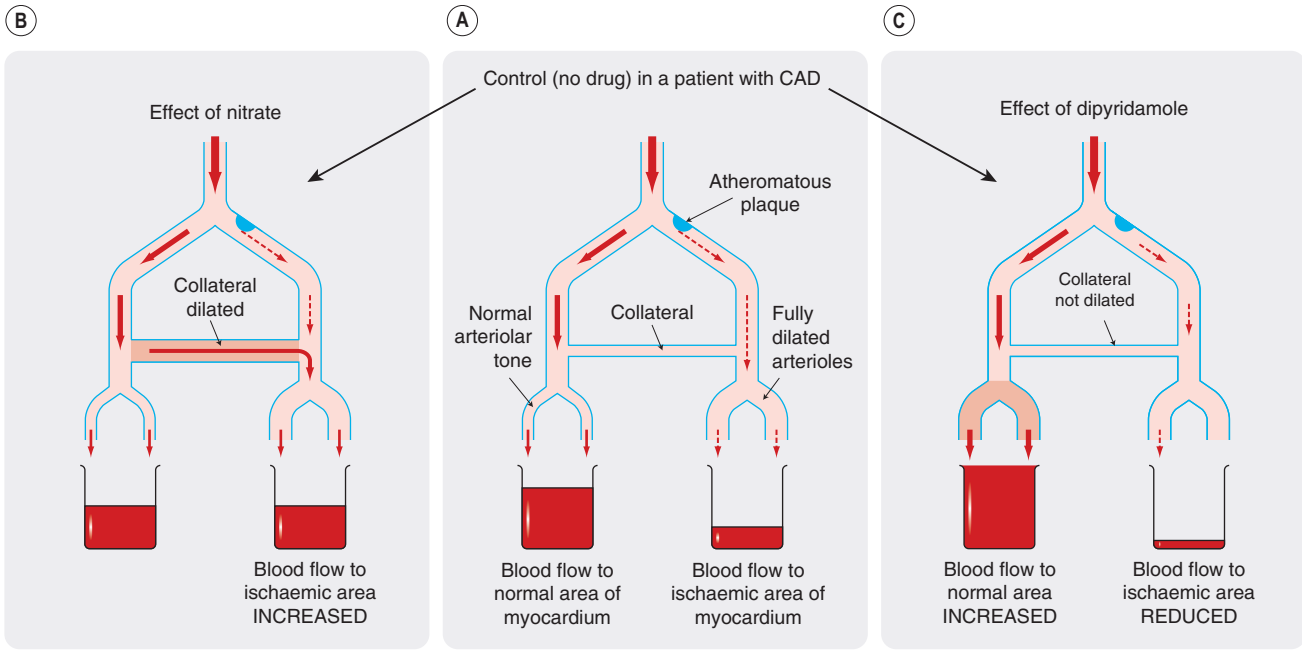


Fig. 21.11 Comparison of the effects of organic nitrates and an arteriolar vasodilator (dipyridamole) on the coronary circulation. [**A**] Control. [**B**] Nitrates dilate the collateral vessel, thus allowing more blood through to the underperfused region (mostly by diversion from the adequately perfused area). [**C**] Dipyridamole dilates arterioles, increasing flow through the normal area at the expense of the ischaemic area (in which the arterioles are anyway fully dilated). CAD, coronary artery disease.

ORGANIC NITRATES

The ability of organic nitrates (see also Chs 20 and 22) to relieve angina was discovered by Lauder Brunton, a distinguished British physician, in 1867. He had found that angina could be partly relieved by bleeding, and also knew that **amyl nitrite**, which had been synthesised 10 years earlier, caused flushing and tachycardia, with a fall in blood pressure, when its vapour was inhaled. He thought that the effect of bleeding resulted from hypotension, and found that amyl nitrite inhalation worked much better. Amyl nitrite has now been replaced by **glyceryl trinitrate** (GTN).[5] Several related organic nitrates, of which the most important is **isosorbide mononitrate**, have a prolonged action.

Actions

Organic nitrates relax smooth muscle (especially vascular smooth muscle, but also other types including oesophageal and biliary smooth muscle). They relax veins, with a consequent reduction in central venous pressure (reduced preload). In healthy subjects, this reduces stroke volume; venous pooling occurs on standing and can cause postural hypotension and dizziness. Therapeutic doses have less effect on small resistance arteries than on veins, but there is a marked effect on larger muscular arteries. This reduces pulse wave reflection from arterial branches (as appreciated in the 19th century by Murrell but neglected for many years thereafter), and consequently reduces central (aortic) pressure and cardiac afterload (see Ch. 22 for the role of these factors in determining cardiac work). The direct dilator effect on coronary arteries opposes coronary artery spasm in variant angina. With larger doses, resistance arteries and arterioles dilate, and arterial pressure falls. Nevertheless, coronary flow is increased as a result of coronary vasodilatation. Myocardial oxygen consumption is reduced because of the reductions in both cardiac preload and afterload. This, together with the increased coronary blood flow, causes a large increase in the oxygen content of coronary sinus blood. Studies in experimental animals have shown that glyceryl trinitrate diverts blood from normal to ischaemic areas of myocardium. The mechanism involves dilatation of collateral vessels that bypass narrowed coronary artery segments (Fig. 21.11).

▼ It is interesting to compare this effect with that of other vasodilators, notably **dipyridamole**, which dilate arterioles but not collaterals. Dipyridamole is at least as effective as nitrates in increasing coronary flow in normal subjects but actually *worsens* angina. This is probably because arterioles in an ischaemic region are fully dilated by the ischaemia, and drug-induced dilatation of the arterioles in normal areas has the effect of diverting blood away from the ischaemic areas (Fig. 21.11), producing what is termed a vascular *steal*. This effect is exploited in a pharmacological 'stress' test for coronary arterial disease, in which dipyridamole is administered intravenously to patients in whom this diagnosis is suspected but who cannot exercise, while monitoring myocardial perfusion and the ECG.

In summary, the antianginal action of nitrates involves:

- reduced cardiac oxygen consumption, because of reduced cardiac preload and afterload
- redistribution of coronary flow towards ischaemic areas via collaterals
- relief of coronary spasm.

In addition to its effects on smooth muscle, nitric oxide increases the rate of relaxation of cardiac muscle (dubbed a '*lusiotropic*' action). It is probable that organic nitrates

[5]Nobel discovered how to stabilise GTN with kieselguhr, enabling him to exploit its explosive properties in dynamite, the manufacture of which earned him the fortune with which he endowed the eponymous prizes.

mimic this action, which could be important in patients with impaired diastolic function, a common accompaniment of hypertension and of heart failure.

Mechanism of action

Organic nitrates are metabolised with release of nitric oxide. At concentrations achieved during therapeutic use, this involves an enzymic step and possibly a reaction with tissue sulfhydryl (–SH) groups. Nitric oxide activates soluble guanylyl cyclase (see Ch. 20), increasing formation of cGMP, which activates protein kinase G (Ch. 4) and leads to a cascade of effects in smooth muscle culminating in dephosphorylation of myosin light chains, sequestration of intracellular Ca^{2+} and consequent relaxation.

Tolerance and unwanted effects

Repeated administration of nitrates to smooth muscle preparations in vitro results in diminished relaxation, possibly partly because of depletion of free –SH groups, although attempts to prevent tolerance by agents that restore tissue –SH groups have not been clinically useful. Tolerance to the antianginal effect of nitrates does not occur to a clinically important extent with ordinary formulations of short-acting drugs (e.g. glyceryl trinitrate), but does occur with longer acting drugs (e.g. isosorbide mononitrate) or when glyceryl trinitrate is administered by prolonged intravenous infusion or by frequent application of slow-release transdermal patches (see below).

The main adverse effects of nitrates are a direct consequence of their main pharmacological actions, and include postural hypotension and headache. This was the cause of 'Monday morning sickness' among workers in explosives factories. Tolerance to these effects develops quite quickly but wears off after a brief nitrate-free interval (which is why the symptoms appeared on Mondays and not later in the week). Formation of *methaemoglobin*, an oxidation product of haemoglobin that is ineffective as an oxygen carrier, seldom occurs when nitrates are used clinically but is induced deliberately with **amyl nitrite** in the treatment of *cyanide poisoning*, because methaemoglobin binds and inactivates cyanide ions.

Pharmacokinetic and pharmaceutical aspects

Glyceryl trinitrate is rapidly inactivated by hepatic metabolism. It is well absorbed from the mouth and is taken as a tablet under the tongue or as a sublingual spray, producing its effects within a few minutes. If swallowed, it is ineffective because of first-pass metabolism. Given sublingually, the trinitrate is converted to di- and mononitrates. Its effective duration of action is approximately 30 min. It is appreciably absorbed through the skin, and a more sustained effect can be achieved by applying it as a transdermal patch. Once a bottle of the tablets has been opened, its shelf-life is quite short because the volatile active substance evaporates; spray preparations avoid this problem.

Isosorbide mononitrate is longer acting than glyceryl trinitrate because it is absorbed and metabolised more slowly but has similar pharmacological actions. It is swallowed rather than taken sublingually, and is taken twice a day for prophylaxis (usually in the morning and at lunch, to allow a nitrate-free period during the night, when patients are not exerting themselves, to avoid tolerance). It is also available in slow-release form for once-daily use in the morning.

Organic nitrates

- Important compounds include **glyceryl trinitrate** and longer-acting **isosorbide mononitrate**.
- These drugs are powerful vasodilators, acting on veins to reduce cardiac preload and reducing arterial wave reflection to reduce afterload.
- Act via nitric oxide, to which they are metabolised. Nitric oxide stimulates cGMP formation and hence activates protein kinase G, affecting both contractile proteins (myosin light chains) and Ca^{2+} regulation.
- Tolerance occurs experimentally and is important clinically with frequent use of long-acting drugs or sustained-release preparations.
- Effectiveness in angina results partly from reduced cardiac load and partly from dilatation of collateral coronary vessels, causing more effective distribution of coronary flow. Dilatation of constricted coronary vessels is particularly beneficial in variant angina.
- Serious unwanted effects are uncommon; headache and postural hypotension may occur initially. Overdose can, rarely, cause methaemoglobinaemia.

Clinical uses of organic nitrates

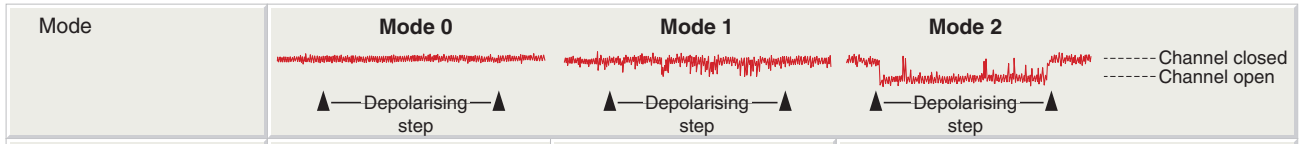
- Stable angina:
  - prevention (e.g. daily **isosorbide mononitrate**, or **glyceryl trinitrate** sublingually immediately before exertion)
  - treatment (sublingual glyceryl trinitrate).
- Unstable angina: intravenous glyceryl trinitrate.
- Acute heart failure: intravenous glyceryl trinitrate.
- Chronic heart failure: isosorbide mononitrate, with hydralazine in patients of African origin (Ch. 22).
- Uses related to relaxation of other smooth muscles (e.g. uterine, biliary) are being investigated.

POTASSIUM CHANNEL ACTIVATORS

Nicorandil combines activation of the potassium K_{ATP} channel (see Ch. 4) with nitrovasodilator (nitric oxide donor) actions. It is both an arterial and a venous dilator, and causes the expected unwanted effects of headache, flushing and dizziness. It is used for patients who remain symptomatic despite optimal management with other drugs, often while they await surgery or angioplasty.

β-ADRENOCEPTOR ANTAGONISTS

β-Adrenoceptor antagonists (see Ch. 14) are important in prophylaxis of angina, and in treating patients with unstable angina. They work for these indications by reducing cardiac oxygen consumption. In addition, they reduce the risk of death following myocardial infarction, probably via their antidysrhythmic action. Any effects on coronary vessel diameter are of minor importance, although these drugs are avoided in variant angina because of the



Mode	Mode 0	Mode 1	Mode 2
Opening probability	Zero	Low	High
Favoured by	DHP antagonists		DHP agonists
% of time normally spent in this mode	<1%	~70%	~30%

Fig. 21.12 Mode behaviour of calcium channels. The traces are patch clamp recordings (see Ch. 3) of the opening of single calcium channels (downward deflections) in a patch of membrane from a cardiac muscle cell. A depolarising step is imposed close to the start of each trace, causing an increase in the opening probability of the channel. When the channel is in mode 1 (centre), this causes a few brief openings to occur; in mode 2 (right), the channel stays open for most of the time during the depolarising step; in mode 0 (left), it fails to open at all. Under normal conditions, the channel spends most of its time in modes 1 and 0, and only rarely enters mode 2. DHP, dihydropyridine. (Redrawn from Hess et al. 1984 Nature 311: 538–544.)

theoretical risk that they will increase coronary spasm. Their very diverse clinical uses are summarised in the clinical box.

CALCIUM ANTAGONISTS

The term 'calcium antagonist' is used for drugs that block cellular entry of Ca^{2+} through calcium channels rather than its intracellular actions (Ch. 4). Some authors use the term 'Ca^{2+} entry blockers' to make this distinction clearer. Therapeutically important calcium antagonists act on L-type channels. L-type calcium antagonists comprise three chemically distinct classes: *phenylalkylamines* (e.g. **verapamil**), *dihydropyridines* (e.g. **nifedipine**, **amlodipine**) and *benzothiazepines* (e.g. **diltiazem**).

Mechanism of action: types of calcium channel

The properties of voltage-gated calcium channels have been studied by voltage clamp and patch clamp techniques (see Ch. 3). Drugs of each of the three chemical classes mentioned above all bind the α_1 subunit of the L-type calcium channel but at distinct sites. These interact allosterically with each other and with the gating machinery of the channel to prevent its opening (see below), thus reducing Ca^{2+} entry. Many calcium antagonists show properties of use dependence (i.e. they block more effectively in cells in which the calcium channels are most active; see the discussion of class I antidysrhythmic drugs above). For the same reason, they also show voltage-dependent blocking actions, blocking more strongly when the membrane is depolarised, causing calcium channel opening and inactivation.

▼ Dihydropyridines affect calcium channel function in a complex way, not simply by physical plugging of the pore. This became clear when some dihydropyridines, exemplified by BAY K 8644, were found to bind to the same site but to do the opposite; that is, to promote the opening of voltage-gated calcium channels. Thus BAY K 8644 *increases* the force of cardiac contraction, and *constricts* blood vessels; it is competitively antagonised by nifedipine. Calcium channels can exist in one of three distinct states, termed 'modes' (Fig. 21.12). When a channel is in mode 0, it does not open in response to depolarisation; in mode 1, depolarisation produces a low opening probability, and each opening is brief. In mode 2, depolarisation produces a very high opening probability, and single openings are prolonged. Under normal conditions, about 70% of the channels at any one moment exist in mode 1, with only 1% or less in mode 2; each channel switches randomly and quite slowly between the three modes. Dihydropyridine antagonists bind selectively to channels in mode 0, thus favouring this non-opening state, whereas agonists bind selectively to channels in mode 2 (Fig. 21.12). This type of two-directional modulation resembles the phenomenon seen with the GABA/benzodiazepine interaction (Ch. 43), and invites speculation about possible endogenous dihydropyridine-like mediator(s) with a regulatory effect on Ca^{2+} entry.

Mibefradil blocks T- as well as L-type channels at therapeutic concentrations, but was withdrawn from therapeutic use because it caused adverse drug interactions by interfering with drug metabolism. **Ethosuximide** (a carbonic anhydrase inhibitor used to treat absence seizures, Ch. 44) also blocks T channels in thalamic and reticular neurones.

Pharmacological effects

The main effects of calcium antagonists, as used therapeutically, are on cardiac and smooth muscle. **Verapamil** preferentially affects the heart, whereas most of the dihydropyridines (e.g. **nifedipine**) exert a greater effect on smooth muscle than on the heart. **Diltiazem** is intermediate in its actions.

Cardiac actions

The antidysrhythmic effects of verapamil and diltiazem have been discussed above. Calcium antagonists can cause AV block and cardiac slowing by their actions on conducting tissues, but this is offset by a reflex increase in sympathetic activity secondary to their vasodilator action. For example, nifedipine typically causes reflex tachycardia; diltiazem causes little or no change in heart rate and verapamil slows the heart rate. Calcium antagonists also have a negative inotropic effect, from their inhibition of Ca^{2+} entry during the action potential plateau. Verapamil has the most marked negative inotropic action, and is contraindicated in heart failure, whereas amlodipine does not worsen cardiovascular mortality in patients with severe but stable chronic heart failure.

Vascular smooth muscle

Calcium antagonists cause generalised arterial/arteriolar dilatation, thereby reducing blood pressure, but do not much affect the veins. They affect all vascular beds, although regional effects vary considerably between different drugs. They cause coronary vasodilatation and are used in patients with coronary artery spasm (variant

angina). Other types of smooth muscle (e.g. biliary tract, urinary tract and uterus) are also relaxed by calcium antagonists, but these effects are less important therapeutically than their actions on vascular smooth muscle, although they do cause adverse effects (see below).

Protection of ischaemic tissues

There are theoretical reasons (see Fig. 21.8) why calcium antagonists might exert a cytoprotective effect in ischaemic tissues and thus be of use in treating heart attack and stroke (see Ch. 39). However, randomised clinical trials have been disappointing, with little or no evidence of beneficial (or harmful) effects of calcium antagonists on cardiovascular morbidity or mortality in patient groups other than patients with hypertension, in whom calcium antagonists have beneficial effects comparable with those of other drugs that lower blood pressure to similar extents (see Ch. 22). **Nimodipine** is partly selective for cerebral vasculature and is sometimes used to reduce cerebral vasospasm following subarachnoid haemorrhage.

Pharmacokinetics

Calcium antagonists in clinical use are all well absorbed from the gastrointestinal tract, and are given by mouth except for some special indications, such as following subarachnoid haemorrhage, for which intravenous preparations are available. They are extensively metabolised. Pharmacokinetic differences between different drugs and different pharmaceutical preparations are clinically important, because they determine the dose interval and also the intensity of some of the unwanted effects, such as headache and flushing (see below). Amlodipine has a long elimination half-life and is given once daily, whereas nifedipine, diltiazem and verapamil have shorter elimination half-lives and are either given more frequently or are formulated in various slow-release preparations to permit once-daily dosing.

Unwanted effects

Most of the unwanted effects of calcium antagonists are extensions of their main pharmacological actions. Short-acting dihydropyridines cause flushing and headache because of their vasodilator action, and in chronic use, dihydropyridines often cause ankle swelling related to arteriolar dilatation and increased permeability of postcapillary venules. Verapamil can cause constipation, probably because of effects on calcium channels in gastrointestinal nerves or smooth muscle. Effects on cardiac rhythm (e.g. heart block) and force of contraction (e.g. worsening heart failure) are discussed above.

Apart from these predictable effects, calcium channel antagonists, as a class, appear rather free from idiosyncratic adverse effects.

Calcium antagonists

- Block Ca^{2+} entry by preventing opening of voltage-gated L-type calcium channels.
- There are three main L-type antagonists, typified by verapamil, diltiazem and dihydropyridines (e.g. nifedipine).
- Mainly affect heart and smooth muscle, inhibiting the Ca^{2+} entry caused by depolarisation in these tissues.
- Selectivity between heart and smooth muscle varies: verapamil is relatively cardioselective, nifedipine is relatively smooth muscle selective, and diltiazem is intermediate.
- Vasodilator effect (mainly dihydropyridines) is mainly on resistance vessels, reducing afterload. Calcium antagonists dilate coronary vessels, which is important in variant angina.
- Effects on heart (verapamil, diltiazem): antidysrhythmic action (mainly atrial tachycardias), because of impaired atrioventricular conduction; reduced contractility.
- Clinical uses:
  - antidysrhythmic (mainly verapamil)
  - angina (e.g. diltiazem)
  - hypertension (mainly dihydropyridines).
- Unwanted effects include headache, constipation (verapamil) and ankle oedema (dihydropyridines). There is a risk of causing cardiac failure or heart block, especially with verapamil.

Clinical uses of calcium antagonists

- Dysrhythmias (verapamil):
  - to slow ventricular rate in rapid atrial fibrillation
  - to prevent recurrence of supraventricular tachycardia (SVT) (intravenous administration of verapamil to terminate SVT attacks has been replaced by use of adenosine).
- Hypertension: usually a dihydropyridine drug (e.g. amlodipine or slow-release nifedipine; Ch. 22).
- To prevent angina (e.g. dihydropyridine or diltiazem).

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22 The vascular system

OVERVIEW

This chapter is concerned with the pharmacology of blood vessels. The walls of arteries, arterioles, venules and veins contain smooth muscle whose contractile state is controlled by circulating hormones and by mediators released locally from sympathetic nerve terminals (Ch. 12) and endothelial cells. These work mainly by regulating Ca^{2+} in vascular smooth muscle cells, as described in Chapter 4. In the present chapter, we first consider the control of vascular smooth muscle by the endothelium and by the renin–angiotensin system, followed by the actions of vasoconstrictor and vasodilator drugs. Finally, we briefly consider clinical uses of vasoactive drugs in some important diseases, namely hypertension (pulmonary as well as systemic), heart failure, shock, peripheral vascular disease and Raynaud's disease. The use of vasoactive drugs to treat angina is covered in Chapter 21.

INTRODUCTION

In this chapter we briefly describe the structure and function of the vascular system. Actions of drugs on the vascular system can be broken down into effects on:

- total systemic ('peripheral') vascular resistance, one of the main determinants of arterial blood pressure
- the resistance of individual vascular beds, which determines the local distribution of blood flow to and within different organs; such effects are relevant to the drug treatment of angina (Ch. 21), Raynaud's phenomenon, pulmonary hypertension and circulatory shock
- aortic compliance and pulse wave reflection, which are relevant to the treatment of cardiac failure and angina
- venous tone and blood volume (the 'fullness' of the circulation), which together determine the central venous pressure and are relevant to the treatment of cardiac failure and angina; diuretics (which reduce blood volume) are discussed in Chapter 28
- atheroma (Ch. 23) and thrombosis (Ch. 24)
- new vessel formation (angiogenesis)—important, for example, in diabetic retinopathy (Ch. 30) and in treating malignant disease (Ch. 55).

Drug effects considered in this chapter are caused by actions on vascular smooth muscle cells. Like other muscles, vascular smooth muscle contracts when cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) rises, but the coupling between $[Ca^{2+}]_i$ and contraction is less tight than in striated or cardiac muscle (Ch. 4). Vasoconstrictors and vasodilators act by increasing or reducing $[Ca^{2+}]_i$, and/or by altering the sensitivity of the contractile machinery to $[Ca^{2+}]_i$. Figure 4.10 summarises cellular mechanisms that are involved in the control of smooth muscle contraction and relaxation. The control of vascular smooth muscle tone by various mediators is described in other chapters (noradrenaline in Ch. 14, 5-HT in Ch. 15, prostanoids in Ch. 17, nitric oxide [NO] in Ch. 20, cardiac natriuretic peptides in Ch. 21, antidiuretic hormone in Ch. 32). Here we focus on endothelium-derived mediators and on the renin–angiotensin–aldosterone system, before describing the actions of vasoactive drugs and their uses in some important clinical disorders (hypertension, heart failure, shock, peripheral vascular disease and Raynaud's disease).

VASCULAR STRUCTURE AND FUNCTION

Blood is ejected with each heartbeat from the left ventricle into the aorta, whence it flows rapidly to the organs via large conduit arteries. Successive branching leads via muscular arteries to arterioles (endothelium surrounded by a layer of smooth muscle only one cell thick) and capillaries (naked tubes of endothelium), where gas and nutrient exchanges occur. Capillaries coalesce to form postcapillary venules, venules and progressively larger veins leading, via the vena cava, to the right heart. Deoxygenated blood ejected from the right ventricle travels through the pulmonary artery, pulmonary capillaries and pulmonary veins back to the left atrium.[1] Small muscular arteries and arterioles are the main resistance vessels, while veins are capacity vessels that contain a large fraction of the total blood volume. In terms of cardiac function, therefore, arteries and arterioles regulate the *afterload*, while veins and pulmonary vessels regulate the *preload* of the ventricles.

Viscoelastic properties of large arteries determine arterial compliance (i.e. the degree to which the volume of the arterial system increases as the pressure increases). This is an important factor in a circulatory system that is driven by an intermittent pump such as the heart. Blood ejected from the left ventricle is accommodated by distension of the aorta, which absorbs the pulsations and delivers a relatively steady flow to the tissues. The greater the compliance of the aorta, the more effectively are fluctuations damped out,[2] and the smaller the oscillations of arterial pressure with each heartbeat (i.e. the difference between the systolic and diastolic pressure, known as the 'pulse pressure'). Reflection of the pressure wave from branch points in the vascular tree also sustains arterial pressure

[1]William Harvey (physician to King Charles I) inferred the circulation of the blood on the basis of superbly elegant quantitative experiments long before the invention of the microscope enabled visual confirmation of the tiny vessels he had predicted. This intellectual triumph did his medical standing no good at all, and Aubrey wrote that 'he fell mightily in his practice, and was regarded by the vulgar as crack-brained'. Plus ça change …

[2]This cushioning action is called the 'windkessel' effect. The same principle was used to deliver a steady rather than intermittent flow from old-fashioned fire pumps.

during diastole. In young people, this helps to preserve a steady perfusion of vital organs, such as the kidney, during diastole.

However, excessive reflection can pathologically augment aortic systolic pressure. This results from stiffening of the aorta due to loss of elastin during ageing, especially in people with hypertension. Elastin is replaced by inelastic collagen. Cardiac work (see Ch. 21) can be reduced by increasing arterial compliance or by reducing arterial wave reflection, even if the cardiac output and mean arterial pressure are unchanged. Over around 55 years of age, pulse pressure and aortic stiffness are important risk factors for cardiac disease.

CONTROL OF VASCULAR SMOOTH MUSCLE TONE

Two important physiological systems regulate vascular tone, namely the vascular endothelium and the renin–angiotensin system.

THE VASCULAR ENDOTHELIUM

A new chapter in our understanding of vascular control opened with the discovery that vascular endothelium acts not only as a passive barrier between plasma and extracellular fluid, but also as a source of numerous potent mediators. These actively control the contraction of the underlying smooth muscle as well as influencing platelet and mononuclear cell function: the roles of the endothelium in haemostasis and thrombosis are discussed in Chapter 24. Several distinct classes of mediator are involved (Fig. 22.1).

- *Prostanoids* (see Ch. 17). The discovery by Bunting, Gryglewski, Moncada and Vane (1976) of prostaglandin PGI_2 (prostacyclin) ushered in this era. This mediator, acting on IP receptors (Ch. 17), relaxes smooth muscle and inhibits platelet aggregation by activating adenylyl cyclase. Endothelial cells from microvessels also synthesise PGE_2, which is a direct vasodilator and inhibits noradrenaline release from sympathetic nerve terminals, while lacking the effect of PGI_2 on platelets. Prostaglandin endoperoxide intermediates (PGG_2, PGH_2) are endothelium-derived contracting factors acting via thromboxane (TX) TP receptors.
- *Nitric oxide (NO)* (see Ch. 20). *Endothelium-derived relaxing factor* (EDRF) was described by Furchgott and Zawadzki in 1980, and identified as NO by the groups of Moncada and of Ignarro (see Fig. 20.2). These discoveries enormously expanded our understanding of the role of the endothelium. NO activates guanylyl cyclase. It is released continuously in resistance vessels,

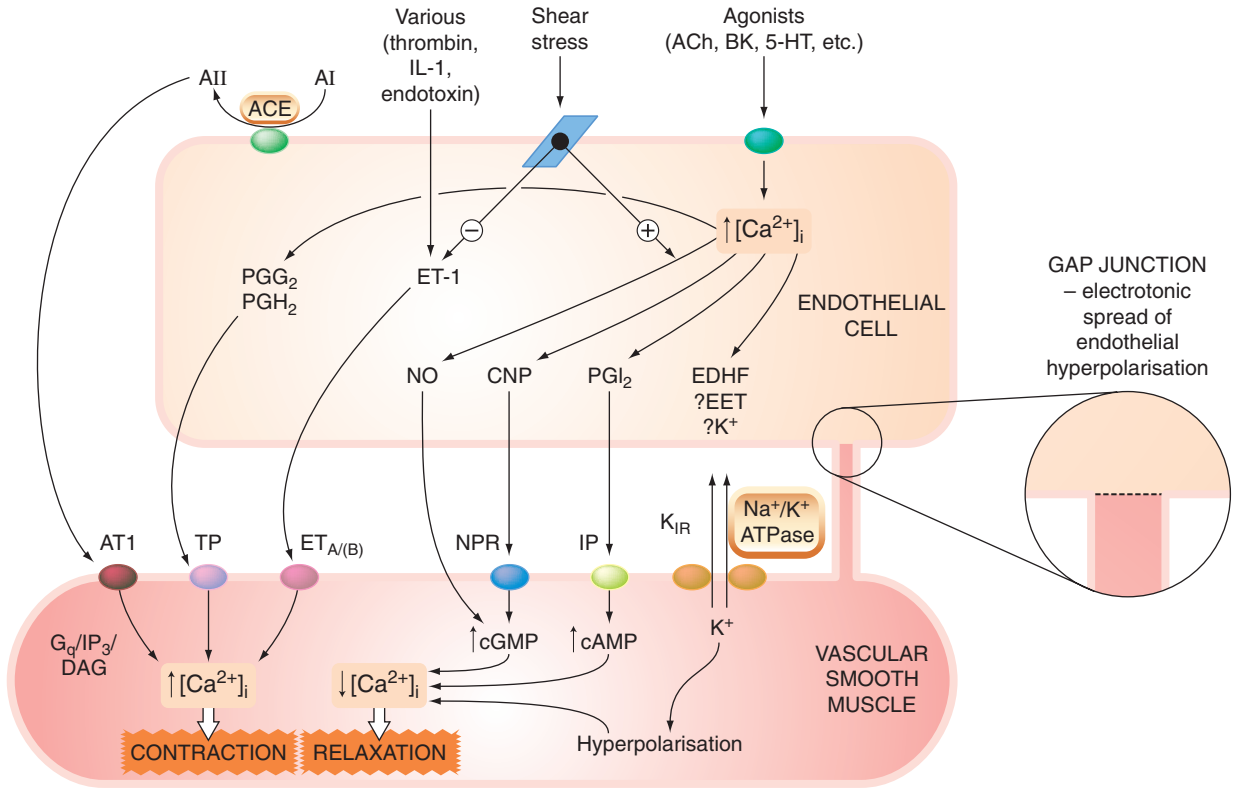

Fig. 22.1 Endothelium-derived mediators. The schematic shows some of the more important endothelium-derived contracting and relaxing mediators; many (if not all) of the vasoconstrictors also cause smooth muscle mitogenesis, while vasodilators commonly inhibit mitogenesis. 5-HT, 5-hydroxytryptamine; A, angiotensin; ACE, angiotensin-converting enzyme; ACh, acetylcholine; AT_1, angiotensin AT_1 receptor; BK, bradykinin; CNP, C-natriuretic peptide; DAG, diacylglycerol; EDHF, endothelium-derived hyperpolarising factor; EET, epoxyeicosatetraenoic acid; ET-1, endothelin-1; $ET_{A/(B)}$, endothelium A (and B) receptors; G_q, G-protein; IL-1, interleukin-1; IP, I prostanoid receptor; IP_3, inosinol 1,4,5-trisphosphate; K_{IR}, inward rectifying potassium channel; Na^+/K^+ ATPase, electrogenic pump; NPR, natriuretic peptide receptor; PG, prostaglandin; TP, T prostanoid receptor.

Vascular smooth muscle

- Vascular smooth muscle is controlled by mediators secreted by sympathetic nerves (Chs 21 and 14) and vascular endothelium, and by circulating hormones.
- Smooth muscle cell contraction is initiated by a rise in $[Ca^{2+}]_i$, which activates myosin light-chain kinase, causing phosphorylation of myosin, or by sensitisation of the myofilaments to Ca^{2+} by inhibition of myosin phosphatase (see Ch. 4).
- Agents cause contraction via one or more mechanism:
  - release of intracellular Ca^{2+} via inositol trisphosphate
  - depolarising the membrane, opening voltage-gated calcium channels and causing Ca^{2+} entry
  - increasing sensitivity to Ca^{2+} via actions on myosin light-chain kinase and/or myosin phosphatase (Ch. 4, Fig. 4.9).
- Agents cause relaxation by:
  - inhibiting Ca^{2+} entry through voltage-gated calcium channels either directly (e.g. **nifedipine**) or indirectly by hyperpolarising the membrane (e.g. potassium channel activators such as the active metabolite of **minoxidil**)
  - increasing intracellular cAMP or cGMP; cAMP inactivates myosin light-chain kinase and facilitates Ca^{2+} efflux, cGMP opposes agonist-induced increases in $[Ca^{2+}]_i$.

giving rise to vasodilator tone and contributing to the physiological control of blood pressure. As well as causing vascular relaxation, it inhibits vascular smooth muscle cell proliferation, inhibits platelet adhesion and aggregation, and inhibits monocyte adhesion and migration; consequently, it may protect blood vessels from atherosclerosis and thrombosis (see Chs 23 and 24).

- *Peptides*. The endothelium secretes several vasoactive peptides. *C-natriuretic peptide* (Ch. 21) and *adrenomedulin* (a vasodilator peptide originally discovered in an adrenal tumour—phaeochromocytoma—but expressed in many tissues, including vascular endothelium) are vasodilators working, respectively, through cGMP and cAMP. *Angiotensin II*, formed by angiotensin-converting enzyme (ACE) on the surface of endothelial cells (see below), and *endothelin* are potent endothelium-derived vasoconstrictor peptides.
- *Endothelium-derived hyperpolarisation factors (EDHFs)*. PGI_2 and NO each hyperpolarise vascular smooth muscle, and this can contribute to their relaxant effects. Endothelium-dependent dilatation in response to several mediators (including acetylcholine and bradykinin) persists in some vessels despite complete inhibition of prostaglandin and NO synthesis. Several endothelium-derived mediators have been implicated, including *epoxyeicosatrienoic acids* (EETs—derived from endothelial cytochrome P450 enzymes), various lipoxygenase (LOX) products, *hydrogen peroxide* (H_2O_2), *carbon monoxide* (CO), *hydrogen sulphide* (H_2S), and *C-natriuretic peptide* (CNP)—see Félétou & Vanhoutte (2009). These authors define an additional EDHF distinct from these mediators, and dependent on calcium-activated potassium (K_{Ca}) channels in endothelial cells. As the name implies, these channels are activated by an increase in endothelial cell $[Ca^{2+}]_i$.

In addition to secreting vasoactive mediators, endothelial cells express several enzymes and transport mechanisms that act on circulating hormones and are important targets of drug action. ACE is a particularly important example (see below).

Many endothelium-derived mediators are mutually antagonistic, conjuring an image of opposing rugby football players swaying back and forth in a scrum; in moments of exasperation, one sometimes wonders whether all this makes sense or whether the designer simply could not make up her mind! An important distinction is made between mechanisms that are tonically active in resistance vessels under basal conditions, as is the case with the noradrenergic nervous system (Ch. 14), NO (Ch. 20) and endothelin (see below), and those that operate mainly in response to injury, inflammation, etc., as with PGI_2. Some of the latter group may be functionally redundant, perhaps representing vestiges of mechanisms that were important to our evolutionary forebears, or they may simply be taking a breather on the touchline and are ready to rejoin the fray if called on by the occurrence of some vascular insult. Evidence for such a 'back-up' role comes, for example, from mice that lack the IP receptor for PGI_2, and that have a normal blood pressure and do not develop spontaneous thrombosis, but are more susceptible to vasoconstrictor and thrombotic stimuli than their wild-type litter mates (Murata et al., 1997).

THE ENDOTHELIUM IN ANGIOGENESIS

As touched on in Chapter 8, the barrier function of vascular endothelium differs markedly in different organs, and its development during angiogenesis is controlled by several growth factors, including *vascular endothelial growth factor (VEGF)* and various tissue-specific factors such as endocrine gland VEGF. These are involved in repair processes and in pathogenesis (e.g. tumour growth and in neovascularisation in the eye—an important cause of blindness in patients with diabetes mellitus). These factors and their receptors are potentially fruitful targets for drug development and new therapies (including gene therapies; Ch. 59).

ENDOTHELIN

Discovery, biosynthesis and secretion

Hickey et al. described a vasoconstrictor factor produced by cultured endothelial cells in 1985. This was identified as *endothelin*, a 21-residue peptide, by Yanagisawa et al. (1988), who achieved the isolation, analysis and cloning of the gene for this peptide, which at that time was the most potent vasoconstrictor known,[3] in an impressively short space of time.

▼ Three genes encode different sequences (ET-1, ET-2 and ET-3), each with a distinctive 'shepherd's crook' structure produced by two internal disulfide bonds. These isoforms are differently expressed in

[3]Subsequently an 11-amino acid peptide (*urotensin*) was isolated from the brains of bony fish and found to be 50–100 times more potent a vasoconstrictor than endothelin in some blood vessels. It and its receptor are expressed in human tissue but its function, if any, in man remains enigmatic.

Table 22.1 Distribution of endothelins and endothelin receptors in various tissues[a]

Tissues	Endothelin			Endothelin receptor	
	1	2	3	ET_A	ET_B
Vascular tissue Endothelium	++++				+
Smooth muscle	+			++	
Brain	+++		+	+	+++
Kidney	++	++	+	+	++
Intestines	+	+	+++	+	+++
Adrenal gland	+		+++	+	++

[a] Levels of expression of endothelins or the receptor mRNA and/or immunoreactive endothelins: ++++, highest; +++, high; ++, moderate; +, low.
(Adapted from Masaki T 1993 Endocr Rev 14: 256–268.)

organs such as brain and adrenal glands (Table 22.1), suggesting that endothelins have functions beyond the cardiovascular system, and this is supported by observations of mice in which the gene coding for ET-1 is disrupted (see below). ET-1 is the only endothelin present in endothelial cells, and is also expressed in many other tissues. Its synthesis and actions are summarised schematically in Figure 22.2. ET-2 is much less widely distributed: it is present in kidney and intestine. ET-3 is present in brain, lung, intestine and adrenal gland. ET-1 is synthesised from a 212-residue precursor molecule (prepro-ET), which is processed to 'big ET-1' and finally cleaved by an endothelin-converting enzyme to yield ET-1. Cleavage occurs not at the usual Lys–Arg or Arg–Arg position, but at a Trp–Val pair, implying a very atypical endopeptidase. The converting enzyme is a metalloprotease and is inhibited by **phosphoramidon** (a pharmacological tool but not used therapeutically). Big ET-1 is converted to ET-1 intracellularly and also on the surface of endothelial and smooth muscle cells.

Stimuli of endothelin synthesis include many vasoconstrictor mediators released by trauma or inflammation, including activated platelets, endotoxin, thrombin, various cytokines and growth factors, angiotensin II, antidiuretic hormone (ADH), adrenaline, insulin, hypoxia and low shear stress. Inhibitors of ET synthesis include NO, natriuretic peptides, PGE_2, PGI_2, heparin and high shear stress.

Release mechanisms of ET-1 are poorly understood. There is evidence that preformed ET-1 can be stored in endothelial cells, although probably not in granules. ET-1 concentration in plasma is low (< 5 pmol/l) compared with concentrations that activate endothelin receptors, but concentrations in the extracellular space between endothelium and vascular smooth muscle are presumably much higher, and endothelin receptor antagonists (see below) cause vasodilatation when infused directly into the brachial artery, consistent with tonic ET-1-mediated vasoconstrictor activity in resistance vasculature. ET-1 has an elimination half life of < 5 min, despite a much longer duration of action, and clearance occurs mainly in the lung and kidneys.

Endothelin receptors and responses

There are two types of endothelin receptor, designated ET_A and ET_B (Table 22.2), both of which are G-protein coupled (Ch. 3). The predominant overall response is vasoconstriction.

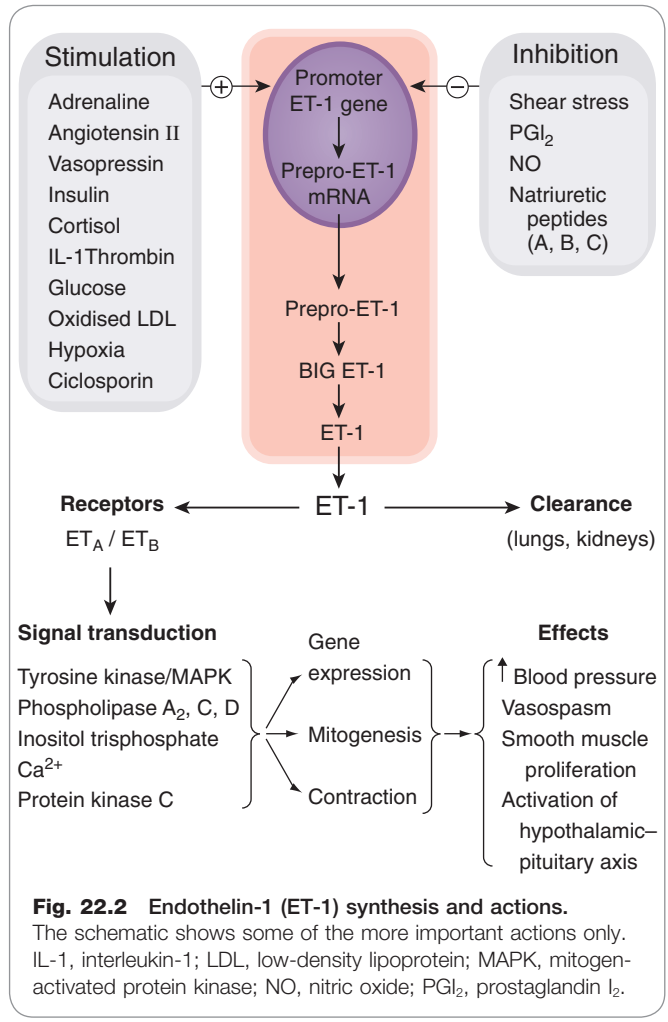

Fig. 22.2 Endothelin-1 (ET-1) synthesis and actions. The schematic shows some of the more important actions only. IL-1, interleukin-1; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PGI_2, prostaglandin I_2.

Table 22.2 Endothelin receptors

Receptor	Affinity	Pharmacological response
ET_A	ET-1 = ET-2 > ET-3	Vasoconstriction, bronchoconstriction, stimulation of aldosterone secretion
ET_B	ET-1 = ET-2 = ET-3	Vasodilatation, inhibition of ex vivo platelet aggregation

(From Masaki T 1993 Endocr Rev 14: 256–268.)

▼ Endothelin-1 preferentially activates ET_A receptors. Messenger RNA for the ETA receptor is expressed in many human tissues, including vascular smooth muscle, heart, lung and kidney. It is not expressed in endothelium. ET_A-mediated responses include vasoconstriction, bronchoconstriction and aldosterone secretion. ET_A receptors are coupled to phospholipase C, which stimulates Na^+/H^+ exchange, protein kinase C and mitogenesis, as well as causing vasoconstriction through inositol trisphosphate-mediated Ca^{2+} release (Ch. 3). There are several partially selective ET_A-receptor antagonists, including BQ-123 (a cyclic pentapeptide) and several orally active

non-peptide drugs (e.g. **bosentan**, a mixed ET_A/ET_B antagonist used in treating pulmonary arterial hypertension—see below). ET_B receptors are activated to a similar extent by each of the three endothelin isoforms, but *sarafotoxin S6c* (a 21-residue peptide that shares the shepherd's crook structure of the endothelins and was isolated from the venom of the burrowing asp) is a selective agonist and has proved useful as a pharmacological tool for studying the ET_B receptor. Messenger RNA for the ET_B receptor is mainly expressed in brain (especially cerebral cortex and cerebellum), with moderate expression in aorta, heart, lung, kidney and adrenals. In contrast to the ET_A receptor, it is highly expressed in endothelium, where it may initiate *vasodilatation* by stimulating NO and PGI_2 production, but it is also present in vascular smooth muscle, where it initiates vasoconstriction like the ET_A receptor. ET_B receptors play a part in clearing ET-1 from the circulation, and ET antagonists with appreciable affinity for ET_B receptors consequently increase plasma concentrations of ET-1, complicating interpretation of experiments with these drugs.

Functions of endothelin

Endothelin-1 is a *paracrine* mediator rather than a circulating hormone, although it stimulates secretion of several hormones (see below). Administration of an ET_A-receptor antagonist or of phosphoramidon into the brachial artery increases forearm blood flow, and ET_A receptor antagonists lower arterial blood pressure, suggesting that ET-1 contributes to vasoconstrictor tone and the control of peripheral vascular resistance. Endothelins have several other possible functions, including roles in:

- release of various hormones, including atrial natriuretic peptide, aldosterone, adrenaline, and hypothalamic and pituitary hormones
- natriuresis and diuresis via actions of collecting duct-derived ET-1 on ET_B receptors on tubular epithelial cells (Ge et al., 2006)
- thyroglobulin synthesis (the concentration of ET-1 in thyroid follicles is extremely high)
- control of uteroplacental blood flow (ET-1 is present in very high concentrations in amniotic fluid)
- renal and cerebral vasospasm (Fig. 22.3)
- development of the cardiorespiratory systems (if the ET-1 gene is disrupted in mice, pharyngeal arch tissues develop abnormally and homozygotes die of respiratory failure at birth), and ET receptor antagonists are teratogenic, causing cardiorespiratory developmental disorders.

The role of the endothelium in controlling vascular smooth muscle

- Endothelial cells release vasoactive mediators including prostacyclin (PGI_2) and nitric oxide (NO) (vasodilators), and endothelin (vasoconstrictor).
- Many vasodilators (e.g. acetylcholine and bradykinin) act via endothelial NO production. The NO derives from arginine and is produced when $[Ca^{2+}]_i$ increases in the endothelial cell, or the sensitivity of endothelial NO synthase to Ca^{2+} is increased (see Fig. 20.3).
- NO relaxes smooth muscle by increasing cGMP formation.
- Endothelin is a potent and long-acting vasoconstrictor peptide released from endothelial cells by many chemical and physical factors. It is not confined to blood vessels, and it has several functional roles.

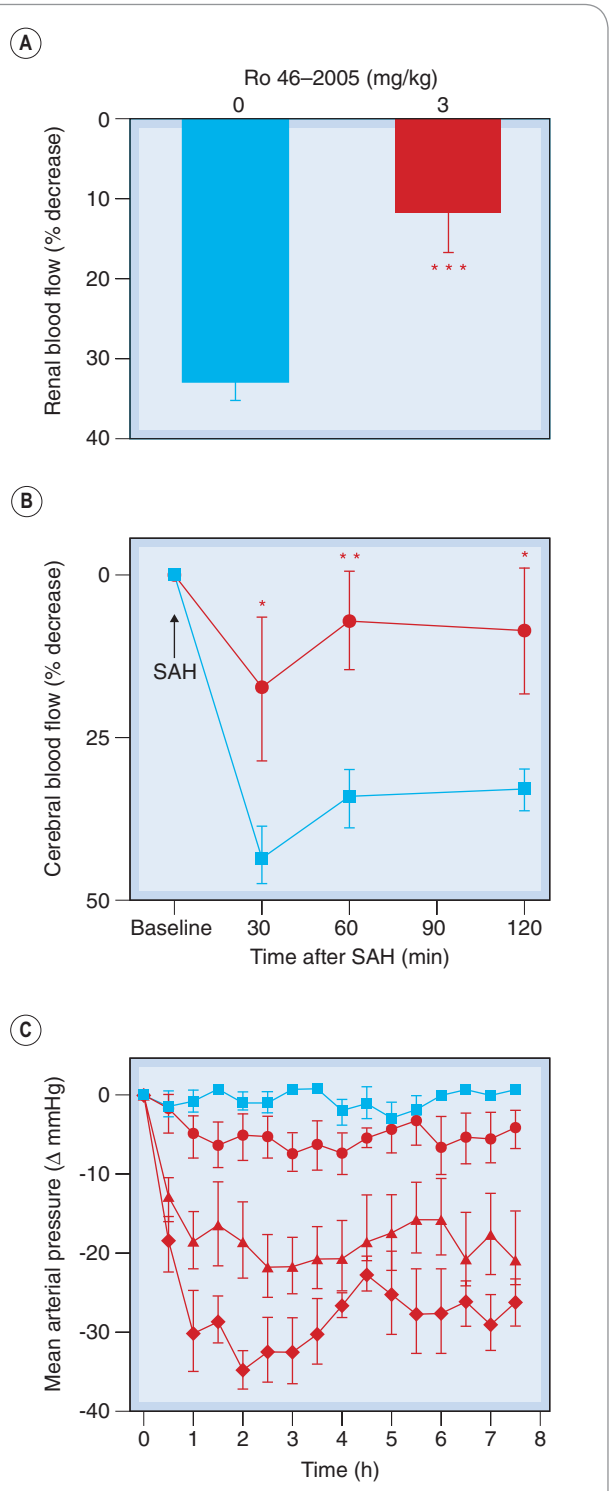

Fig. 22.3 In vivo effects of a potent non-peptide endothelin-1 ET_A- and ET_B-receptor antagonist, Ro 46-2005, in three animal models. [**A**] Prevention by Ro 46-2005 of postischaemic renal vasoconstriction in rats. [**B**] Prevention by Ro 46-2005 of the decrease in cerebral blood flow after subarachnoid haemorrhage (SAH) in rats treated with placebo (blue) or with Ro 46-2005 (red). [**C**] Effect of orally administered Ro 46-2005 on mean arterial pressure in sodium-depleted squirrel monkeys treated with placebo (blue) or increasing doses of antagonist (red: • < ▲ < ◆. (From Clozel M et al. 1993 Nature 365: 759–761.)

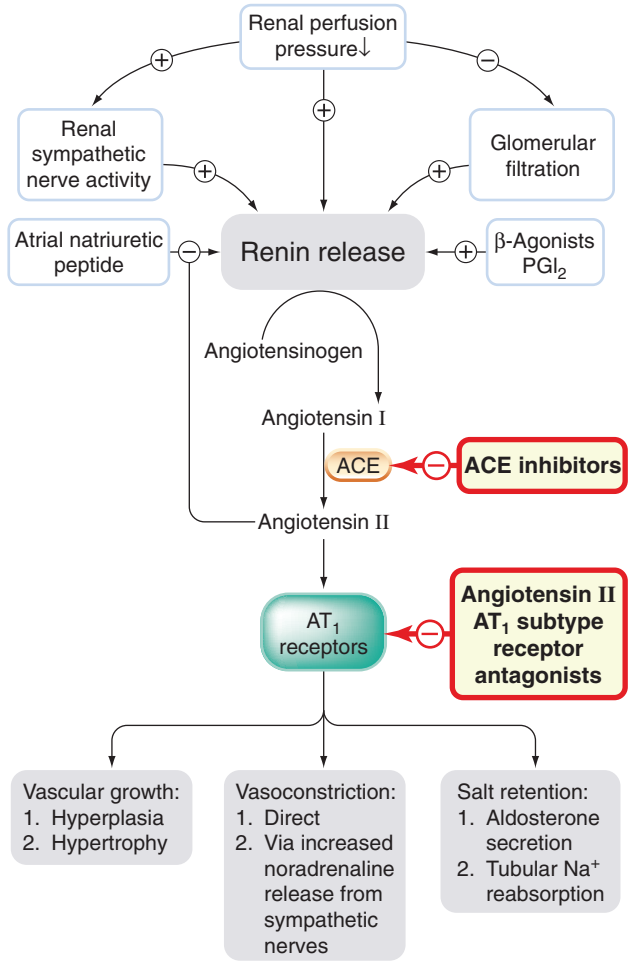

Fig. 22.4 Control of renin release and formation, and action of angiotensin II. Sites of action of drugs that inhibit the cascade are shown. ACE, angiotensin-converting enzyme; AT_1, angiotensin II receptor subtype 1.

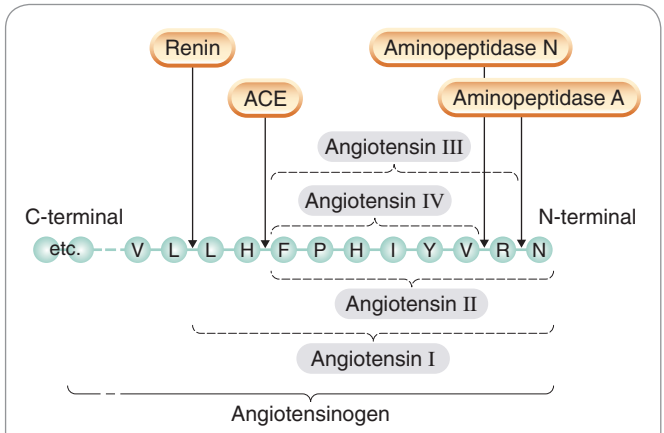

Fig. 22.5 Formation of angiotensins I–IV from the N-terminal of the precursor protein angiotensinogen.

THE RENIN–ANGIOTENSIN SYSTEM

The renin–angiotensin system synergises with the sympathetic nervous system, for example by increasing the release of noradrenaline from sympathetic nerve terminals. It stimulates aldosterone secretion and plays a central role in the control of Na^+ excretion and fluid volume, as well as of vascular tone.

The control of renin secretion (Fig. 22.4) is only partly understood. It is a proteolytic enzyme that is secreted by the *juxtaglomerular apparatus* (see Fig. 28.2) in response to various physiological stimuli including reduced renal perfusion pressure, or reduced Na^+ concentration in distal tubular fluid which is sensed by the *macula densa* (a specialised part of the distal tubule apposed to the juxtaglomerular apparatus). Renal sympathetic nerve activity, β-adrenoceptor agonists and PGI_2 all stimulate renin secretion directly, whereas angiotensin II causes feedback inhibition. Atrial natriuretic peptide (Ch. 21) also inhibits renin secretion. Renin is cleared rapidly from plasma. It acts on *angiotensinogen* (a plasma globulin made in the liver), splitting off a decapeptide, *angiotensin I*.

Angiotensin I has no appreciable activity per se, but is converted by *angiotensin-converting enzyme* (ACE) to an octapeptide, *angiotensin II*, which is a potent vasoconstrictor. Angiotensin II is a substrate for enzymes (aminopeptidase A and N) that remove single amino acid residues, giving rise, respectively, to angiotensin III and angiotensin IV (Fig. 22.5). These had been regarded as of little importance, but it is now known that angiotensin III stimulates aldosterone secretion and is involved in thirst. Angiotensin IV also has distinct actions, probably via its own receptor, including release of *plasminogen activator inhibitor-1* from the endothelium (Ch. 21). Receptors for angiotensin IV have a distinctive distribution, including the hypothalamus.

Angiotensin-converting enzyme is a membrane-bound enzyme on the surface of endothelial cells, and is particularly abundant in the lung, which has a vast surface area of vascular endothelium.[4] The common isoform of ACE is also present in other vascular tissues, including heart, brain, striated muscle and kidney, and is not restricted to endothelial cells.[5] Consequently, local formation of angiotensin II can occur in different vascular beds, and it provides local control independent of blood-borne angiotensin II. ACE inactivates bradykinin (see Ch. 19) and several other peptides. This may contribute to the pharmacological actions of ACE inhibitors, as discussed below. The main actions of angiotensin II are mediated via AT_1 and/or AT_2 receptors, which belong to the family of G-protein-coupled receptors. Effects mediated by AT1 receptors include:

- generalised vasoconstriction, especially marked in efferent arterioles of the renal glomeruli
- increased noradrenaline release, reinforcing sympathetic effects
- proximal tubular reabsorption of Na^+
- secretion of aldosterone from the adrenal cortex (see Ch. 32)
- growth of cardiac and vascular cells.[6]

[4]Approximately that of a football field.

[5]A different isoform of ACE is also present in testis, and male mice lacking this ACE have markedly reduced fertility.

[6]These effects are initiated by the G-protein-coupled AT_1 receptor acting via the same intracellular tyrosine phosphorylation pathways as are used by cytokines, for example the Jak/Stat pathway (Ch. 3).

Table 22.3 Classification of vasoactive drugs that act indirectly

Site	Mechanism	Examples	See Chapter
Vasoconstrictors			
Sympathetic nerves	Noradrenaline (norepinephrine) release	Tyramine	14
	Blocks noradrenaline reuptake	Cocaine	14
Endothelium	Endothelin release	Angiotensin II (in part)	This chapter
Vasodilators			
Sympathetic nerves	Inhibits noradrenaline release	Prostaglandin E_2, guanethidine	12, 14
Endothelium	Nitric oxide release	Acetylcholine, substance P	20
Central nervous system	Vasomotor inhibition	Anaesthetics	40
Enzymes	Angiotensin-converting enzyme inhibition	Captopril	This chapter

AT_2 receptors are expressed during fetal life and in distinct brain regions in adults. They may be involved in growth, development and exploratory behaviour. Cardiovascular effects of AT_2 receptors (inhibition of cell growth and lowering of blood pressure) are relatively subtle and oppose those of AT_1 receptors.

The renin–angiotensin–aldosterone pathway contributes to the pathogenesis of heart failure, and several leading classes of therapeutic drug act on it at different points (see below).

VASOACTIVE DRUGS

Drugs can affect vascular smooth muscle by acting either directly on smooth muscle cells, or indirectly, for example on endothelial cells, on sympathetic nerve terminals or on the central nervous system (CNS) (Table 22.3). Mechanisms of directly acting vasoconstrictors and vasodilators are summarised in Figure 4.10. Many indirectly acting drugs are discussed in other chapters (see Table 22.3). We concentrate here on agents that are not covered elsewhere.

VASOCONSTRICTOR DRUGS

The α_1-adrenoceptor agonists and drugs that release noradrenaline from sympathetic nerve terminals or inhibit its reuptake (sympathomimetic amines) cause vasoconstriction and are discussed in Chapter 14. Some eicosanoids (e.g. *thromboxane* A_2; see Chs 17 and 24) and several peptides, notably *endothelin*, *angiotensin* and *ADH*, are also predominantly vasoconstrictor. **Sumatriptan** and ergot alkaloids acting on certain 5-hydroxytryptamine receptors (5-HT_2 and 5-HT_{1D}) also cause vasoconstriction (Ch. 15).

ANGIOTENSIN II

The physiological role of the renin–angiotensin system is described above. Angiotensin II is roughly 40 times as potent as noradrenaline in raising blood pressure. Like α_1-adrenoceptor agonists, it constricts mainly cutaneous, splanchnic and renal vasculature, with less effect on blood flow to brain and skeletal muscle. It has no routine clinical uses, its therapeutic importance lying in the fact that other drugs (e.g. **captopril** and **losartan**, see below) affect the cardiovascular system by reducing its production or action.

ANTIDIURETIC HORMONE

Antidiuretic hormone (ADH, also known as vasopressin) is a posterior pituitary peptide hormone (Ch. 32). It is important for its antidiuretic action on the kidney (Ch. 28) but is also a powerful vasoconstrictor in skin and some other vascular beds. Its effects are initiated by two distinct receptors (V_1 and V_2). Water retention is mediated through V_2 receptors, occurs at low plasma concentrations of ADH and involves activation of adenylyl cyclase in renal collecting ducts. Vasoconstriction is mediated through V_1 receptors, requires higher concentrations of ADH and involves activation of phospholipase C (see Ch. 3). ADH causes generalised vasoconstriction, including the coeliac, mesenteric and coronary vessels. It also affects other (e.g. gastrointestinal and uterine) smooth muscle and causes abdominal cramps for this reason. It is sometimes used to treat patients with bleeding oesophageal varices and portal hypertension before more definitive treatment, although many gastroenterologists prefer to use **octreotide** (unlicensed indication; see Ch. 32) for this. It may also have a place in treating hypotensive shock (see below).

ENDOTHELIN

Endothelins are discussed above in the context of their physiological roles; as explained above, they have vasodilator and vasoconstrictor actions, but vasoconstriction predominates. Intravenous administration causes transient vasodilatation followed by profound and long-lived vasoconstriction. The endothelins are even more potent vasoconstrictors than angiotensin II. As yet, they have no clinical uses, and ET antagonists are licensed only for the rare disease primary pulmonary hypertension (see below).

VASODILATOR DRUGS

Vasodilator drugs play a major role in the treatment of common conditions including hypertension, cardiac failure and angina pectoris, as well as several less common but severe diseases including pulmonary hypertension and Raynaud's disease.

Vasoconstrictor substances

- The main groups are sympathomimetic amines (direct and indirect; Ch. 14), certain eicosanoids (especially thromboxane A_2; Ch. 17), peptides (angiotensin II, antidiuretic hormone [ADH] and endothelin; Ch. 19) and a group of miscellaneous drugs (e.g. ergot alkaloids; Ch. 15).
- Clinical uses include local applications (e.g. nasal decongestion, co-administration with local anaesthetics). Sympathomimetic amines and ADH are used in circulatory shock. Adrenaline is life-saving in anaphylactic shock and in cardiac arrest. ADH may be used to stop bleeding from oesophageal varices in patients with portal hypertension caused by liver disease.

DIRECTLY ACTING VASODILATORS

Targets on which drugs act to relax vascular smooth muscle include plasma membrane voltage-dependent calcium channels, sarcoplasmic reticulum channels (Ca^{2+} release or reuptake) and enzymes that determine Ca^{2+} sensitivity of the contractile proteins (see Fig. 4.10).[7]

Calcium antagonists

L-type calcium antagonists are discussed in Chapter 21. As well as their actions on the heart they cause generalised arterial vasodilatation, although individual agents exhibit distinct patterns of regional potency. Dihydropyridines (e.g. **nifedipine**) act preferentially on vascular smooth muscle, whereas **verapamil** acts directly on the heart (negative chronotropic and inotropic effects) in addition to causing vasodilatation; **diltiazem** is intermediate in specificity. Consequently, rapid-acting dihydropyridines usually produce reflex tachycardia, whereas verapamil and diltiazem do not.

Drugs that activate potassium channels

Some drugs (e.g. **minoxidil**, **diazoxide**) relax smooth muscle by opening K_{ATP} channels (see Fig. 22.6). This hyperpolarises the cells and switches off voltage-dependent calcium channels.[8] Potassium channel activators work by antagonising the action of intracellular ATP on these channels.

Minoxidil is a very potent and long-acting vasodilator, used as a drug of last resort in treating severe hypertension unresponsive to other drugs. It causes hirsutism (its active metabolite is actually used as a rub-on cream to treat baldness). It also causes marked salt and water retention, and is usually prescribed with a loop diuretic. It causes reflex tachycardia, and a β-adrenoceptor antagonist is used to prevent this. **Nicorandil** (Ch. 21) combines K_{ATP} channel activation with NO donor activity, and is used in refractory angina. **Levosimendan** combines K_{ATP} channel activation with sensitisation of the cardiac contractile mechanism to Ca^{2+} by binding troponin C (Ch. 21), and is used in decompensated heart failure (see below).

Drugs that act via cyclic nucleotides

Cyclase activation

Many drugs relax vascular smooth muscle by increasing the cellular concentration of either cGMP or cAMP. For example, NO, nitrates and the natriuretic peptides act through cGMP (see Chs 20 and 21); BAY41-2272, a pyrazolopyridine, activates soluble guanylyl cyclase via an NO-independent site (see Ch. 20). The *β_2 agonists*, *adenosine* and *PGI_2* increase cytoplasmic cAMP (see Ch. 14). *Dopamine* has mixed vasodilator and vasoconstrictor actions. It selectively dilates renal vessels, where it increases cAMP by activating adenylyl cyclase. It is the precursor of *noradrenaline* (Ch. 14), and is also a transmitter in its own right in the brain (Ch. 38) and probably also in the periphery (Ch. 12). Dopamine, when administered as an intravenous infusion, produces a mixture of cardiovascular effects resulting from agonist actions on α- and β-adrenoceptors, as well as on dopamine receptors. Blood pressure increases slightly, but the main effects are vasodilatation in the renal circulation and increased cardiac output. Dopamine was widely used in intensive care units in patients in whom renal failure associated with decreased renal perfusion appeared imminent; despite its beneficial effect on renal haemodynamics, it does not, however, improve survival in these circumstances and this use is obsolete (Liang et al., 2008). **Nesiritide**, a recombinant form of human B-type natriuretic peptide (BNP) (see Ch. 21), has been approved in the USA for the treatment of acutely decompensated heart failure, but a pooled analysis of randomised controlled trials has suggested that it too may increase mortality, and indications for its use remain controversial (Potter et al., 2009).

Nitroprusside (nitroferricyanide) is a powerful vasodilator with little effect outside the vascular system. It reacts with tissue sulfhydryl groups under physiological conditions to yield NO. Unlike the organic nitrates, which preferentially dilate capacitance vessels and muscular arteries, it acts equally on arterial and venous smooth muscle. Its clinical usefulness is limited because it must be given intravenously. In solution, particularly when exposed to light, nitroprusside hydrolyses with formation of cyanide. The intravenous solution must therefore be made up freshly from dry powder and protected from light. Nitroprusside is rapidly converted to thiocyanate in the body, its plasma half-life being only a few minutes, so it must be given as a continuous infusion with careful monitoring to avoid hypotension. Prolonged use causes thiocyanate accumulation and toxicity (weakness, nausea and inhibition of thyroid function); consequently, nitroprusside is useful only for short-term treatment (usually up to 72 h maximum). It is used in intensive care units for hypertensive emergencies, to produce controlled hypotension during surgery, and to reduce cardiac work during the reversible cardiac dysfunction that occurs after cardiopulmonary bypass surgery.

Phosphodiesterase inhibition

Phosphodiesterases (PDEs; see Ch. 3) include at least 14 distinct isoenzymes. Methylxanthines (e.g. **theophylline**)

[7] A pyridine drug, Y27632, causes vasorelaxation by inhibiting a Rho-associated protein kinase, thereby selectively inhibiting smooth muscle contraction by inhibiting Ca^{2+} sensitisation.

[8] K_{ATP} channels in pancreatic islet B cells form an important link between the metabolic state of the cell and membrane function, and sulfonylurea drugs cause insulin secretion by mimicking the action of ATP on these channels (see Ch. 30). Conversely, potassium channel activators such as **diazoxide** (Fig. 22.6) increase blood glucose by inhibiting insulin secretion from the pancreas.

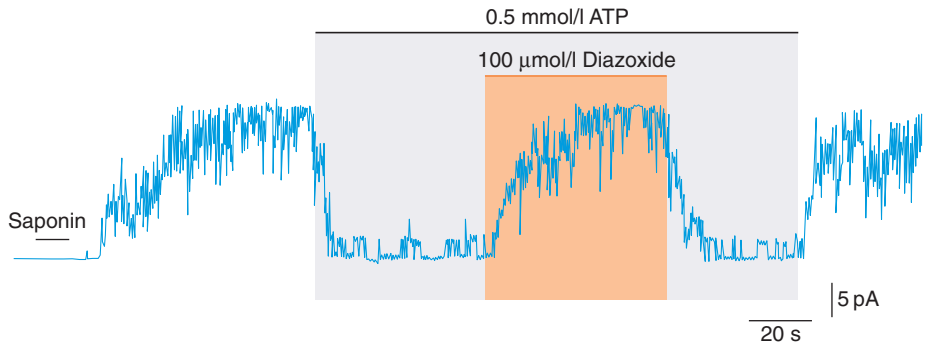

Fig. 22.6 ATP-sensitive potassium channels. Patch clamp (see Ch. 3) record from insulin-secreting pancreatic B cell: saponin permeabilised the cell, with loss of intracellular ATP, causing the channels to open (upward deflection) until they were inhibited by ATP. Addition of diazoxide, a vasodilator drug (which also inhibits insulin secretion; see text) reopens the channels. In smooth muscle, this causes hyperpolarisation and relaxation. (Redrawn from Dunne et al. 1990 Br J Pharmacol 99: 169.)

and **papaverine** are non-selective PDE inhibitors (and have other actions, too). Methylxanthines exert their main effects on bronchial smooth muscle and on the CNS, and are discussed in Chapters 27 and 47. In addition to inhibiting PDE, some methylxanthines are also purine receptor antagonists (Ch. 16). Papaverine is produced by opium poppies (see Ch. 41) and is chemically related to **morphine**. However, pharmacologically it is quite unlike morphine, its main action being to relax smooth muscle. Its mechanism is poorly understood but seems to involve a combination of PDE inhibition and block of calcium channels. Selective PDE type III inhibitors (e.g. **milrinone**) increase cAMP in cardiac muscle. They have a positive inotropic effect but, despite short-term haemodynamic improvement, increase mortality in heart failure, possibly by causing dysrhythmias. **Cilostazol**, a related drug, improves symptoms in patients with peripheral vascular disease (see below). **Dipyridamole**, as well as enhancing the actions of adenosine (see Ch. 16), also causes vasodilatation by inhbiting phosphodiesterase. It is used to prevent stroke, but can provoke angina. Selective *PDE type V* inhibitors (e.g. **sildenafil**) inhibit the breakdown of cGMP. Penile erection is caused by increased activity in nitrergic nerves in the pelvis. These release NO (Ch. 20), which activates guanylyl cyclase in smooth muscle in the corpora cavernosa. Taken by mouth about an hour before sexual stimulation, sildenafil increases penile erection by potentiating this pathway. It has revolutionised treatment of erectile dysfunction (see Ch. 34) and has therapeutic potential in other situations, including pulmonary hypertension (see below) via potentiation of NO-mediated effects.

VASODILATORS WITH UNKNOWN MECHANISM OF ACTION

Hydralazine

Hydralazine acts mainly on arteries and arterioles, causing a fall in blood pressure accompanied by reflex tachycardia and an increased cardiac output. It interferes with the action of inositol trisphosphate on Ca^{2+} release from the sarcoplasmic reticulum. Its original clinical use was in hypertension. It is still used for short-term treatment of severe hypertension in pregnancy but can cause an immune disorder resembling systemic lupus erythematosus,[9] so alternative agents are now usually preferred for long-term treatment of hypertension. It has a place in treating heart failure in patients of African origin in combination with a long-acting organic nitrate (see below).

Vasodilator drugs

- Vasodilators act:
  - to increase local tissue blood flow
  - to reduce arterial pressure
  - to reduce central venous pressure.
- Net effect is a reduction of cardiac preload (reduced filling pressure) and of afterload (reduced vascular resistance), hence reduction of cardiac work.
- Main uses are:
  - antihypertensive therapy (e.g. AT_1 antagonists, calcium antagonists and α_1-adrenoceptor antagonists)
  - treatment/prophylaxis of angina (e.g. calcium antagonists, nitrates)
  - treatment of cardiac failure (e.g. angiotensin-converting enzyme inhibitors, AT_1 antagonists).

Ethanol

Ethanol (see Ch. 48) dilates cutaneous vessels, causing the familiar drunkard's flush. Several general anaesthetics (e.g. **propofol**) cause vasodilatation as an unwanted effect (Ch. 40).

INDIRECTLY ACTING VASODILATOR DRUGS

The two main groups of indirectly acting vasodilator drugs are inhibitors of:

1. sympathetic vasoconstriction
2. the renin–angiotensin–aldosterone system.

[9]An autoimmune disease affecting one or more tissues, including joints, skin and pleural membranes. It is characterised by antibodies directed against DNA.

Table 22.4 Summary of drugs that inhibit the renin–angiotensin–aldosterone system

Class	Drug[a]	Pharmacokinetics	Adverse effects[b]	Uses	Notes
ACE inhibitors	Captopril	Short acting $t_{1/2}$ ~2 h Dose 2–3 times daily	Cough Hypotension Proteinuria Taste disturbance	Hypertension Heart failure After MI	ACEIs are cleared mainly by renal excretion
	Enalapril	Pro-drug—active metabolite enalaprilat $t_{1/2}$ ~11 h Dose 1–2 times daily	Cough Hypotension Reversible renal impairment (in patients with renal artery stenosis)	As captopril	Lisinopril, perindopril, ramipril, trandalopril are similar Some are licensed for different uses (e.g. stroke, left ventricular hypertrophy)
Angiotensin receptor blockers (ARBs)	Valsartan	$t_{1/2}$ ~6 h	Hypotension Reversible renal impairment (in patients with renal artery stenosis)	Hypertension Heart failure	ARBs are cleared by hepatic metabolism
	Losartan	Long-acting metabolite $t_{1/2}$ ~8 h	As valsartan	As valsartan Diabetic nephropathy	Irbesartan is similar, with $t_{1/2}$ ~10–15 h
	Candesartan	$t_{1/2}$ 5–10 h Long acting because receptor complex is stable	As valsartan	As valsartan	Given as prodrug ester (candesartan cilexetil)
Renin inhibitor	Aliskiren	Low oral bioavailability $t_{1/2}$ 24 h	As valsartan, also diarrhoea	Essential hypertension	Licensed in 2007, the first drug of this type
Aldosterone antagonists	Eplerenone	$t_{1/2}$ 3–5 h	As valsartan, especially hyperkalemia Nausea, diarrhoea	Heart failure after MI	
	Spironolactone	Prodrug converted to canrenone, which has $t_{1/2}$ ~24 h	As eplerenone Also oestrogenic effects (gynaecomastia, menstrual irregularity, erectile dysfunction)	Primary hyperaldosteronism Heart failure Oedema and ascites (e.g. in hepatic cirrhosis)	

[a] All drugs listed are orally active.
[b] Adverse effects common to all drugs listed include hyperkalemia (especially in patients with impaired renal function) and teratogenesis.
ACEI, angiotensin-converting enzyme inhibitor; MI, myocardial infarction.

The central control of sympathetically mediated vasoconstriction is believed to involve not only α_2 adrenoceptors but also another class of receptor, termed the *imidazoline I_1 receptor*, present in the brain stem in the rostral ventrolateral medulla. Drugs can inhibit the sympathetic pathway at any point from the CNS to the peripheral sympathetic nerve terminal (see Ch. 14). In addition, many vasodilators (e.g. acetylcholine, bradykinin, substance P) exert some or all of their effects by stimulating biosynthesis of vasodilator prostaglandins or of NO (or of both) by vascular endothelium (see above and Ch. 20), thereby causing functional antagonism of the constrictor tone caused by sympathetic nerves and angiotensin II.

The renin–angiotensin–aldosterone system (RAAS—see Table 22.4 for a summary of selective antagonists) can be inhibited at several points:

- renin release: β-adrenoceptor antagonists inhibit renin release (although their other actions can result in a small increase in peripheral vascular resistance)
- renin activity: renin inhibitors
- ACE: ACE inhibitors (ACEIs)
- angiotensin II receptors: AT_1-receptor antagonists (ARBs)
- aldosterone receptors: aldosterone-receptor antagonists.

Renin inhibitors

Orally active renin inhibitors reduce plasma renin activity. One such drug, **aliskiren**, is licensed for essential hypertension.

Angiotensin-converting enzyme inhibitors

The first ACEI to be marketed was **captopril** (Fig. 22.7), an early example of successful drug design based on a chemical knowledge of the target molecule. Various small peptides had been found to be weak inhibitors of the enzyme.[10]

[10]The lead compound was a nonapeptide derived from the venom of *Bothrops jacaraca*—a South American snake. It was originally characterised as a bradykinin-potentiating peptide (ACE inactivates bradykinin).

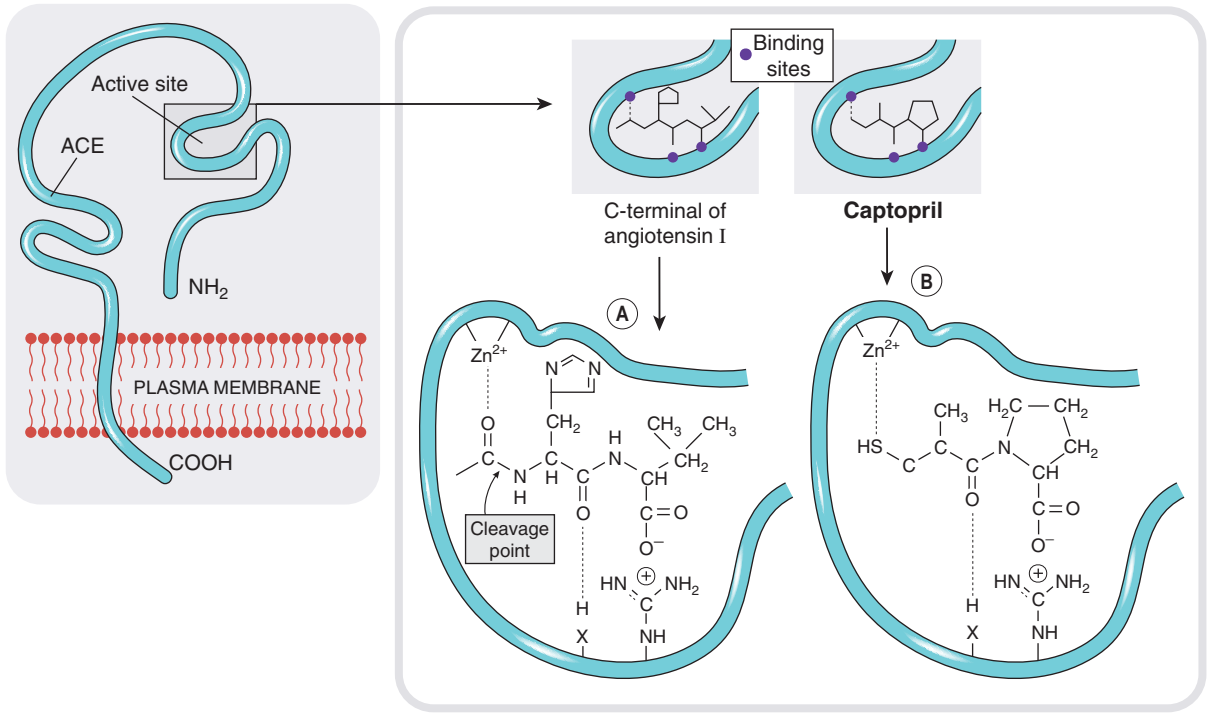

Fig. 22.7 **The active site of angiotensin-converting enzyme.** [**A**] Binding of angiotensin I. [**B**] Binding of the inhibitor captopril, which is an analogue of the terminal dipeptide of angiotensin I.

Captopril was designed to combine the steric properties of such peptide antagonists in a non-peptide molecule that was active when given by mouth. Captopril has a short plasma half-life (about 2 h) and must be given 2 or 3 times daily. Later ACE inhibitors (Table 22.4), which are widely used in the clinic, have a longer duration of action.

Pharmacological effects

ACE inhibitors cause only a small fall in arterial pressure in healthy human subjects who are consuming the amount of salt contained in a usual Western diet, but a much larger fall in hypertensive patients, particularly those in whom renin secretion is enhanced (e.g. in patients receiving diuretics). ACEIs affect capacitance and resistance vessels, and reduce cardiac load as well as arterial pressure. They do not affect cardiac contractility, so cardiac output normally increases. They act preferentially on angiotensin-sensitive vascular beds, which include those of the kidney, heart and brain. This selectivity may be important in sustaining adequate perfusion of these vital organs in the face of reduced perfusion pressure. Critical renal artery stenosis[11] represents an exception to this, where ACE inhibition results in a fall in glomerular filtration rate (see below).

Clinical uses of ACE inhibitors are summarised in the clinical box.

Unwanted effects

Adverse effects (Table 22.4) directly related to ACE inhibition are common to all drugs of this class. These include hypotension, especially after the first dose and especially in patients with heart failure who have been treated with loop diuretics, in whom the renin–angiotensin system is highly activated. A dry cough, possibly the result of accumulation of bradykinin (Ch. 17), is the commonest persistent adverse effect. Kinin accumulation may also underly *angioedema* (painful swelling in tissues which can be life-threatening if it involves the airway). Patients with severe bilateral renal artery stenosis predictably develop renal failure if treated with ACEIs, because glomerular filtration is normally maintained, in the face of low afferent arteriolar pressure, by angiotensin II, which selectively constricts efferent arterioles; hyperkalaemia may be severe owing to reduced aldosterone secretion. Such renal failure is reversible provided that it is recognised promptly and treatment with ACEI discontinued.

Clinical uses of angiotensin-converting enzyme inhibitors

- Hypertension.
- Cardiac failure.
- Following myocardial infarction (especially when there is ventricular dysfunction).
- In people at high risk of ischaemic heart disease.
- Diabetic nephropathy.
- Progressive renal insufficiency.

Angiotensin II receptor antagonists

Losartan, **candesartan**, **valsartan** and **irbesartan** (sartans) are non-peptide, orally active AT_1 receptor antagonists

[11]Severe narrowing of the renal artery caused, for example, by atheroma (Ch. 23).

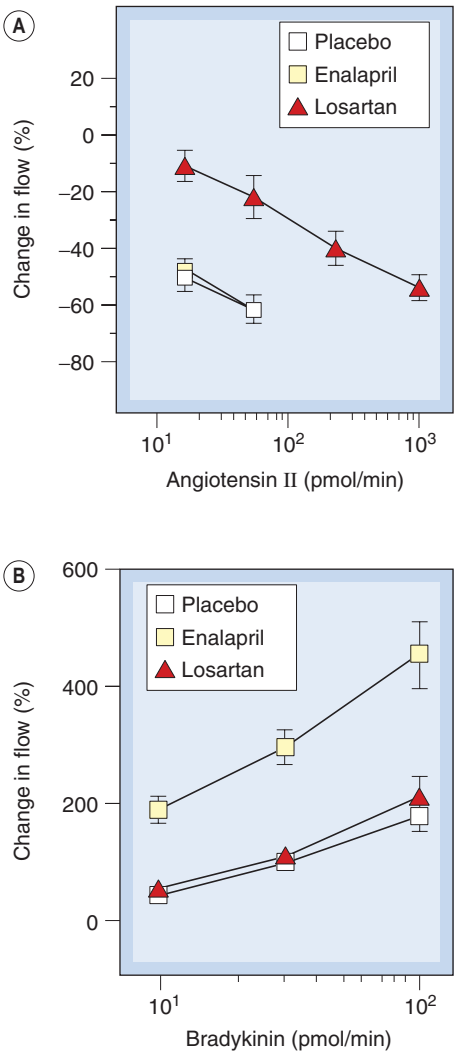

Fig. 22.8 Comparison of effects of angiotensin-converting enzyme inhibition and angiotensin receptor blockade in the human forearm vasculature. [**A**] Effect of brachial artery infusion of angiotensin II on forearm blood flow after oral administration of placebo, enalapril (10 mg) or losartan (100 mg). [**B**] Effect of brachial artery infusion of bradykinin, as in [**A**]. (From Cockcroft J R et al. 1993 J Cardiovasc Pharmacol 22: 579–584.)

(ARBs). ARBs differ pharmacologically from ACEIs (Fig. 22.8) but behave similarly to ACEIs in clinical practice, apart from not causing cough—consistent with the 'bradykinin accumulation' explanation of this side effect, mentioned above. ACE is not the only enzyme capable of forming angiotensin II, *chymase* (which is not inhibited by ACE inhibitors) providing one alternative route. It is not known if alternative pathways of angiotensin II formation are important in vivo, but if so, then ARBs could be more effective than ACE inhibitors in such situations. It is not known whether any of the beneficial effects of ACE inhibitors are bradykinin/NO mediated, so it is unwise to assume that ARBs will necessarily share all the therapeutic properties of ACE inhibitors. However, there is considerable overlap in the clinical indications for ARBs and ACEIs (Table 22.4).

Types of vasodilator drug

Directly acting vasodilators

- Calcium antagonists (e.g. **nifedipine**, **diltiazem**, **verapamil**): block Ca^{2+} entry in response to depolarisation. Common adverse effects include ankle swelling and (especially with verapamil) constipation.
- K_{ATP} channel activators (e.g. **minoxidil**): open membrane potassium channels, causing hyperpolarisation. Ankle swelling and increased hair growth are common.
- Drugs that increase cytoplasmic cyclic nucleotide concentrations by:
  - increasing adenylyl cyclase activity, for example prostacyclin (**epoprostenol**), β_2-adrenoceptor agonists, adenosine
  - increasing guanylyl cyclase activity: nitrates (e.g. **glyceryl trinitrate**, **nitroprusside**)
  - inhibiting phosphodiesterase activity (e.g. **sildenafil**).

Indirectly acting vasodilators

- Drugs that interfere with the sympathetic nervous system (e.g. α_1-adrenoceptor antagonists). Postural hypotension is a common adverse effect.
- Drugs that block the renin–angiotensin system:
  - renin inhibitors (e.g. **aliskiren**)
  - angiotensin-converting enzyme inhibitors (e.g. **ramipril**); dry cough may be troublesome
  - AT_1 receptor antagonists (e.g. **losartan**).
- Drugs or mediators that stimulate endothelial NO release (e.g. acetylcholine, bradykinin).
- Drugs that block the endothelin system:
  - endothelin synthesis (e.g. phosphoramidon)
  - endothelin receptor antagonists (e.g. **bosentan**).

Vasodilators whose mechanism is uncertain

- Miscellaneous drugs including alcohol, **propofol** (Ch. 40) and **hydralazine**.

Clinical uses of angiotensin II subtype 1 receptor antagonists (sartans)

The AT_1 antagonists are extremely well tolerated but are teratogenic. Their uses include the following:

- Hypertension, especially in:
  - young patients (who have higher renin than older ones)
  - diabetic patients
  - hypertension complicated by left ventricular hypertrophy.
- Heart failure.
- Diabetic nephropathy.

CLINICAL USES OF VASOACTIVE DRUGS

It is beyond the scope of this book to provide a detailed account of the clinical uses of vasoactive drugs, but it is nonetheless useful to consider briefly the treatment of certain important disorders, namely:

- systemic hypertension
- heart failure
- shock
- peripheral vascular disease
- Raynaud's disease
- pulmonary hypertension.

SYSTEMIC HYPERTENSION

Systemic hypertension is a common disorder that, if not effectively treated, increases the risk of coronary thrombosis, strokes and renal failure. Until about 1950, there was no effective treatment, and the development of antihypertensive drugs has been a major therapeutic success story. Systemic blood pressure is an excellent 'surrogate marker' for increased cardiovascular risk in that there is good evidence from randomised controlled trials that common antihypertensive drugs (diuretics, ACEIs, calcium antagonists) combined with lifestyle changes not only lowers blood pressure but also reduces the extra risks of heart attacks and strokes associated with high blood pressure.

Correctable causes of hypertension include phaeochromocytoma,[12] steroid-secreting tumours of the adrenal cortex or narrowing (coarctation) of the aorta, but most cases involve no obvious cause and are grouped as *essential hypertension* (so-called because it was originally, albeit incorrectly, thought that the raised blood pressure was 'essential' to maintain adequate tissue perfusion). Increased cardiac output may be an early feature, but by the time essential hypertension is established (commonly in middle life) there is usually increased peripheral resistance and the cardiac output is normal. Blood pressure control is intimately related to the kidneys, as demonstrated in humans requiring renal transplantation: hypertension 'goes with' the kidney from a hypertensive donor, and donating a kidney from a normotensive to a hypertensive corrects hypertension in the recipient (see also Ch. 28). Persistently raised arterial pressure leads to hypertrophy of the left ventricle and remodelling of resistance arteries, with narrowing of the lumen, and predisposes to atherosclerosis.

Figure 22.9 summarises physiological mechanisms that control arterial blood pressure and shows sites at which antihypertensive drugs act, notably the sympathetic nervous system, the renin–angiotensin–aldosterone system and endothelium-derived mediators. Remodelling of resistance arteries in response to raised pressure reduces the ratio of lumen diameter to wall thickness and increases the peripheral vascular resistance. The role of cellular growth factors (including angiotensin II) and inhibitors of growth (e.g. NO) in the evolution of these structural changes is of great interest to vascular biologists, and is potentially important for ACEIs and ARBs.

Reducing arterial blood pressure greatly improves the prognosis of patients with hypertension. Controlling hypertension (which is asymptomatic) without producing unacceptable side effects is therefore an important clinical need, which is, in general, well catered for by modern drugs. Treatment involves non-pharmacological measures (e.g. increased exercise, reduced dietary salt and saturated fat with increased fruit and fibre, and weight and alcohol reduction) followed by the staged introduction of drugs, starting with those of proven benefit and least likely to produce side effects. Some of the drugs that were used to lower blood pressure in the early days of antihypertensive therapy, including *ganglion blockers*, *adrenergic neuron blockers* and **reserpine** (see Ch. 14), produced a fearsome array of adverse effects and are now obsolete. The preferred regimens have changed progressively as better-tolerated drugs have become available. One rational strategy with some evidence to support it, and recommended by the current British Hypertension Society guidelines, is to start treatment with either an ACEI or an AT_1 antagonist in patients who are likely to have normal or raised plasma renin (i.e. younger white people), and with either a thiazide diuretic or a calcium antagonist in older people and people of African origin (who are more likely to have low plasma renin). If the target blood pressure is not achieved but the drug is well tolerated, then a drug of the other group is added. It is best not to increase the dose of any one drug excessively, as this often causes adverse effects and engages homeostatic control mechanisms (e.g. renin release by a diuretic) that limit efficacy.

β-Adrenoceptor antagonists are less well tolerated than ACEIs or ARBs, and the evidence supporting their routine use is less strong than for other classes of antihypertensive drugs. They are useful for hypertensive patients with some additional indication for β blockade, such as angina or heart failure.

Addition of a third or fourth drug (e.g. to ARB/diuretic or ARB/calcium antagonist combination) is often needed, and a long-acting α_1-adrenoceptor antagonist (Ch. 14) such as **doxazosin** is one option in this setting. The α_1 antagonists additionally improve symptoms of benign prostatic hypertrophy,[13] common in older men , albeit at the expense of some postural hypotension, which is the main unwanted effect of these agents. Doxazosin is used once daily and has a mild but theoretically desirable effect on plasma lipids (reducing the ratio of low- to high-density lipoproteins; see Ch. 23). **Spironolactone** (a competitive antagonist of aldosterone; Ch. 32) has staged something of a comeback in treating severe hypertension. Careful monitoring of plasma K^+ concentration is required, because spironolactone inhibits urinary K^+ excretion as well as causing oestrogen-related adverse effects, but it is usually well tolerated in low doses. **Methyldopa** is now used mainly for hypertension during pregnancy because of the lack of documented adverse effects on the baby (in contrast to ACEIs, ARBs and standard β-adrenoceptor antagonists, which are contraindicated during pregnancy). **Clonidine** (a centrally acting α_2 agonist) is now seldom used. **Moxonidine**, a centrally acting agonist at imidazoline I_1 receptors that causes less drowsiness than α_2 agonists, is licensed for mild or moderate hypertension, but there is little evidence from clinical end-point trials to support its use. **Minoxidil**, combined with a diuretic and

[12]Catecholamine-secreting tumours of chromaffin tissue, usually the adrenal medulla (Ch. 13).

[13]Difficulty starting the stream, poor stream, terminal dribbling and needing to get up often in the night to pass urine—all depressingly common in ageing men.

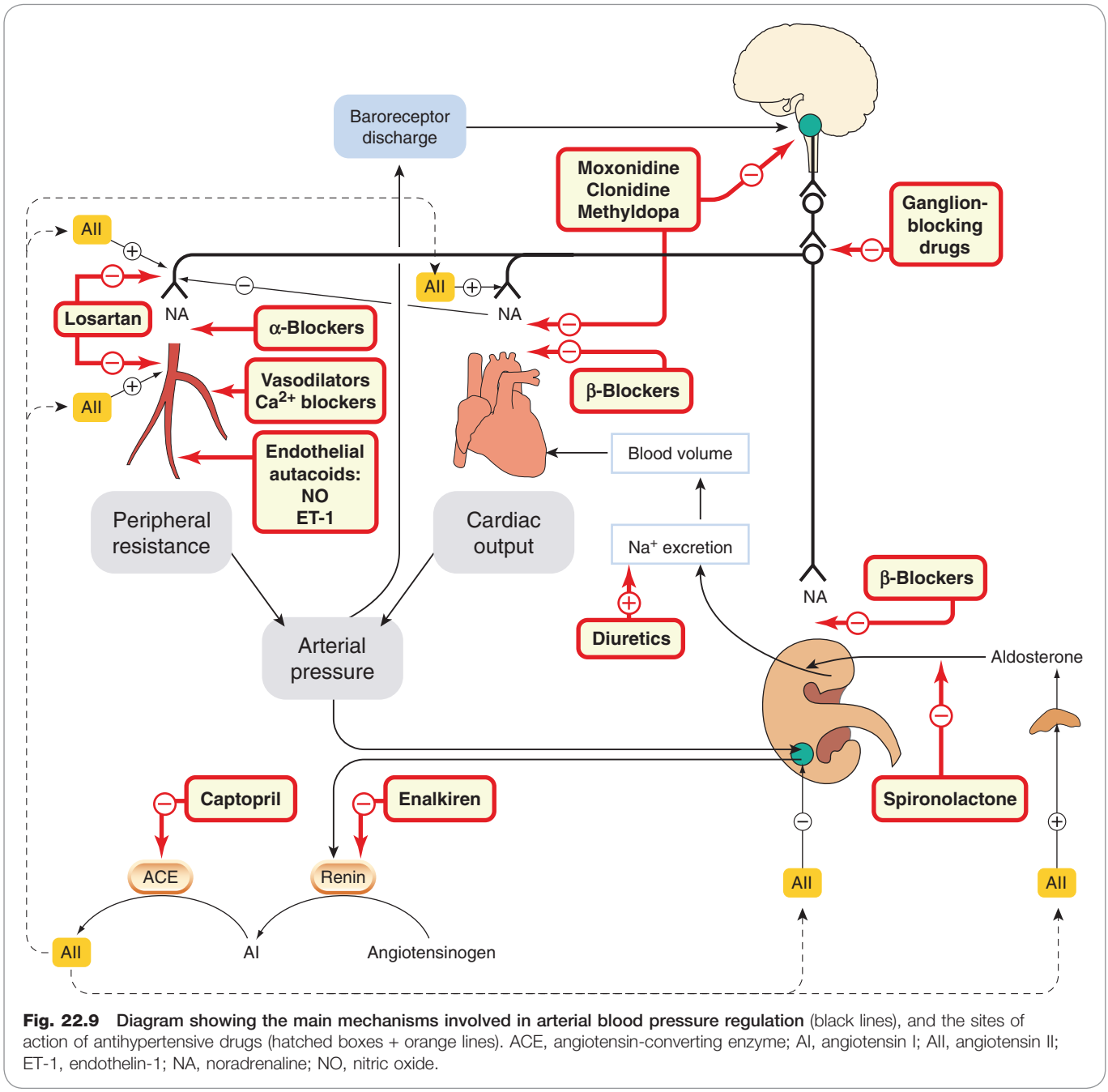

Fig. 22.9 Diagram showing the main mechanisms involved in arterial blood pressure regulation (black lines), and the sites of action of antihypertensive drugs (hatched boxes + orange lines). ACE, angiotensin-converting enzyme; AI, angiotensin I; AII, angiotensin II; ET-1, endothelin-1; NA, noradrenaline; NO, nitric oxide.

β-adrenoceptor antagonist, is sometimes effective where other drugs have failed in severe hypertension resistant to other drugs. **Fenoldopam**, a selective dopamine D_1 receptor agonist, is approved in the USA for the short-term management in hospital of severe hypertension. Its effect is similar in magnitude to that of intravenous nitroprusside, but it lacks thiocyanate-associated toxicity and is slower in onset and offset.

Commonly used antihypertensive drugs and their common adverse effects are summarised in Table 22.5.

HEART FAILURE

Heart failure is a clinical syndrome characterised by symptoms of breathlessness and/or fatigue, usually with signs of fluid overload (edema, crackles heard when listening to the chest). The underlying physiological abnormality (see also Ch. 21) is a cardiac output that is inadequate to meet the metabolic demands of the body, initially during exercise but, as the syndrome progresses, also at rest. It may be caused by disease of the myocardium itself (most commonly secondary to coronary artery disease), or by circulatory factors such as volume overload (e.g. leaky valves, or arteriovenous shunts caused by congenital defects) or pressure overload (e.g. stenosed—i.e. narrowed—valves, arterial or pulmonary hypertension). Some of these underlying causes are surgically correctable, and in some either the underlying disease (e.g. hyperthyroidism; Ch. 33), or an aggravating factor such as anaemia (Ch. 25) or atrial fibrillation (Ch. 21), is treatable with drugs. Here, we focus on

Table 22.5 Common antihypertensive drugs and their adverse effects

Drug	Adverse effects[a]		
	Postural hypotension	Impotence	Other
Thiazide diuretics (e.g. **bendroflumethiazide**)	±	++	Urinary frequency, gout, glucose intolerance, hypokalemia, hyponatremia
ACE inhibitors (e.g. **enalapril**)	±	–	Cough, first-dose hypotension, teratogenicity, reversible renal dysfunction (in presence of renal artery stenosis)
AT_1 antagonists (e.g. **losartan**)	–	–	Teratogenicity, reversible renal dysfunction (in presence of renal artery stenosis)
Ca^{2+} antagonists (e.g. **nifedipine**)	–	±	Ankle oedema
β-adrenoceptor antagonists (e.g. **metoprolol**)	–	+	Bronchospasm, fatigue, cold hands and feet, bradycardia
α_1-adrenoceptor antagonists (e.g. **doxazosin**)	++	–	First-dose hypotension

[a] ± indicates that the adverse effect occurs in special circumstances only (e.g. postural hypotension occurs with a thiazide diuretic only if the patient is dehydrated for some other reason or is taking some additional drug).

drugs used to treat heart failure irrespective of the underlying cause.

When cardiac output is insufficient to meet metabolic demand, an increase in fluid volume occurs, partly because increased venous pressure causes increased formation of tissue fluid, and partly because reduced renal blood flow activates the renin–angiotensin–aldosterone system, causing Na^+ and water retention. Irrespective of the cause, the outlook for adults with cardiac failure is grim: 50% of those with the most severe grade are dead in 6 months, and of those with 'mild/moderate' disease, 50% are dead in 5 years. Non-drug measures, including dietary salt restriction and exercise training in mildly affected patients,[14] are important, but drugs are needed to improve symptoms of oedema, fatigue and breathlessness, and to improve prognosis.

A highly simplified diagram of the sequence of events is shown in Figure 22.10. A common theme is that several of the feedbacks that are activated are 'counter-regulatory' — i.e. they make the situation worse not better. This occurs because the body fails to distinguish the haemodynamic state of heart failure from haemorrhage, in which release of vasoconstrictors such as angiotensin II and ADH would be appropriate.[15] ACEIs and ARBs, β-adrenoceptor and aldosterone antagonists interrupt these counter-regulatory neurohormonal mechanisms and have each been shown to prolong life in heart failure, although prognosis remains poor despite optimal management.

Drugs used to treat heart failure act in various complementary ways to do the following.

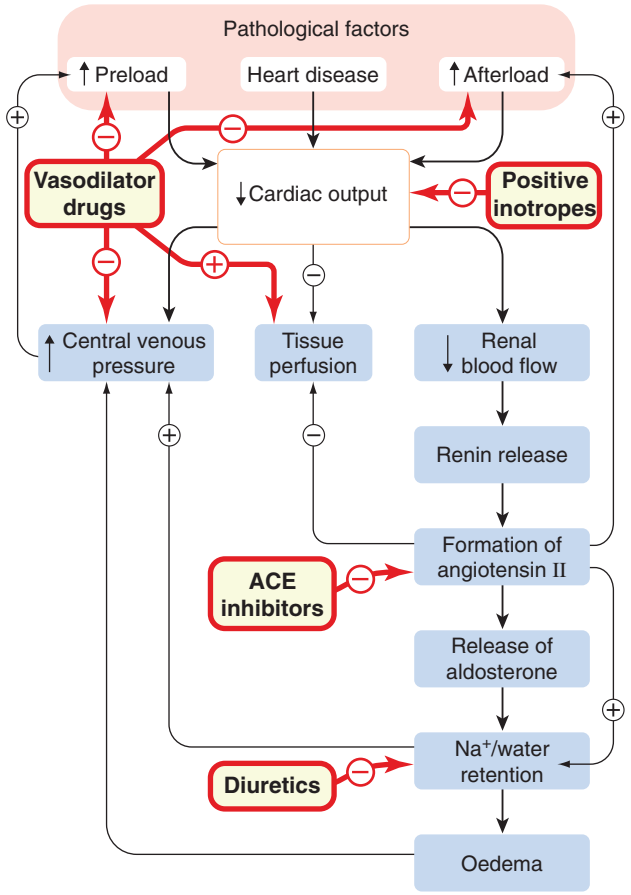

Fig. 22.10 Simplified scheme showing the pathogenesis of heart failure, and the sites of action of some of the drugs used to treat it. The symptoms of heart failure are produced by reduced tissue perfusion, oedema and increased central venous pressure. ACE, angiotensin-converting enzyme.

[14]Bed rest used to be recommended but results in deconditioning, and regular exercise has been shown to be beneficial in patients who can tolerate it.

[15]Natural selection presumably favoured mechanisms that would benefit young hunter gatherers at risk of haemorrhage; middle-aged or elderly people at high risk of heart failure are past their reproductive prime.

Increase natriuresis. Diuretics, especially loop diuretics (Ch. 28), are important in increasing salt and water excretion, especially if there is pulmonary oedema. In chronic heart failure, drugs that have been shown to improve prognosis were all studied in patients treated with diuretics.

Inhibit the renin–angiotensin–aldosterone system. The renin–angiotensin–aldosterone system is inappropriately activated in patients with cardiac failure, especially when they are treated with diuretics. The β-adrenoceptor antagonists inhibit renin secretion and are used in clinically stable patients with chronic heart failure (see below). ACEIs and ARBs block the formation of angiotensin II and inhibit its action, respectively, thereby reducing vascular resistance, improving tissue perfusion and reducing cardiac afterload. They also cause natriuresis by inhibiting secretion of aldosterone and by reducing the direct stimulatory effect of angiotensin II on reabsorption of Na^+ and HCO_3^- in the early part of the proximal convoluted tubule. Most important of all, they prolong life. The question of whether ACEIs and ARBs can usefully be combined is being evaluated. Angiotensin II is not the only stimulus to aldosterone secretion, and during chronic treatment with ACEIs, circulating aldosterone concentrations return towards pretreatment values (a phenomenon known as 'aldosterone escape'). This provided a rationale for studying the effect of combining **spironolactone** (an aldosterone antagonist; see Ch. 32) with ACEI treatment, and this further reduces mortality. **Eplerenone** is an aldosterone antagonist with less oestrogen-like adverse effects than sprironolactone; it too has been shown to improve survival in patients with heart failure when added to conventional therapy. Patients with impaired renal function were excluded from these trials, and careful monitoring of plasma K^+ concentration is important when they are treated with an ACEI or an ARB in combination with an aldosterone antagonist.

Block β adrenoceptors. Heart failure is accompanied by potentially harmful activation of the sympathetic nervous system as well as of the renin–angiotensin system, providing a rationale for using β-adrenoceptor antagonists. Most clinicians were very wary of this approach because of the negative inotropic action of these drugs, but when started in low doses that are increased slowly, **metoprolol**, **carvedilol** and **bisoprolol** each improves survival when added to optimal treatment in clinically stable patients with chronic heart failure.

Antagonise ADH. ADH (see above) is released in heart failure and may contribute to undesirable vasoconstriction (via V_{1A} receptors) and hyponatraemia (via V_2 receptors).[16] Two non-peptide vasopressin receptor antagonists ('vaptans') have been licensed by the Food and Drug Administration and many more are in development (Finley et al., 2008). **Conivaptan** is a non-selective V_{1A} / V_2 antagonist licensed for treatment of the syndrome of inappropriate ADH secretion (SIADH) and intravenously for short-term treatment of hypervolaemic (or euvolaemic) heart failure. **Tolvaptan** is a selective V_2 receptor antagonist approved for oral treatment of clinically significant hypervolaemic (or euvolaemic) hyponatraemia. Neither has been shown to improve long-term survival in heart failure, and their possible place in therapy is currently the subject of intense investigation (Jessup et al., 2009).

Relax vascular smooth muscle. Glyceryl trinitrate (Ch. 21) is infused intravenously to treat acute cardiac failure. Its venodilator effect reduces venous pressure, and its effects on arterial compliance and wave reflection reduce cardiac work. The combination of **hydralazine** (to reduce afterload) with a long-acting organic nitrate (to reduce preload) in patients with chronic heart failure improved survival in a randomised controlled trial, but the results suggested that the benefit was restricted to African-American patients. This ethnic group is genetically very heterogeneous, and it is unknown what other groups will benefit from such treatment.

Increase the force of cardiac contraction. Cardiac glycosides (Ch. 21) are used either in patients with heart failure who also have chronic rapid atrial fibrillation, or in patients who remain symptomatic despite treatment with a diuretic and ACEI. **Digoxin** does not reduce mortality in heart failure patients in sinus rhythm who are otherwise optimally treated, but does improve symptoms and reduce the need for hospital admission. In contrast, PDE inhibitors (see Ch. 21) increase cardiac output, but increase mortality in heart failure, probably through cardiac dysrhythmias. **Dobutamine** (a β_1-selective adrenoceptor agonist; see Ch. 21) is used intravenously when a rapid response is needed in the short term, for example following heart surgery.

Drugs used in chronic heart failure

- Loop diuretics, for example **furosemide** (Ch. 28).
- Angiotensin-converting enzyme inhibitors (e.g. **ramipril**).
- Angiotensin II subtype 1 receptor antagonists (e.g. **valsartan**, **candesartan**).
- β-adrenoceptor antagonists (e.g. **metoprolol**, **bisoprolol**, **carvedilol**), introduced in low dose in stable patients.
- Aldosterone receptor antagonists (e.g. **spironolactone**, Ch. 28; and **eplerenone**).
- **Digoxin** (see Ch. 21), especially for heart failure associated with established rapid atrial fibrillation. It is also indicated in patients who remain symptomatic despite optimal treatment.
- Organic nitrates (e.g. **isosorbide mononitrate**) reduce preload, and **hydralazine** reduces afterload. Used in combination, these prolong life in African-Americans.

SHOCK AND HYPOTENSIVE STATES

Shock is a medical emergency characterised by inadequate perfusion of vital organs, usually because of a very low arterial blood pressure. This leads to anaerobic metabolism and hence to increased lactate production. Mortality is very high, even with optimal treatment in an intensive care unit. Shock can be caused by various insults, including haemorrhage, burns, bacterial infections, anaphylaxis (Ch. 17) and myocardial infarction (Fig. 22.11). The common factor is reduced effective circulating blood volume (hypovolaemia) caused either directly by bleeding or by movement of fluid from the plasma to the gut lumen or extracellular

[16]Inappropriate secretion of ADH causes hyponatraemia because the kidney retains water while continuing to excrete sodium ions, whereas drinking, which is largely determined by habit in addition to thirst, continues. This leads to reduction of the plasma sodium concentration as a result of dilution.

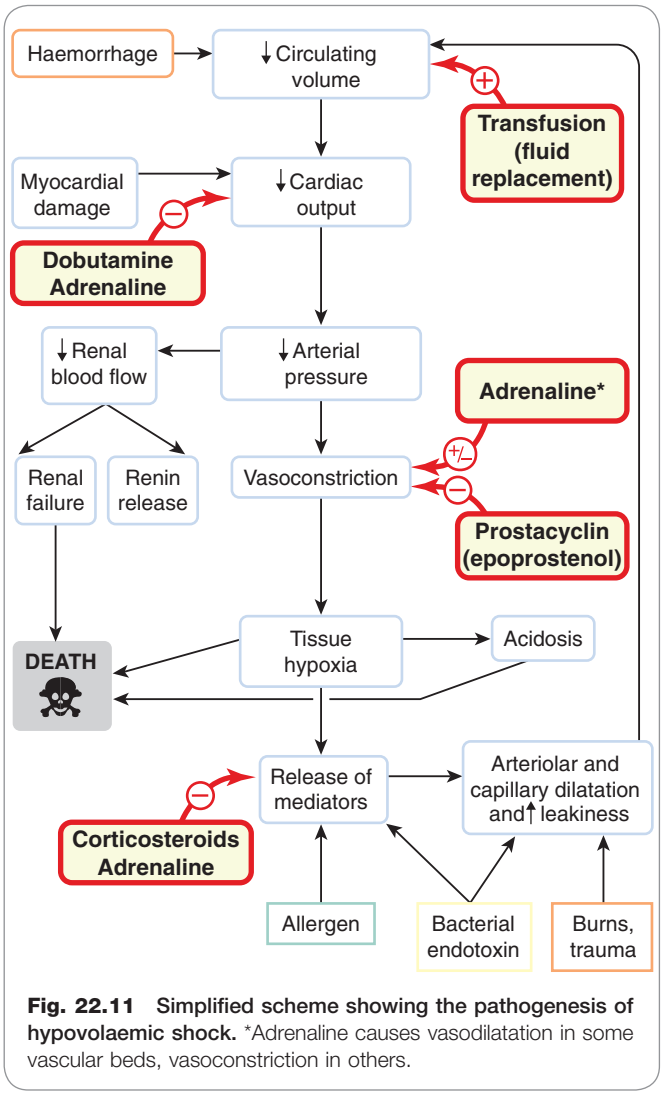

Fig. 22.11 Simplified scheme showing the pathogenesis of hypovolaemic shock. *Adrenaline causes vasodilatation in some vascular beds, vasoconstriction in others.

fluid. The physiological (homeostatic) response to this is complex: vasodilatation in a vital organ (e.g. brain, heart or kidney) favours perfusion of that organ, but at the expense of a further reduction in blood pressure, which leads to reduced perfusion of other organs. Survival depends on a balance between vasoconstriction in non-essential vascular beds and vasodilatation in vital ones. The dividing line between the normal physiological response to blood loss and clinical shock is that in shock tissue hypoxia produces secondary effects that magnify rather than correct the primary disturbance. Therefore patients with established shock have profound and inappropriate vasodilatation in non-essential organs, and this is difficult to correct with vasoconstrictor drugs. The release of mediators (e.g. histamine, 5-hydroxytryptamine, bradykinin, prostaglandins, cytokines including interleukins and tumour necrosis factor, NO and undoubtedly many more as-yet-unidentified substances) that cause capillary dilatation and leakiness is the opposite of what is required to improve function in this setting. Mediators promoting vasodilatation in shock converge on two main mechanisms:

1. Activation of ATP-sensitive potassium channels in vascular smooth muscle by reduced cytoplasmic ATP and increased lactate and protons.
2. Increased synthesis of NO, which activates myosin light-chain phosphatase and activates K_{Ca} channels (see above).

A third key mechanism seems to be a relative *deficiency* of ADH, which is secreted acutely in response to haemorrhage but subsequently declines, probably because of depletion from the neurohypophysis (see Ch. 32)—contrast this with the situation in *chronic* heart failure discussed above where *excess* (rather than deficient) ADH may contribute to problems.

Patients with shock are not a homogeneous population, making it hard to perform valid clinical trials, and in contrast to hypertension and heart failure there is very little evidence to support treatment strategies based on hard clinical end points (such as improved survival). Hypoperfusion leads to multiple organ failure (including renal failure), and intensive therapy specialists spend much effort supporting the circulations of such patients with cocktails of vasoactive drugs designed to optimise flow to vital organs. Trials of antagonists designed to block or neutralise endotoxin, interleukins, tumour necrosis factor and the inducible form of NO synthase have so far been disappointing. *Volume replacement* is of benefit if there is hypovolaemia; *antibiotics* are essential if there is persistent bacterial infection; **adrenaline** can be life-saving in anaphylaxis; a preparation of recombinant activated protein C, **drotrecogin alpha** (activated) (see Ch. 24) improves mortality in severe septic shock with multiple organ failure and is licensed for this indication; **vasopressin** may be effective in increasing blood pressure even when there is resistance to adrenaline; *corticosteroids* suppress the formation of NO and of prostaglandins but are not of proven benefit once shock is established; **epoprostenol** (PGI_2) may be useful in patients with inappropriate platelet activation (e.g. *meningococcal sepsis*); positive inotropic agents, including adrenaline and **dobutamine**, may help in individual patients, as may **levosimendan** (Mebazaa et al., 2007).

PERIPHERAL VASCULAR DISEASE

When atheroma involves peripheral arteries, the first symptom is usually pain in the calves on walking (claudication), followed by pain at rest, and in severe cases gangrene of the feet or legs. Treatment is often surgical. Other vascular beds (e.g. coronary, cerebral and renal) are often also affected by atheromatous disease in patients with peripheral vascular disease. Drug treatment includes antiplatelet drugs (e.g. **aspirin**, **clopidogrel**; see Ch. 24), a statin (e.g. **simvastatin**; see Ch. 23) and an ACEI (e.g. **ramipril**; see above). These reduce the excess risk of ischaemic coronary and cerebral events. Additionally, several placebo-controlled studies have demonstrated that **cilostazol**, a type III PDE inhibitor (see above), improves pain-free and maximum walking distance in such patients, but its effect on mortality is unknown.

RAYNAUD'S DISEASE

Inappropriate vasoconstriction of small arteries and arterioles gives rise to Raynaud's phenomenon (blanching of the fingers during vasoconstriction, followed by blueness

owing to deoxygenation of the static blood and redness from reactive hyperaemia following return of blood flow). This can be mild, but if severe causes ulceration and gangrene of the fingers. It can occur in isolation (Raynaud's disease) or in association with a number of other diseases, including several so-called connective tissue diseases (e.g. systemic sclerosis, systemic lupus erythematosus). Treatment of Raynaud's phenomenon hinges on stopping smoking (crucially) and on avoiding the cold; β-adrenoceptor antagonists are contraindicated. Vasodilators (e.g. **nifedipine**; see Ch. 21) are of some benefit in severe cases, and evidence from several small studies suggests that other vasodilators (e.g. PGI_2, CGRP) can have surprisingly prolonged effects, but are difficult to administer.

PULMONARY HYPERTENSION

After birth, pulmonary vascular resistance is much lower than systemic vascular resistance, and systolic pulmonary artery pressure in adults is normally approximately 20 mmHg.[17]

Pulmonary artery pressure is much less easy to measure than is systemic pressure, often requiring cardiac catheterisation, so only severe and symptomatic pulmonary hypertension usually gets diagnosed. Pulmonary hypertension usually causes some regurgitation of blood from the right ventricle to the right atrium. This tricuspid regurgitation can be used to estimate the pulmonary artery pressure indirectly by ultrasonography. Pulmonary hypertension may be *idiopathic* (i.e. of unknown cause, analogous to essential hypertension in the systemic circulation), or associated with some other disease. Increased pulmonary pressure can result from an increased cardiac output (such as occurs, for example, in patients with hepatic cirrhosis—where vasodilatation may accompany intermittent subclinical exposure to bacterial endotoxin—or in patients with congenital connections between the systemic and pulmonary circulations). Vasoconstriction and/or structural narrowing of the pulmonary resistance arteries increase pulmonary arterial pressure, even if cardiac output is normal. In some situations, both increased cardiac output and increased pulmonary vascular resistance are present.

In contrast to systemic hypertension, pulmonary hypertension associated with other diseases is much more common than idiopathic pulmonary hypertension, which is a rare, severe and progressive disease. Endothelial dysfunction (see above, and also Chs 23 and 24) is implicated in its aetiology. Drugs (e.g. anorexic drugs including **dexfenfluramine**, now withdrawn) and toxins (e.g. *monocrotaline*) can cause pulmonary hypertension. Occlusion of the pulmonary arteries, for example with *recurrent pulmonary emboli* (Ch. 24), is a further cause, and *anticoagulation* (see Ch. 24) is an important part of treatment. Aggregates of deformed red cells in patients with *sickle cell anaemia* (Ch. 25) can also occlude small pulmonary arteries.

Increased pulmonary vascular resistance may, alternatively, result from vasoconstriction and/or structural changes in the walls of pulmonary resistance arteries. Many of the diseases (e.g. systemic sclerosis) associated with Raynaud's phenomenon mentioned in the section above are also associated with pulmonary hypertension. Vasoconstriction may precede cellular proliferation and medial hypertrophy which causes wall thickening in the pulmonary vasculature. Treatment with vasodilators (e.g. **nifedipine**) is used. Vasodilators with an antiproliferative action (e.g. **epoprostenol**, drugs that *potentiate NO*, or *antagonise endothelin*) are more promising.

Drugs used in treating pulmonary arterial hypertension and clinical disorders for which vasoactive drugs are important are shown in the clinical boxes.

Drugs used in pulmonary hypertension

Drugs are used where indicated to treat any underlying cause; in addition, consider the following:

- Oral anticoagulants (Ch. 24).
- Diuretics (Ch. 28).
- Oxygen.
- Digoxin (Ch. 21).
- Calcium channel blockers.
- Endothelin receptor antagonists (e.g. **bosentan**, **ambrisentan**, **sitaxentan**) by mouth for less severe stages of disease.
- Prostanoid analogues (**iloprost**, **treprostinil**, **beraprost**) by parenteral routes of administration, e.g. subcutaneous or inhaled, for more severe stages of disease.
- **Epoprostenol** (Ch. 17). This is given as a long-term intravenous infusion, and improves survival (Fig. 22.12).
- Inhaled NO is administered in intensive care, for example for pulmonary hypertensive crises in newborn babies.
- Phosphodiesterase V inhibitor: **sildenafil** is licensed for this indication.

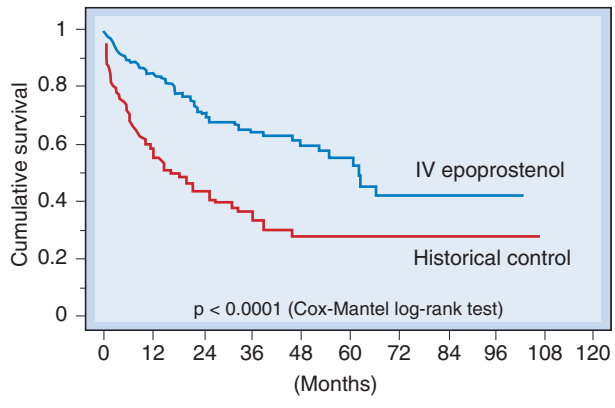

Fig. 22.12 Survival in primary pulmonary hypertension. Survival in 178 patients treated with intravenous epoprostenol versus a historical control group of 135 patients matched for disease severity. (Adapted from Sitbon O et al. 2002 Prog Cardiovasc Dis 45: 115.)

[17]In fetal life, pulmonary vascular resistance is high; failure to adapt appropriately at birth is associated with prematurity, lack of pulmonary surfactant and hypoxaemia. The resulting pulmonary hypertension is treated by paediatric intensive care specialists with measures including replacement of surfactant and ventilatory support, sometimes including inhaled NO—see Ch. 20.

Clinical disorders for which vasoactive drugs are important

- Systemic hypertension:
  - secondary to underlying disease (e.g. renal or endocrine)
  - primary 'essential' hypertension, an important risk factor for atheromatous disease (Ch. 23). Treatment reduces the excess risk of stroke or myocardial infarction, the main classes of drugs being (a) angiotensin-converting enzyme (ACE) inhibitors or AT_1 receptor antagonists; (b) β-adrenoceptor antagonists; (c) calcium antagonists; and (d) diuretics.
- Cardiac failure. Several diseases (most commonly ischaemic heart disease) impair the ability of the heart to deliver an output adequate to meet metabolic needs. Symptoms of oedema can be improved with diuretics. Life expectancy is reduced but can be improved by treatment of haemodynamically stable patients with:
  - ACE inhibitors and/or AT_1 receptor antagonists
  - β-adrenoceptor antagonists (e.g. **carvedilol**, **bisoprolol**)
  - aldosterone antagonists (e.g. **spironolactone**).
- Shock. Several diseases (e.g. overwhelming bacterial infections, Ch. 50; anaphylactic reactions, Ch. 26) lead to inappropriate vasodilatation, hypotension and reduced tissue perfusion with raised circulating concentrations of lactic acid. Pressors (e.g. **adrenaline**) are used.
- Peripheral vascular disease. Atheromatous plaques in the arteries of the legs are often associated with atheroma in other vascular territories. Statins (Ch. 23) and antiplatelet drugs (Ch. 24) are important.
- Raynaud's disease. Inappropriate vasoconstriction in small arteries in the hands causes blanching of the fingers followed by blueness and pain. **Nifedipine** or other vasodilators are used.
- Pulmonary hypertension, which can be:
  - idiopathic (a rare disorder): **epoprostenol**, **iloprost**, **bosentan** and **sildenafil** are of benefit in selected patients
  - associated with hypoxic lung disease.

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Atherosclerosis and lipoprotein metabolism

23

OVERVIEW

Atheromatous disease is ubiquitous and underlies the commonest causes of death (myocardial infarction caused by thrombosis—Ch. 24—on ruptured atheromatous plaque in a coronary artery) and disability (stroke, heart failure) in industrial societies. Hypertension is one of the most important risk factors for atheroma, and is discussed in Chapter 22. Here, we consider other risk factors, especially dyslipidaemia,[1] which, like hypertension, is amenable to drug therapy. We describe briefly the processes of atherogenesis and of lipid transport as a basis for understanding the actions of lipid-lowering drugs. Important agents (statins, fibrates, cholesterol absorption inhibitors, nicotinic acid derivatives, fish oil derivatives) are described, with emphasis on the statins, which reduce the incidence of arterial disease and prolong life.

INTRODUCTION

In this chapter we summarise the pathological process of atherogenesis and approaches to the prevention of atherosclerotic disease. Lipoprotein transport forms the basis for understanding drugs used to treat dyslipidaemia. We emphasise the *statins*, which have been a major success story, not only lowering plasma cholesterol but also reducing cardiovascular events by approximately 25–50% and prolonging life. However, some patients cannot tolerate them, and they are not effective in all patients. Evidence that other drugs that influence dyslipidaemia improve clinical outcomes is less secure than for the statins, and two recent setbacks described below call into question the reliability of changes in circulating lipid concentrations in response to drugs as surrogates predicting clinical improvement. In the absence of hard evidence of clinical improvement, other classes of lipid-lowering drugs remain second line to statins, so there is rather a lot of 'small print' in this section.

ATHEROGENESIS

Atheroma is a focal disease of the intima of large and medium-sized arteries. Lesions evolve over decades, during most of which time they are clinically silent, the occurrence of symptoms signalling advanced disease. Presymptomatic lesions are often difficult to detect non-invasively, although ultrasound is useful in accessible arteries (e.g. the carotids), and associated changes such as reduced aortic compliance and arterial calcification can be detected by measuring, respectively, aortic pulse wave velocity and coronary artery calcification. Until recently, there have been no good subprimate models, but transgenic mice (see Ch. 7) deficient in apolipoproteins or receptors that play key roles in lipoprotein metabolism have transformed this scene. Nevertheless, most of our current understanding of atherogenesis comes from human epidemiology and pathology, and from clinical investigations.

Epidemiological studies have identified numerous risk factors for atheromatous disease. Some of these cannot be altered (e.g. a family history of ischaemic heart disease), but others are modifiable (see Table 23.1) and are potential targets for therapeutic drugs. Clinical trials have shown that improving risk factors can reduce the consequences of atheromatous disease. Many risk factors (e.g. type 2 diabetes, dyslipidaemia, cigarette smoking) cause endothelial dysfunction (see Ch. 22), evidenced by reduced vasodilator responses to acetylcholine or to increased blood flow (so-called 'flow-mediated dilatation', responses that are inhibited by drugs that block nitric oxide [NO] synthesis; Ch. 20). Healthy endothelium produces NO and other mediators that protect against atheroma, so it is likely that metabolic cardiovascular risk factors act by causing endothelial dysfunction.

Atherogenesis involves the following processes:

1. *Endothelial dysfunction*, with altered NO (Ch. 20) biosynthesis, predisposes to atherosclerosis.
2. *Injury* of dysfunctional endothelium leads to expression of adhesion molecules. This encourages monocyte attachment and migration of monocytes from the lumen into the intima. Lesions have a predilection for regions of disturbed flow such as the origins of aortic branches.
3. *Low-density lipoprotein (LDL) cholesterol* is transported into the vessel wall. Endothelial cells and monocytes/macrophages generate free radicals that oxidise LDL (oxLDL), resulting in lipid peroxidation.
4. The *oxLDL* is taken up by macrophages via 'scavenger' receptors. Such macrophages are called *foam cells* because of their 'foamy' histological appearance, resulting from accumulation of cytoplasmic lipid, and are characteristic of atheroma. Uptake of oxLDL activates macrophages and releases proinflammatory cytokines.
5. Subendothelial collections of foam cells and T lymphocytes form *fatty streaks*.
6. Cholesterol can be *mobilised from the artery wall* and transported in plasma in the form of high-density lipoprotein (HDL) cholesterol, a protective mechanism termed 'reverse cholesterol transport'.
7. Activated platelets, macrophages and endothelial cells release cytokines and growth factors, causing proliferation of smooth muscle and deposition of connective tissue components. This *inflammatory fibroproliferative response* leads to a dense fibrous cap

[1]The term dyslipidaemia is preferred to hyperlipidaemia because a low plasma concentration of high-density lipoprotein cholesterol is believed to be harmful and is a therapeutic target.

Table 23.1 Modifiable risk factors for atheromatous disease

Raised low-density lipoprotein cholesterol
Reduced high-density lipoprotein cholesterol
Hypertension (Ch. 22)
Diabetes mellitus (Ch. 30)
Cigarette smoking (Ch. 48)
Obesity (Ch. 31)
Physical inactivity
Raised C-reactive protein[a]
Raised coagulation factors (e.g. factor VII, fibrinogen)
Raised homocysteine
Raised lipoprotein(a)[b]

[a] Strongly associated with atheromatous disease but unknown if this is causal.
[b] Potentially modifiable but strongly genetically determined: nicotinic acid does lower lipoprotein(a).

overlying a lipid-rich core, the whole structure comprising the atheromatous plaque.

8. Plaque can *rupture*, forming a substrate for *thrombosis* (see Ch. 24, Figs 24.1 and 24.10). The presence of large numbers of macrophages predisposes to plaque rupture, whereas vascular smooth muscle and matrix proteins stabilise the plaque.

To understand how drugs prevent atheromatous disease, it is necessary briefly to review lipoprotein transport.

LIPOPROTEIN TRANSPORT

Lipids and cholesterol are transported in the bloodstream as complexes of lipid and protein known as *lipoproteins*. These consist of a central core of hydrophobic lipid (including triglycerides and cholesteryl esters) encased in a hydrophilic coat of polar phospholipid, free cholesterol and *apoprotein*. There are four main classes of lipoprotein, differing in the relative proportion of the core lipids and in the type of apoprotein (various kinds of apoA and apoB, see below). Apoproteins bind to receptors specific for each that mediate uptake of lipoprotein particles into liver, blood or other tissues. Lipoproteins differ in size and density, and this latter property, measured originally by ultracentrifugation but now commonly estimated by simpler methods, is the basis for their classification into:

- HDL particles (contain apoA1 and apoA2), diameter 7–20 nm
- LDL particles (contain apoB-100), diameter 20–30 nm
- very-low-density lipoprotein (VLDL) particles (contain apoB-100), diameter 30–80 nm
- chylomicrons (contain apoB-48), diameter 100–1000 nm.

Each class of lipoprotein has a specific role in lipid transport, and there are different pathways for exogenous and for endogenous lipids, as well as a pathway for reverse cholesterol transport (Fig. 23.1). In the *exogenous pathway*, cholesterol and triglycerides absorbed from the ileum are transported as chylomicrons in lymph and then blood, to capillaries in muscle and adipose tissue. Here, triglycerides are hydrolysed by lipoprotein lipase, and the tissues take up the resulting free fatty acids and glycerol. The chylomicron remnants, still containing their full complement of cholesteryl esters, pass to the liver, bind to receptors on hepatocytes and undergo endocytosis. Cholesterol liberated in hepatocytes is stored, oxidised to bile acids, secreted unaltered in bile, or can enter the endogenous pathway.

In the *endogenous pathway*, cholesterol and newly synthesised triglycerides are transported from the liver as VLDL to muscle and adipose tissue, where triglyceride is hydrolysed to fatty acids and glycerol; these enter the tissues as described above. During this process, the lipoprotein particles become smaller but retain a full complement of cholesteryl esters and become LDL particles. LDL provides the source of cholesterol for incorporation into cell membranes and for synthesis of steroids (see Chs 32 and 34) but is also key in atherogenesis. Cells take up LDL by endocytosis via *LDL receptors* that recognise apoB-100. Cholesterol can return to plasma from the tissues in HDL particles (reverse cholesterol transport). Cholesterol is esterified with long-chain fatty acids in HDL particles, and the resulting cholesteryl esters are transferred to VLDL or LDL particles by a transfer protein present in the plasma and known as *cholesteryl ester transfer protein* (CETP). Lipoprotein(a), or Lp(a), is a species of LDL that is associated with atherosclerosis and is localised in atherosclerotic lesions. Lp(a) contains a unique apoprotein, apo(a), with structural similarities to plasminogen (Ch. 24). Lp(a) competes with and inhibits the binding of plasminogen to its receptors on the endothelial cell. Plasminogen is normally the substrate for plasminogen activator, which is secreted by and bound to endothelial cells, generating the fibrinolytic enzyme *plasmin* (see Fig. 24.10). The effect of the binding of Lp(a) is that less plasmin is generated, fibrinolysis is inhibited and thrombosis promoted.

▼ There is current interest in four lipid transfer proteins that have been implicated in atherogenesis (reviewed by Stein & Stein, 2005). *ACAT* (acyl coenzyme A: cholesterol acyltransferase), which is expressed in two forms, catalyses the intracellular synthesis of cholesteryl ester in macrophages, adrenal cortex, gut and liver. *LCAT* (lecithin cholesterol acyltransferase) catalyses cholesteryl ester synthesis in HDL particles. *CETP* and *PLTP* (phospholipid transfer protein) are involved in transfer of cholesterol between different classes of lipoprotein particle in plasma. **Tamoxifen**, used in the treatment and prevention of breast cancer (Chs 34 and 55), is a potent ACAT inhibitor (de Medina et al., 2004).

DYSLIPIDAEMIA

Dyslipidaemia may be primary or secondary. The *primary* forms are due to a combination of diet and genetics (often but not always polygenic). They are classified into six phenotypes (the Frederickson classification; Table 23.2). An especially great risk of ischaemic heart disease occurs in a subset of primary type IIa hyperlipoproteinaemia caused by single-gene defects of LDL receptors; this is known as *familial hypercholesterolaemia* (FH), and the plasma cholesterol concentration in affected adults is typically > 8 mmol/l in heterozygotes and 12–25 mmol/l in homozygotes. Study of FH enabled Brown & Goldstein (1986) to define the LDL receptor pathway of cholesterol homeostasis (for which they shared a Nobel Prize). Drugs used to treat primary dyslipidaemia are described below.

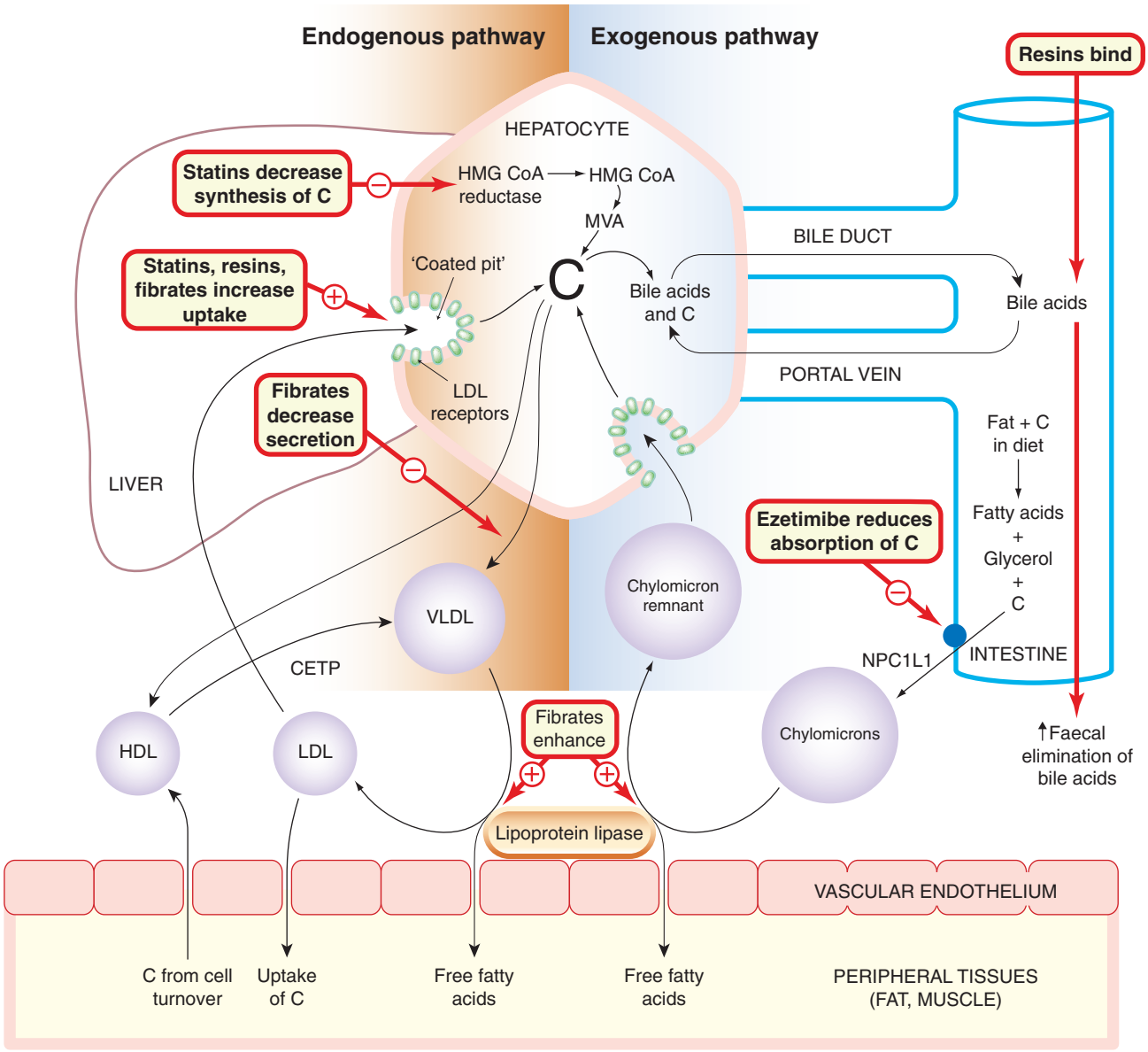

Fig. 23.1 Schematic diagram of cholesterol transport in the tissues, with sites of action of the main drugs affecting lipoprotein metabolism. C, cholesterol; CETP, cholesteryl ester transport protein; HDL, high-density lipoprotein; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL, low-density lipoprotein; MVA, mevalonate; NPC1L1, a cholesterol transporter in the brush border of enterocytes; VLDL, very-low-density lipoprotein.

Table 23.2 Frederickson/World Health Organization classification of hyperlipoproteinaemia

Type	Lipoprotein elevated	Cholesterol	Triglycerides	Atherosclerosis risk	Drug treatment
I	Chylomicrons	+	+++	NE	None
IIa	LDL	++	NE	High	Statin ± ezetimibe
IIb	LDL + VLDL	++	++	High	Fibrates, statin, nicotinic acid
III	βVLDL	++	++	Moderate	Fibrates
IV	VLDL	+	++	Moderate	Fibrates
V	Chylomicrons + VLDL	+	++	NE	Fibrate, niacin, fish oil and statin combinations

+, increased concentration; LDL, low-density lipoprotein; NE, not elevated; VLDL, very-low-density lipoprotein; βVLDL, a qualitatively abnormal form of VLDL identified by its pattern on electrophoresis.

Lipoprotein metabolism and dyslipidaemia

Lipids, including cholesterol and triglycerides, are transported in the plasma as lipoproteins, of which there are four classes:

- Chylomicrons transport triglycerides and cholesterol from the gastrointestinal tract to the tissues, where triglyceride is split by lipoprotein lipase, releasing free fatty acids and glycerol. These are taken up in muscle and adipose tissue. Chylomicron remnants are taken up in the liver, where cholesterol is stored, secreted in bile, oxidised to bile acids or converted into:
  - very-low-density lipoproteins (VLDLs), which transport cholesterol and newly synthesised triglycerides to the tissues, where triglycerides are removed as before, leaving:
  - intermediate-density and low-density lipoprotein (LDL) particles with a large component of cholesterol; some LDL cholesterol is taken up by the tissues and some by the liver, by endocytosis via specific LDL receptors.
- High-density lipoprotein (HDL) particles adsorb cholesterol derived from cell breakdown in tissues (including arteries) and transfer it to VLDL and LDL particles via cholesterol ester transport protein (CETP).
- Dyslipidaemias can be primary, or secondary to a disease (e.g. hypothyroidism). They are classified according to which lipoprotein particle is abnormal into six phenotypes (the Frederickson classification). The higher the LDL cholesterol and the lower the HDL cholesterol, the higher the risk of ischaemic heart disease.

Secondary forms of dyslipidaemia are a consequence of other conditions, such as diabetes mellitus, alcoholism, nephrotic syndrome, chronic renal failure, hypothyroidism, liver disease and administration of drugs, for example **isotretinoin** (an isomer of vitamin A given by mouth as well as topically in the treatment of severe acne), **tamoxifen** (Mikhailidis et al., 1997), **ciclosporine** (Ch. 26) and *protease inhibitors* used to treat infection with human immunodeficiency virus (Ch. 51). Secondary forms are treated where possible by correcting the underlying cause.

PREVENTION OF ATHEROMATOUS DISEASE

Drug treatment is often justified, to supplement healthy habits. Treatment of hypertension (Ch. 22) and, to a lesser extent, diabetes mellitus (Ch. 30) reduces the incidence of symptomatic atheromatous disease, and antithrombotic drugs (Ch. 24) reduce arterial thrombosis. Reducing LDL is also effective and is the main subject of this present chapter, but several other steps in atherogenesis are also potential targets for pharmacological attack.

▼ ***Angiotensin-converting enzyme inhibitors*** (Ch. 22) improve endothelial function and prolong life in patients with atheromatous disease. Other drugs that also increase NO biosynthesis or availability are under investigation.

Measures to increase HDL: moderate alcohol consumption increases HDL, and epidemiological evidence favours moderate alcohol consumption in older people.[2] Regular exercise also increases circulating HDL; drug treatment to increase HDL is of uncertain benefit. Fibrates and nicotinic acid derivatives—see below—modestly increase HDL, and reduce LDL and triglycerides. In subjects with low HDL, inhibition of cholesteryl ester transfer protein (CETP) with **torcetrapib** markedly increased HDL, but also increased blood pressure and was associated with a 60% *increase* in all-cause mortality (leading to abrupt discontinuation of its development). It is unclear if this is a class effect, but **anacetrapib** markedly increases HDL without increasing blood pressure; its effect on mortality is not yet known. ApoA-I Milano is a variant of apolipoprotein A-I identified in individuals in rural Italy with very low levels of HDL but almost no cardiovascular disease. Infusion of recombinant ApoA-I Milano–phospholipid complexes causes rapid regression of atherosclerosis in animal models, and administered intravenously caused regression of atherosclerosis in patients with acute coronary syndrome. It is expensive to produce and must be administered intravenously, but the strategy continues to be a focus of intense interest (see review by Duffy & Rader, 2009).

Antioxidants (e.g. vitamin C and vitamin E) are of interest, both because of evidence that they improve endothelial function in patients with increased oxidant stress, and because of epidemiological evidence that a diet rich in antioxidants is associated with reduced risk of coronary artery disease. Results from clinical trials have been negative, however, and several antioxidants reduce HDL. **Oestrogen**, used to prevent symptoms of the menopause (Ch. 34) and to prevent postmenopausal osteoporosis, has antioxidant properties and exerts other vascular effects that could be beneficial. Epidemiological evidence suggested that women who use such hormone replacement might be at reduced risk of atheromatous disease, but controlled trials showed significant *adverse* effects on cardiovascular mortality (Ch. 34 and see commentary by Dubey et al., 2004).

Anti-inflammatory approaches: drug treatment to lower *C-reactive protein* has been mooted, but it is possible that elevated C-reactive protein is a marker of vascular inflammation rather than playing an active part in disease progression. Other anti-inflammatory measures are being investigated; for example, *acyl coenzyme A, cholesterol acyltransferase (ACAT) inhibitors*.

Other novel therapies in development include drugs that inhibit squalene synthesis, microsomal transport protein (MTP) inhibitors and drugs that alter apoB. Among drugs that alter apoB, **mipomersen** is notable as an antisense oligonucleotide complementary to the coding region for apoB-100 of mRNA. It is an interfering RNA (iRNA; see Ch. 59) modified to render it resistant to nuclease enzymes. Injected once weekly, it has a marked effect in lowering LDL in patients with homoxyzgous FH, who are highly resistant to drug treatment (Kastelein et al., 2006).

LIPID-LOWERING DRUGS

Several drugs decrease plasma LDL. Drug therapy is used in addition to dietary measures and correction of other modifiable cardiovascular risk factors.

The main agents used clinically are:

- statins: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors
- fibrates
- inhibitors of cholesterol absorption
- nicotinic acid or its derivatives
- fish oil derivatives.

Fish oil lowers plasma triglyceride concentration but can increase plasma cholesterol.

[2]'Sinful, ginful, rum-soaked men, survive for three score years and ten'—or longer, we rather hope…

Atheromatous disease

- Atheroma is a focal disease of large and medium-sized arteries. Atheromatous plaques occur in most people, progress insidiously over many decades, and underlie the commonest causes of death (myocardial infarction) and disability (e.g. stroke) in industrialised countries.
- Fatty streaks are the earliest structurally apparent lesion and progress to fibrous and/or fatty plaques. Symptoms occur only when blood flow through the vessel is reduced below that needed to meet the metabolic demands of tissues downstream from the obstruction.
- Important modifiable risk factors include hypertension (Ch. 22), dyslipidaemia (this chapter) and smoking (Ch. 48).
- The pathophysiology is of chronic inflammation in response to injury. Endothelial dysfunction leads to loss of protective mechanisms, monocyte/macrophage and T-cell migration, uptake of low-density lipoprotein (LDL) cholesterol and its oxidation, uptake of oxidised LDL by macrophages, smooth muscle cell migration and proliferation, and deposition of collagen.
- Plaque rupture leads to platelet activation and thrombosis (Ch. 24).

STATINS: HMG-CoA REDUCTASE INHIBITORS

The rate-limiting enzyme in cholesterol synthesis is HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonic acid (see Fig. 23.1). **Simvastatin**, **lovastatin** and **pravastatin** are specific, reversible, competitive HMG-CoA reductase inhibitors with K_i values of approximately 1 nmol/l. **Atorvastatin** and **rosuvastatin** are long-lasting inhibitors. Decreased hepatic cholesterol synthesis upregulates LDL receptor synthesis, increasing LDL clearance from plasma into liver cells. The main biochemical effect of statins is therefore to reduce plasma LDL. There is also some reduction in plasma triglyceride and increase in HDL. Several large randomised placebo-controlled trials of the effects of HMG-CoA reductase inhibitors on morbidity and mortality have been positive.

▼ The Scandinavian Simvastatin Survival Study (4S) recruited patients with ischaemic heart disease and plasma cholesterol of 5.5–8.0 mmol/l: simvastatin lowered serum LDL by 35% and death by 30% (Fig. 23.2). This was accounted for by a 42% reduction in death from coronary disease over the median follow-up period of 5.4 years. Other large trials have confirmed reduced mortality both in patients with established ischaemic heart disease (e.g. the Cholesterol and Recurrent Events [CARE] trial) and in healthy people at risk of coronary disease, with a wide range of plasma cholesterol values and other risk factors, and treated with different statins (e.g. the West of Scotland Coronary Prevention Study [WOSCOPS], the Heart Protection Study and the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). Intensive lowering of LDL with atorvastatin 80 mg had a greater effect on event rate than did a 10 mg dose, but with a greater incidence of abnormally raised plasma transaminase activity (LaRosa et al., 2005). In secondary prevention trials of statins, cardiovascular event rate is approximately linearly related to the achieved plasma LDL over a concentration range from approximately 1.8 to 4.9 mmol/l, and the event rate falls on the same line in placebo- and statin-treated patients.

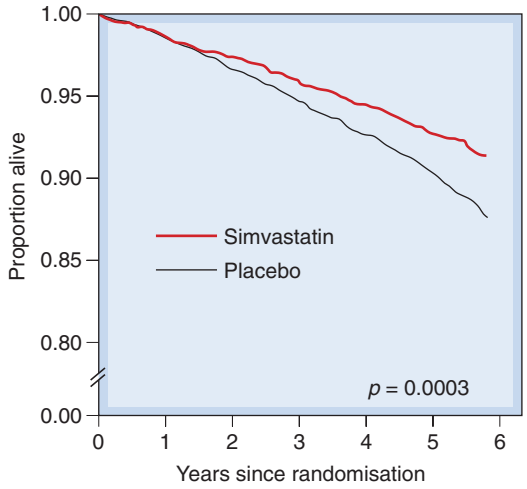

Fig. 23.2 Survival in patients with coronary heart disease and serum cholesterol 5.5–8.0 mmol/l treated either with placebo or with simvastatin. The relative risk of death in the simvastatin group was 0.70 (95% confidence intervals 0.58–0.85). (Based on 4S study 1994 Lancet 344: 1383–1389.)

Other actions of statins

Products of the mevalonate pathway react with protein ('lipidation', which is the addition to a protein of hydrophobic groups such as prenyl or farnesyl moieties). Several important membrane-bound enzymes (e.g. endothelial NO synthase; see Ch. 20) are modified in this way. The fatty groups serve as anchors, localising the enzyme in organelles such as caveoli and Golgi apparatus. Consequently, there is currently great interest in actions of statins that are unrelated, or indirectly related, to their effect on plasma LDL (sometimes referred to as *pleiotropic* effects). Some of these actions are undesirable (e.g. HMG-CoA reductase guides migrating primordial germ cells, and statin use is contraindicated during pregnancy), but some offer therapeutic promise, for example in Alzheimer's disease where a role for statins is controversial (see review by Querfurth & LaFerla, 2010) and prevention of prostate cancer (Shannon et al., 2005). Such actions include:

- improved endothelial function
- reduced vascular inflammation
- reduced platelet aggregability
- increased neovascularisation of ischaemic tissue
- increased circulating endothelial progenitor cells
- stabilisation of atherosclerotic plaque
- antithrombotic actions
- enhanced fibrinolysis
- inhibition of germ cell migration during development
- immune suppression
- protection against sepsis.

The extent to which these effects contribute to the anti-atheromatous actions of statins is unknown.

Pharmacokinetics

Short-acting statins are given by mouth at night to reduce peak cholesterol synthesis in the early morning. They are well absorbed and extracted by the liver, their site of action, and are subject to extensive presystemic metabolism via cytochrome P450 and glucuronidation pathways.

Clinical uses of HMG-CoA reductase inhibitors (statins, e.g. simvastatin, atorvastatin)

- Secondary prevention of myocardial infarction and stroke in patients who have symptomatic atherosclerotic disease (e.g. angina, transient ischaemic attacks, or following myocardial infarction or stroke).
- Primary prevention of arterial disease in patients who are at high risk because of elevated serum cholesterol concentration, especially if there are other risk factors for atherosclerosis. Tables (available for example in the British National Formulary) are used to target treatment to those at greatest risk.
- Atorvastatin lowers serum cholesterol in patients with homozygous familial hypercholesterolaemia.
- In severe drug-resistant dyslipidaemia (e.g. heterozygous familial hypercholesterolaemia), ezetimibe is combined with statin treatment.
- Contraindicated in pregnancy.

Simvastatin is an inactive lactone prodrug; it is metabolised in the liver to its active form, the corresponding β-hydroxy fatty acid.

Adverse effects

Statins are well tolerated; mild unwanted effects include muscle pain (myalgia), gastrointestinal disturbance, raised concentrations of liver enzymes in plasma, insomnia and rash. More serious adverse effects are rare but include severe myositis (rhabdomyolysis) and angio-oedema. Myositis is a class effect of statins, occurs also with other lipid-lowering drugs (especially fibrates) and is dose related.[3] It is commoner in patients with low lean body mass or uncorrected hypothyroidism.

FIBRATES

Several fibric acid derivatives (fibrates) are available, including **bezafibrate**, **ciprofibrate**, **gemfibrozil**, **fenofibrate** and **clofibrate**. These markedly reduce circulating VLDL, and hence triglyceride, with a modest (approximately 10%) reduction in LDL and an approximately 10% increase in HDL. Their mechanism of action is complex (see Fig. 23.1). They are agonists at PPARα nuclear receptors[4] (Ch. 3); in humans, the main effects are to increase transcription of the genes for lipoprotein lipase, apoA1 and apoA5. They increase hepatic LDL uptake. In addition to effects on lipoproteins, fibrates reduce plasma C-reactive protein and fibrinogen, improve glucose tolerance and inhibit vascular smooth muscle inflammation by inhibiting the expression of the transcription factor nuclear factor κB. As with the pleiotropic effects of statins (see above), there is great interest in these actions, although again it is unknown if they are clinically important.

▼ In one study, gemfibrozil reduced coronary heart disease by approximately one-third compared with placebo in middle-aged men with primary hyperlipoproteinaemia, but fibrates have not been shown to improve survival. An HDL intervention trial performed by the US Veterans Affairs Department in some 2500 men with coronary heart disease and low HDL together with low LDL showed that gemfibrozil increased HDL and reduced coronary disease and stroke. Event rates were linked to changes in HDL but not to triglycerides or to LDL, suggesting that increasing HDL with a fibrate reduces vascular risk.

Clinical uses of fibrates (e.g. gemfibrozil, fenofibrate)

- Mixed dyslipidaemia (i.e. raised serum triglyceride as well as cholesterol), provided this is not caused by excessive alcohol consumption. Fenofibrate is uricosuric, which may be useful where hyperuricaemia coexists with mixed dyslipidaemia.
- In patients with low high-density lipoprotein and high risk of atheromatous disease (often type 2 diabetic patients; see Ch. 30).
- Combined with other lipid-lowering drugs in patients with severe treatment-resistant dyslipidaemia. This may, however, increase the risk of rhabdomyolysis.

Adverse effects

Myositis is unusual but can be severe (rhabdomyolysis), with myoglobinuria and acute renal failure. It occurs particularly in patients with renal impairment, because of reduced protein binding and impaired drug elimination. Fibrates should be avoided in such patients and also in alcoholics, who are predisposed to hypertriglyceridaemia but are at risk of rhabdomyolysis.[5] Myositis can also be caused (rarely) by statins (see above), and the combined use of fibrates with this class of drugs is therefore generally inadvisable (although it is sometimes undertaken by specialists). Gastrointestinal symptoms, pruritus and rash are more common than with statins. Clofibrate predisposes to gallstones, and its use is therefore limited to patients who have had a cholecystectomy (i.e. removal of the gall bladder).

DRUGS THAT INHIBIT CHOLESTEROL ABSORPTION

Historically, bile acid-binding resins (e.g. **colestyramine**, **colestipol**) were the only agents available to reduce cholesterol absorption and were among the few means to lower plasma cholesterol. Taken by mouth, they sequester bile acids in the intestine and prevent their reabsorption and enterohepatic recirculation (Fig. 23.1). The concentration of HDL is unchanged, and they cause an unwanted increase in triglycerides.

▼ The American Lipid Research Clinics' trial of middle-aged men with primary hypercholesterolaemia showed that addition of a resin

[3]Cerivastatin, a potent statin introduced at relatively high dose, was withdrawn because of severe myositis occurring particularly in patients treated with gemfibrozil—discussed later in the chapter.

[4]Standing for peroxisome proliferator-activated receptors—don't ask! (Peroxisomes are organelles that are not present in human cells, so something of a misnomer!) Thiazolidinedione drugs used in treating diabetes act on related PPARγ receptors; see Ch. 30.

[5]For several reasons, including a tendency to lie immobile for prolonged periods followed by generalised convulsions—'rum fits'—and delirium tremens.

to dietary treatment caused a mean 13% fall in plasma cholesterol and a 20–25% fall in coronary heart disease over 7 years, but no studies have shown improved survival. Decreased absorption of exogenous cholesterol and increased metabolism of endogenous cholesterol into bile acids in the liver lead to increased expression of LDL receptors on hepatocytes, and hence to increased clearance of LDL from the blood and a reduced concentration of LDL in plasma. Resins are bulky, unpalatable and often cause diarrhoea. They interfere with the absorption of fat-soluble vitamins, and of *thiazide diuretics* (Chs 22 and 28), digoxin (Ch. 21) and warfarin (Ch. 24), which should therefore be taken at least 1 h before or 4–6 h after the resin. With the introduction of statins, their use in treating dyslipidaemia was relegated largely to additional treatment in patients with severe disease (e.g. FH) and (a separate use) treating bile salt-associated symptoms of pruritus (itch) and diarrhoea—see clinical box. **Colesevelam** (introduced recently) is less bulky (daily dose up to 4 g compared with a dose up to 36 g for colestyramine) but more expensive. Subsequently, plant sterols and stanols have been marketed; these are isolated from wood pulp and used to make margarines or yoghurts. They reduce plasma cholesterol to a small extent and are tastier than resins.[6] Their mechanism is unclear; sitostanol in the gut lumen competes with cholesterol for uptake and sitosterol interferes with cholesterol transfer within the enterocyte.

EZETIMIBE

Ezetimibe is one of a group of azetidinone cholesterol absorption inhibitors, and is used as an adjunct to diet and statins in hypercholesterolaemia. It inhibits absorption of cholesterol (and of plant stanols) from the duodenum by blocking a transport protein (NPC1L1) in the brush border of enterocytes, without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids. Because of its high potency compared with resins (a daily dose of 10 mg compared with a dose of resin of up to 36 g of colestyramine), it should represent a very real advance as a substitute for resins as supplementary treatment to statins in patients with severe dyslipidaemia. However, disappointingly, in a study of 720 patients with heterozygous FH comparing simvastatin alone with the combination of simvastatin with ezetimibe, whereas ezetimibe did indeed have the desired effect on LDL (approximately an extra 20% reduction), it did not retard thickening in the inner layers of the carotid artery over 2 years of follow-up (Kastelein et al., 2008). Such thickening is closely linked to atherosclerosis. A larger trial evaluating its effect on cardiovascular outcome is ongoing and eagerly (anxiously?) awaited. The mechanism of ezetimibe is distinct from that of phytosterol and phytostanol esters, which interfere with the micellar presentation of sterols to the cell surface.

Ezetimibe is administered by mouth and is absorbed into intestinal epithelial cells, where it localises to the brush border, which is its presumed site of action. It is also extensively (> 80%) metabolised to an active metabolite. Enterohepatic recycling results in slow elimination. The terminal half-life is approximately 22 h. It enters milk (at least in animal studies) and is contraindicated for women who are breastfeeding. It is generally well tolerated but can cause diarrhoea, abdominal pain or headache; rash and angio-oedema have been reported.

NICOTINIC ACID

Nicotinic acid is a vitamin, and as such is essential for many important metabolic processes. Quite separately from this, it has been used in gram quantities as a lipid-lowering agent. It is converted to nicotinamide, which inhibits hepatic VLDL secretion (see Fig. 23.1), with consequent reductions in circulating triglyceride and LDL including Lp(a), and an increase in HDL. The mechanism is poorly understood but is believed to be initiated by an effect on lipolysis via a G-protein-coupled orphan receptor called HM74A and present in adipocyte membranes (see review by Karpe & Frayn, 2004). It also influences hepatic diacylglycerol transferase. Long-term administration to survivors of myocardial infarction reduced mortality in the Coronary Drug Project trial, but unwanted effects limit its clinical use. A modified-release preparation is better tolerated, and is a real, if modest, advance.

Adverse effects include flushing, palpitations and gastrointestinal disturbance. Flushing is associated with production of PGD_2 (Ch. 17) and is reduced by taking with aspirin or with **laropiprant** (a PGD_2 antagonist)—Figure 23.3. High doses can disturb liver function, impair glucose tolerance, and precipitate gout by increasing circulating urate concentration.

Clinical use of drugs that reduce cholesterol absorption: ezetimibe or bile acid-binding resins (e.g. colestyramine)

- As an addition to a statin when response has been inadequate (ezetimibe).
- For hypercholesterolaemia when a statin is contraindicated.
- Uses unrelated to atherosclerosis, including:
  - pruritus in patients with partial biliary obstruction (bile acid-binding resin)
  - bile acid diarrhoea, for example caused by diabetic neuropathy (bile acid-binding resin).

FISH OIL DERIVATIVES

Omega-3 marine triglycerides reduce plasma triglyceride concentrations but increase cholesterol. Plasma triglyceride concentrations are less strongly associated with coronary artery disease than is cholesterol, but there is epidemiological evidence that eating fish regularly does reduce ischaemic heart disease, and dietary supplementation with ω-3 polyunsaturated fatty acids (PUFAs) improves survival in patients who have recently had a myocardial infarction (GISSI-Prevenzione Investigators, 1999). The mechanism may be the potent antiarrhythmic effects of PUFA (reviewed by Leaf et al., 2003). The mechanism of action of fish oil on plasma triglyceride concentrations is unknown. Fish oil is rich in PUFA, including eicosapentaenoic and docosahexaenoic acid, and it has other potentially important effects including inhibition of platelet function, prolongation of bleeding time, anti-inflammatory effects and reduction of plasma fibrinogen. Eicosapentaenoic acid substitutes for arachidonic acid in cell membranes and gives rise to 3-series prostaglandins and thromboxanes (that is, prostanoids with three double bonds in their side chains rather than the usual two), and 5-series leukotrienes. This probably accounts for their effects on haemostasis, because thromboxane A_3 is much less active as a platelet-aggregating agent than is

[6]This is not, however, saying much.

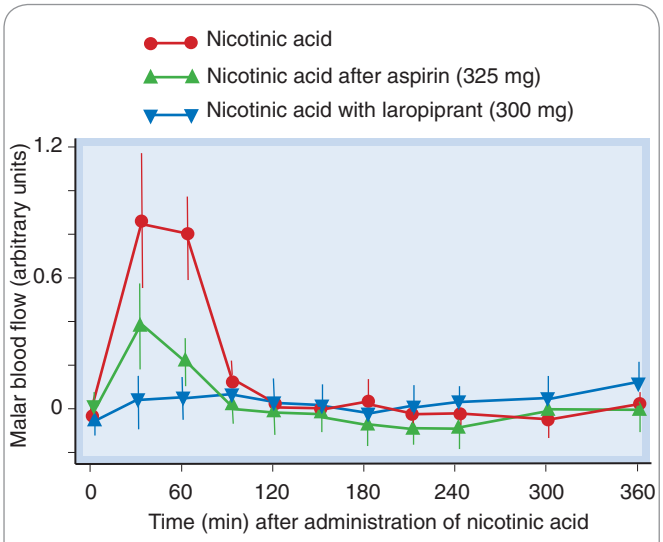

Fig 23.3 Vasodilatation caused by nicotinic acid (1.5 g, extended-release preparation) is attenuated by aspirin or by laropiprant, an antagonist of prostaglandin D_2 (PGD_2). Blood flow in the cheeks of human subjects was measured by laser Doppler perfusion imaging after either placebo or nicotinic acid. Aspirin (325 mg 30 min before nicotinic acid) or laropiprant (300 mg with nicotinic acid) reduced the increase in malar blood flow caused by nicotinic acid. (Redrawn from Lai E et al. 2007 Suppression of niacin-induced vasodilation with an antagonist to prostaglandin D2 receptor subtype 1. Clin Pharmacol Therap 81: 849–857.)

thromboxane A_2, whereas PGI_3 is similar in potency to PGI_2 as an inhibitor of platelet function. The alteration in leukotriene biosynthesis probably underlies the anti-inflammatory effects of fish oil. Fish oil is contraindicated in patients with type IIa hyperlipoproteinaemia because of the increase in LDL that it causes. A preparation of omega 3-acid ethyl esters is licensed in the UK for prevention of recurrent events after myocardial infarction in addition to treatment of hypertriglyceridaemia; it causes less increase in LDL and fewer problems with fishy odour, weight gain and dyspepsia than the older fish oil preparations.

Drugs in dyslipidaemia

The main drugs used in patients with dyslipidaemias are:

- HMG-CoA reductase inhibitors (*statins*, e.g. **simvastatin**): inhibit synthesis of cholesterol, increasing expression of low-density lipoprotein (LDL) receptors on hepatocytes and hence increasing hepatic LDL cholesterol (LDL-C) uptake. They reduce cardiovascular events and prolong life in people at risk, and clinically are the most important class of drugs used in dyslipidaemias. Adverse effects include myalgias (rarely, severe muscle damage) and raised liver enzymes.
- Fibrates (e.g. **gemfibrozil**): activate PPARα receptors, increase activity of lipoprotein lipase, decrease hepatic very-low-density lipoprotein production and enhance clearance of LDL by the liver. They markedly lower serum triglycerides, and modestly increase high-density lipoprotein cholesterol. Adverse effects include muscle damage.
- Agents that interfere with cholesterol absorption, usually as an adjunct to diet plus statin:
  - ezetimibe
  - stanol-enriched foods
  - bile acid-binding resins (e.g. colestyramine, colesevelam).
- Modified-release nicotinic acid. Flushing is the main adverse effect; it can be controlled by aspirin or by laropiprant (a PGD_2 antagonist).
- Fish oil derivatives—omega-3-acid ethyl esters.

Clinical uses of nicotinic acid derivatives

- As adjunct to a statin and diet in dyslipidaemia, especially when associated with low HDL and raised triglycerides.
- When a statin is contraindicated.

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24 Haemostasis and thrombosis

OVERVIEW

This chapter summarises the main features of blood coagulation, platelet function and fibrinolysis. These processes underlie haemostasis and thrombosis, and provide a basis for understanding haemorrhagic disorders (e.g. haemophilia) and thrombotic diseases both of arteries (e.g. thrombotic stroke, myocardial infarction) and of veins (e.g. deep vein thrombosis). Drugs that act on the coagulation cascade, on platelets and on fibrinolysis are considered. Anticoagulants, antiplatelet drugs and fibrinolytic drugs are especially important clinically because of the prevalence of thrombotic disease, and are emphasised for this reason.

INTRODUCTION

Haemostasis is the arrest of blood loss from damaged blood vessels and is essential to life. A wound causes vasoconstriction, accompanied by:

- adhesion and activation of platelets
- formation of fibrin.

Platelet activation leads to the formation of a haemostatic plug, which stops the bleeding and is subsequently reinforced by fibrin. The relative importance of each process depends on the type of vessel (arterial, venous or capillary) that has been injured.

Thrombosis is the pathological formation of a 'haemostatic' plug within the vasculature in the absence of bleeding ('haemostasis in the wrong place'). Over a century ago, Rudolph Virchow defined three predisposing factors—'Virchow's triad': *injury to the vessel wall*—for example, when an atheromatous plaque ruptures or becomes eroded; *altered blood flow*—for example, in the left atrial appendage of the heart during atrial fibrillation, or in the veins of the legs while sitting awkwardly on a long journey; and *abnormal coagulability* of the blood—as occurs, for example, in the later stages of pregnancy or during treatment with certain oral contraceptives (see Ch. 34). Increased coagulability of the blood can be inherited and is referred to as *thrombophilia*. A *thrombus*, which forms in vivo, should be distinguished from a *clot*, which forms in static blood in vitro. Clots are amorphous, consisting of a diffuse fibrin meshwork in which red and white blood cells are trapped indiscriminately. By contrast, arterial and venous thrombi each have a distinct structure.

An *arterial thrombus* (see Fig. 24.1) is composed of so-called white thrombus consisting mainly of platelets in a fibrin mesh. It is usually associated with atherosclerosis and can interrupt blood flow, causing ischaemia or death (infarction) of tissue downstream. Venous thrombus is composed of 'red thrombus' and consists of a small white head and a large jelly-like red tail, similar in composition to a blood clot, which streams away in the flow. Thrombus can break away from its attachment and float through the circulation, forming an embolus; venous emboli usually lodge in the lungs, while thrombus that embolises from the left heart or a carotid artery usually lodges in an artery in the brain or other organs, causing death, stroke or other disaster.

Drug therapy to promote haemostasis (e.g. antifibrinolytic and haemostatic drugs; see below) is indicated when this essential process is defective (e.g. coagulation factors in haemophilia or following excessive anticoagulant therapy), or when it proves difficult to staunch haemorrhage following surgery or for menorrhagia. Drug therapy to treat or prevent thrombosis or thromboembolism is extensively used because such diseases are common as well as serious. Drugs affect haemostasis and thrombosis in three distinct ways, by influencing:

1. blood coagulation (fibrin formation)
2. platelet function
3. fibrin removal (fibrinolysis).

BLOOD COAGULATION

COAGULATION CASCADE

Blood coagulation means the conversion of liquid blood to a gel or clot. The main event is the conversion by thrombin of soluble *fibrinogen* to insoluble strands of *fibrin*, the last step in a complex enzyme cascade. The components (called factors) are present in blood as inactive precursors (zymogens) of proteolytic enzymes and co-factors. They are activated by proteolysis, the active forms being designated by the suffix 'a'. Factors XIIa, XIa, Xa, IXa and thrombin (IIa) are all serine proteases. Activation of a small amount of one factor catalyses the formation of larger amounts of the next factor, which catalyses the formation of still larger amounts of the next, and so on; consequently, the cascade provides a mechanism of amplification.[1] As might be expected, this accelerating enzyme cascade has to be controlled by inhibitors, because otherwise all the blood in the body would solidify within minutes of the initiation of haemostasis. One of the most important inhibitors is *antithrombin III*, which neutralises all the serine proteases in the cascade. Vascular endothelium also actively limits thrombus extension (see below).

Two pathways of fibrin formation were described traditionally (termed 'intrinsic'—because all the components are present in the blood—and 'extrinsic'—because some components come from outside the blood). The intrinsic or 'contact' pathway is activated when shed blood comes into contact with an artificial surface such as glass, but physiologically the system functions as a single *in vivo pathway* (Fig. 24.2). Tissue damage exposes blood to tissue factor,

[1]Coagulation of 100 ml of blood requires 0.2 mg of factor VIII, 2 mg of factor X, 15 mg of prothrombin and 250 mg of fibrinogen.

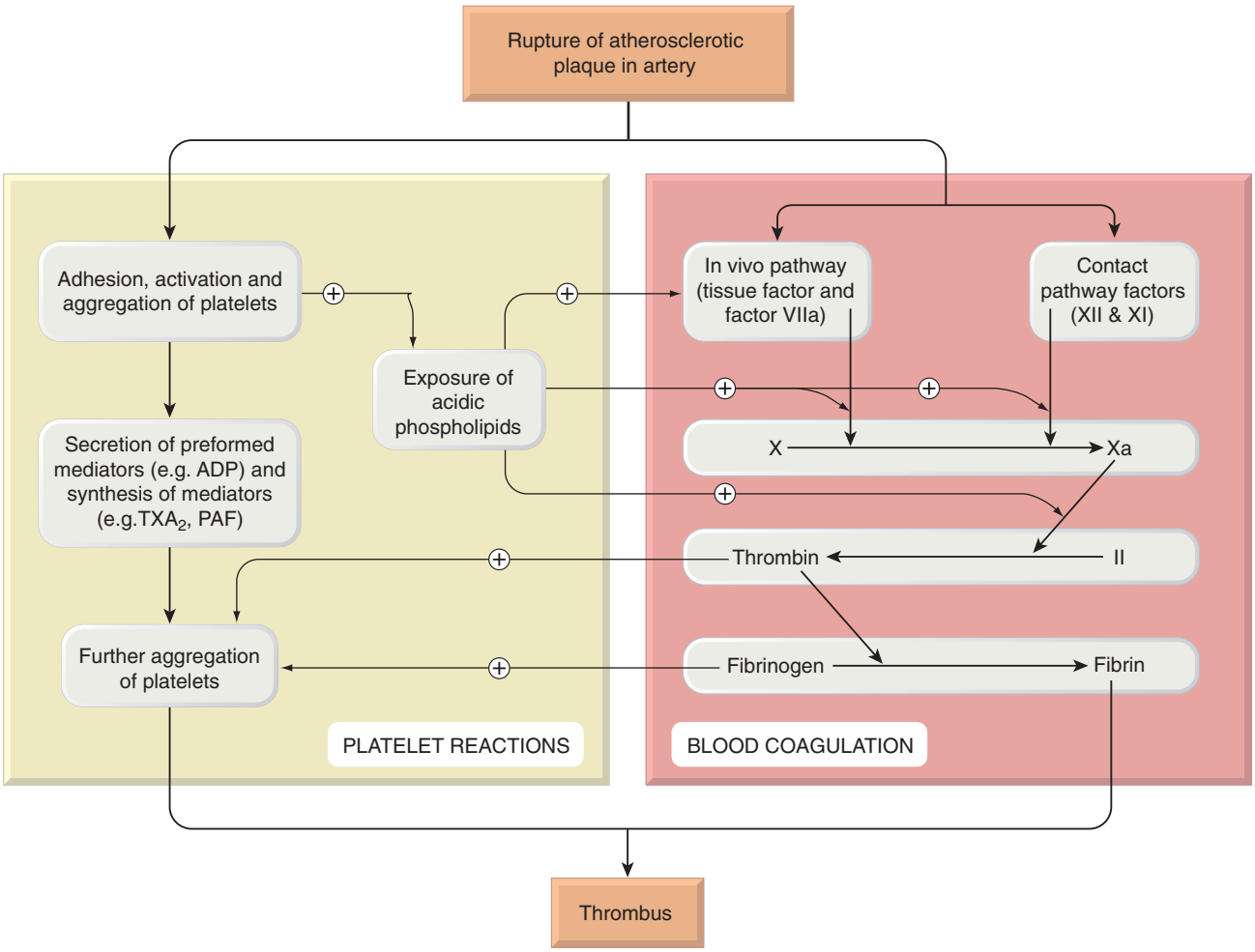

Fig. 24.1 The main events in the formation of an arterial thrombus. Exposure of acidic phospholipids during platelet activation provides a surface on which factors IXa and VIIa interact with factor X; factor Xa then interacts with factor II, as illustrated in more detail in Figure 24.4. Activation of factor XII also initiates the fibrinolytic pathway, which is shown in Figure 24.10. (A similar series of events occurs when there is vascular damage, leading to haemostasis.) PAF, platelet-activating factor; TXA_2, thromboxane A_2.

initiating the process and leading to production of a small amount of thrombin. This acts through several positive feedbacks (on Va, VIIIa and on platelets) that amplify and propagate the process with production of more thrombin.

▼ The in vivo pathway is initiated by 'tissue factor'. This is the cellular receptor for factor VII, which, in the presence of Ca^{2+}, undergoes an active site transition. This results in rapid autocatalytic activation of factor VII to VIIa. The tissue factor–VIIa complex activates factors IX and X. Acidic phospholipids function as *surface catalysts*. They are provided during platelet activation, which exposes acidic phospholipids (especially phosphatidylserine), and these activate various clotting factors, closely juxtaposing them in functional complexes. Platelets also contribute by secreting coagulation factors, including factor Va and fibrinogen. Coagulation is sustained by further generation of factor Xa by IXa–VIIIa–Ca^{2+}–phospholipid complex. This is needed because the tissue factor–VIIa complex is rapidly inactivated in plasma by tissue factor pathway inhibitor and by antithrombin III. Factor Xa, in the presence of Ca^{2+}, phospholipid and factor Va, activates prothrombin to thrombin, the main enzyme of the cascade. The *contact* (intrinsic) pathway commences when factor XII (Hageman factor) adheres to a negatively charged surface and converges with the in vivo pathway at the stage of factor X activation (see Fig. 24.2). The proximal part of this pathway is not crucial for blood coagulation in vivo.[2] The two pathways are not entirely separate even before they converge, and various positive feedbacks promote coagulation.

THE ROLE OF THROMBIN

Thrombin (factor IIa) cleaves fibrinogen, producing fragments that polymerise to form fibrin. It also activates factor XIII, a *fibrinoligase*, which strengthens fibrin-to-fibrin links, thereby stabilising the coagulum. In addition to coagulation, thrombin also causes platelet aggregation, stimulates cell proliferation and modulates smooth muscle contraction. Paradoxically, it can inhibit as well as promote coagulation (see below). Effects of thrombin on platelets and smooth muscle are initiated by interaction with specific protease-activated receptors (PARs; see Ch. 3), which belong to the superfamily of G-protein-coupled receptors. PARs initiate cellular responses that contribute not only to haemostasis and thrombosis, but also to inflammation and

[2]Mr Hageman (the patient deficient in factor XII after whom it was named) died not from excessive bleeding but from a pulmonaory embolism: factor XII deficiency does not give rise to a bleeding disorder.

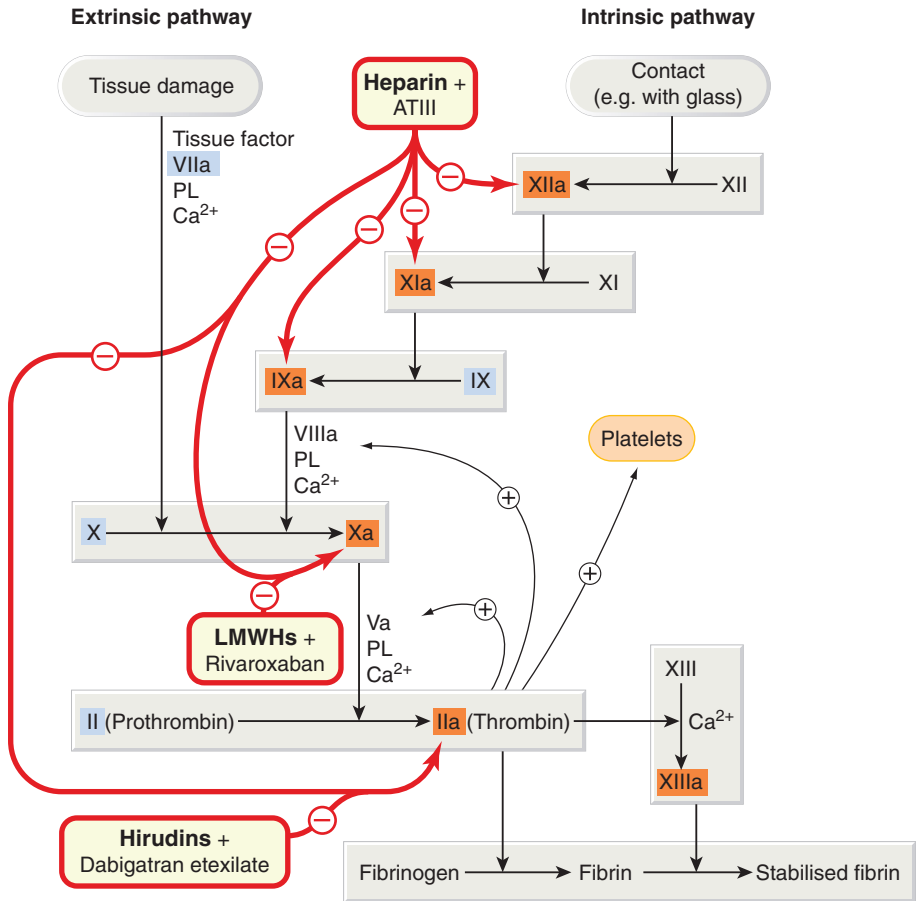

Fig. 24.2 The coagulation cascade: sites of action of anticoagulant drugs. Oral anticoagulants interfere with post-translational γ-carboxylation of factors II, VII, IX and X (shown in blue boxes); see Figure 24.4. Heparins activate antithrombin III. ATIII, antithrombin III; LMWHs, low-molecular-weight heparins; PL, negatively charged phospholipid supplied by activated platelets.

Haemostasis and thrombosis

- Haemostasis is the arrest of blood loss from damaged vessels and is essential to survival. The main phenomena are:
  - platelet adhesion and activation
  - blood coagulation (fibrin formation).
- Thrombosis is a pathological condition resulting from inappropriate activation of haemostatic mechanisms:
  - venous thrombosis is usually associated with stasis of blood; a venous thrombus has a small platelet component and a large component of fibrin
  - arterial thrombosis is usually associated with atherosclerosis, and the thrombus has a large platelet component.
- A portion of a thrombus may break away, travel as an embolus and lodge downstream, causing ischaemia and/or infarction.

perhaps angiogenesis. The signal transduction mechanism is unusual: receptor activation requires proteolysis by thrombin of the extracellular N-terminal domain of the receptor, revealing a new N-terminal sequence that acts as a 'tethered agonist' (see Fig. 3.7).

VASCULAR ENDOTHELIUM IN HAEMOSTASIS AND THROMBOSIS

Vascular endothelium, the container of the circulating blood, can change focally from a non-thrombogenic to a thrombogenic structure in response to different demands. Normally, it provides a non-thrombogenic surface by virtue of surface *heparan sulfate*, a glycosaminoglycan related to heparin, which is, like heparin, a co-factor for antithrombin III. Endothelium thus plays an essential role in preventing intravascular platelet activation and coagulation. However, it also plays an active part in haemostasis, synthesising and storing several key haemostatic components; von Willebrand factor,[3] tissue factor and

[3]Von Willebrand factor is a glycoprotein that is missing in a hereditary haemorrhagic disorder called von Willebrand's disease. It is synthesised by vascular endothelial cells (the presence of immunoreactive von Willebrand factor is an identifying feature of these cells in culture) and is also present in platelets.

plasminogen activator inhibitor (PAI)-1 are particularly important. PAI-1 is secreted in response to *angiotensin IV*, receptors for which are present on endothelial cells, providing a link between the renin–angiotensin system (see Ch. 22) and thrombosis. These prothrombotic factors are involved, respectively, in platelet adhesion and in coagulation and clot stabilisation. However, the endothelium is also implicated in thrombus limitation. Thus it generates prostaglandin (PG) I_2 (prostacyclin; Ch. 17) and nitric oxide (NO; Ch. 20); converts the platelet agonist ADP to adenosine, which inhibits platelet function (Ch. 16); synthesises *tissue plasminogen activator* (tPA; see below); and expresses *thrombomodulin*, a receptor for thrombin. After combination with thrombomodulin, thrombin activates *protein C*, a vitamin K-dependent anticoagulant. Activated protein C, helped by its co-factor protein S, inactivates factors Va and VIIa. This is known to be physiologically important, because a naturally occurring mutation of the gene coding for factor V (factor V Leiden), which confers resistance to activated protein C, results in the commonest recognised form of inherited thrombophilia. A synthetic form of activated protein C, **drotrecogin alpha (activated)**, is licensed for the treatment of severe septic shock with multiple organ failure (Ch. 22).

Endotoxin and cytokines, including tumour necrosis factor, tilt the balance of prothrombotic and antithrombotic endothelial functions towards thrombosis by causing loss of heparan (see above) and expression of tissue factor, and impair endothelial NO function. If other mechanisms limiting coagulation are also faulty or become exhausted, disseminated intravascular coagulation can result. This is a serious complication of sepsis and of certain malignancies, and the main treatment is to correct the underlying disease.

Blood coagulation (fibrin formation)

The clotting system consists of a cascade of proteolytic enzymes and cofactors.

- Inactive precursors are activated in series, each giving rise to more of the next.
- The last enzyme, thrombin, derived from prothrombin (II), converts soluble fibrinogen (I) to an insoluble meshwork of fibrin in which blood cells are trapped, forming the clot.
- There are two limbs in the cascade:
  - the in vivo (extrinsic) pathway
  - the contact (intrinsic) pathway.
- Both pathways result in activation of factor X to Xa, which converts prothrombin to thrombin.
- Calcium ions and a negatively charged phospholipid (PL) are essential for three steps, namely the actions of:
  - factor IXa on X
  - factor VIIa on X
  - factor Xa on II.
- PL is provided by activated platelets adhering to the damaged vessel.
- Some factors promote coagulation by binding to PL and a serine protease factor; for example, factor Va in the activation of II by Xa, or VIIIa in the activation of X by IXa.
- Blood coagulation is controlled by:
  - enzyme inhibitors (e.g. antithrombin III)
  - fibrinolysis.

DRUGS THAT ACT ON THE COAGULATION CASCADE

Drugs are used to modify the cascade either when there is a defect in coagulation or when there is unwanted coagulation.

COAGULATION DEFECTS

Genetically determined deficiencies of clotting factors are not common. Examples are classic haemophilia, caused by lack of factor VIII, and an even rarer form of haemophilia (haemophilia B or Christmas disease) caused by lack of factor IX (also called Christmas factor). Missing factors can be supplied by giving fresh plasma or concentrated preparations of, respectively, factor VIII or factor IX.

Acquired clotting defects are more common than hereditary ones. The causes include liver disease, vitamin K deficiency (universal in neonates) and excessive oral anticoagulant therapy, each of which may require treatment with vitamin K.

VITAMIN K

Vitamin K (for *Koagulation* in German) is a fat-soluble vitamin (Fig. 24.3) occurring naturally in plants (vitamin K_1) and as a series of bacterial menaquinones (vitamin K_2) formed in the gut (see Shearer & Newman, 2008, for a review). It is essential for the formation of clotting factors II, VII, IX and X. These are all glycoproteins with several γ-carboxyglutamic acid (Gla) residues. The interaction of factors Xa and prothrombin (factor II) with Ca^{2+} and phospholipid is shown in Figure 24.4. γ-Carboxylation occurs after the synthesis of the amino acid chain, and the carboxylase enzyme requires reduced vitamin K as a co-factor (Fig. 24.5). Binding does not occur in the absence of γ-carboxylation. Similar considerations apply to the proteolytic activation of factor X by IXa and by VIIa (see Fig. 24.2).

There are several other vitamin K-dependent Gla proteins, including proteins C and S (see above) and osteocalcin in bone: the effect of the vitamin on osteoporosis is under investigation.

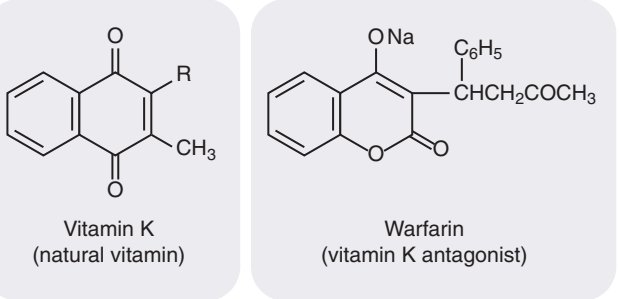

Fig. 24.3 Vitamin K and warfarin. Warfarin, a vitamin K antagonist, is an oral anticoagulant. It competes with vitamin K (note the similarity in their structures) for the reductase enzyme (VKORC1) that activates vitamin K and is the site of its action (see Fig. 24.5).

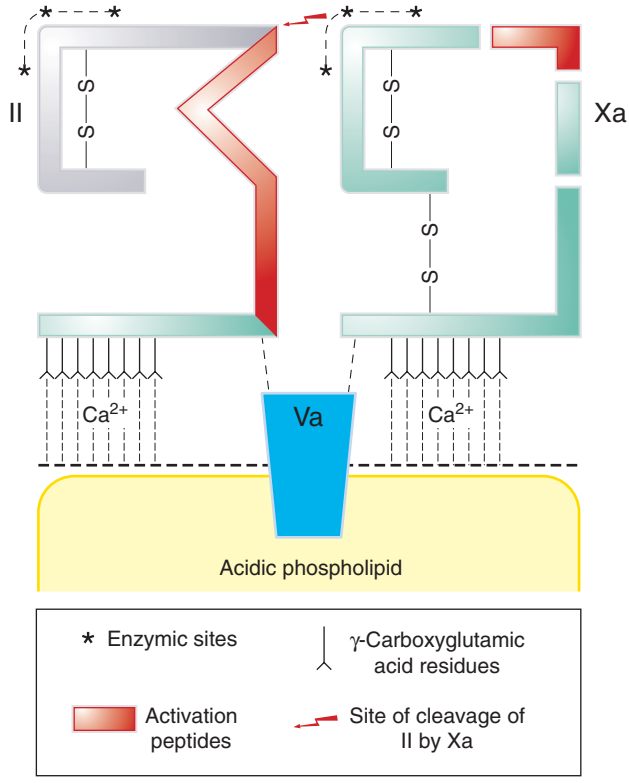

Fig. 24.4 Activation of prothrombin (factor II) by factor Xa. The complex of factor Va with a negatively charged phospholipid surface (supplied by aggregating platelets) forms a binding site for factor Xa and prothrombin (II), which have peptide chains (shown schematically) that are similar to one another. Platelets thus serve as a localising focus. Calcium ions are essential for binding. Xa activates prothrombin, liberating thrombin (shown in grey). (Modified from Jackson C M 1978 Br J Haematol 39: 1.)

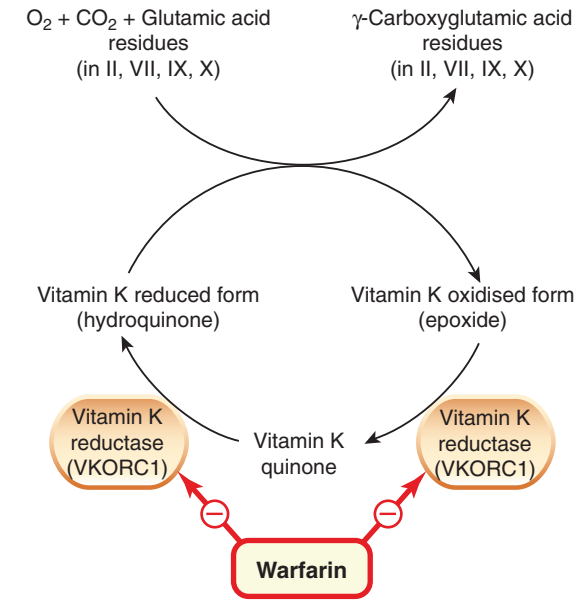

Fig. 24.5 Mechanism of vitamin K and of warfarin. After the peptide chains in clotting factors II, VII, IX and X have been synthesised, reduced vitamin K (the hydroquinone) acts as a co-factor in the conversion of glutamic acid to γ-carboxyglutamic acid. During this reaction, the reduced form of vitamin K is converted to the epoxide, which in turn is reduced to the quinone and then the hydroquinone by vitamin K epoxide reductase component 1 (VKORC1), the site of action of warfarin.

Administration and pharmacokinetic aspects

Natural vitamin K_1 (**phytomenadione**) may be given orally or by injection. If given by mouth, it requires bile salts for absorption, and this occurs by a saturable energy-requiring process in the proximal small intestine. A synthetic preparation, **menadiol sodium phosphate**, is also available. It is water soluble and does not require bile salts for its absorption. This synthetic compound takes longer to act than phytomenadione. There is very little storage of vitamin K in the body. It is metabolised to more polar substances that are excreted in the urine and the bile.

Clinical uses of vitamin K are summarised in the clinical box.

Clinical uses of vitamin K

- Treatment and/or prevention of bleeding:
  - from excessive oral anticoagulation (e.g. by **warfarin**)
  - in babies: to prevent *haemorrhagic disease of the newborn*.
- For vitamin K deficiencies in adults:
  - *sprue*, *coeliac disease*, *steatorrhoea*
  - lack of bile (e.g. with *obstructive jaundice*).

THROMBOSIS

Thrombotic and thromboembolic disease is common and has severe consequences, including myocardial infarction, stroke, deep vein thrombosis and pulmonary embolus. The main drugs used for platelet-rich 'white' thrombi are the antiplatelet drugs and fibrinolytic drugs, which are considered below. The main drugs used to prevent or treat 'red' thrombus are:

- injectable anticoagulants (**heparin** and newer thrombin inhibitors)
- oral anticoagulants (**warfarin** and related compounds; orally active thrombin inhibitors).

Heparins and direct thrombin inhibitors act immediately, whereas warfarin and other vitamin K antagonists take several days to exert their effect. Consequently, if warfarin is used to treat patients with venous thrombosis, an agent that acts immediately is also administered until the effect of warfarin has become established.

HEPARIN (INCLUDING LOW-MOLECULAR-WEIGHT HEPARINS)

Heparin was discovered in 1916 by a second-year medical student at Johns Hopkins Hospital. He was attempting to extract thromboplastic (i.e. coagulant) substances from various tissues during a vacation project, but found instead

a powerful anticoagulant activity.[4] This was named heparin, because it was first extracted from liver.

Heparin is not a single substance but a family of sulfated glycosaminoglycans (mucopolysaccharides). It is present together with histamine in the granules of mast cells. Commercial preparations are extracted from beef lung or hog intestine and, because preparations differ in potency, assayed biologically against an agreed international standard: doses are specified in units of activity rather than of mass.

Heparin fragments (e.g. **enoxaparin**, **dalteparin**) or a synthetic pentasaccharide (**fondaparinux**), referred to as low-molecular-weight heparins (LMWHs), are often used in place of unfractionated heparin, which is reserved for special situations such as patients with renal failure in whom LMWHs are contraindicated (see below).

Mechanism of action

Heparin inhibits coagulation, both in vivo and in vitro, by activating antithrombin III (see above). Antithrombin III inhibits thrombin and other serine proteases by binding to the active serine site. Heparin modifies this interaction by binding, via a unique pentasaccharide sequence, to antithrombin III, changing its conformation and increasing its affinity for serine proteases.

Thrombin is considerably more sensitive to the inhibitory effect of the heparin–antithrombin III complex than is factor Xa. To inhibit thrombin, it is necessary for heparin to bind to the enzyme as well as to antithrombin III; to inhibit factor Xa, it is necessary only for heparin to bind to antithrombin III (Fig. 24.6). Antithrombin III deficiency is very rare but can cause thrombophilia and resistance to heparin therapy.

The LMWHs increase the action of antithrombin III on factor Xa but not its action on thrombin, because the molecules are too small to bind to both enzyme and inhibitor, essential for inhibition of thrombin but not for that of factor Xa (Fig. 24.6).

Administration and pharmacokinetic aspects

Heparin is not absorbed from the gut because of its charge and high molecular weight, and it is therefore given intravenously or subcutaneously (intramuscular injections would cause haematomas).

▼ After intravenous injection of a bolus dose, there is a phase of rapid elimination followed by a more gradual disappearance owing both to saturable processes (involving binding to sites on endothelial cells and macrophages) and to slower non-saturable processes including renal excretion. As a result, once the dose exceeds the saturating concentration, a greater proportion is dealt with by these slower processes, and the apparent half-life increases with increasing dose (saturation kinetics; see Ch. 10).

Heparin acts immediately following intravenous administration, but the onset is delayed by up to 60 min when it is given subcutaneously. The elimination half-life is approximately 40–90 min. In urgent situations, it is therefore usual to start treatment with a bolus intravenous dose, followed by a constant-rate infusion. The *activated partial thromboplastin time* (APTT), or some other in vitro clotting test, is measured and the dose of heparin adjusted to achieve a value within a target range (e.g. 1.5–2.5 times control).

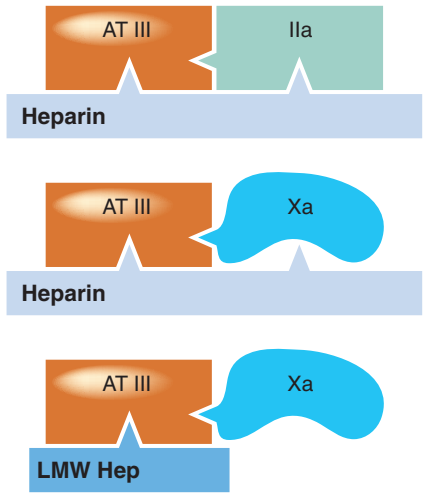

Fig. 24.6 Action of heparins. The schematic shows interactions of heparins, antithrombin III (AT III) and clotting factors. To increase the inactivation of thrombin (IIa) by AT III, heparin needs to interact with both substances (top), but to speed up its effect on factor Xa it need only interact with AT III (middle). Low-molecular-weight heparins (LMW Hep) increase the action of AT III on factor Xa (bottom), but cannot increase the action of AT III on thrombin because they cannot bind both simultaneously. (Modified from Hirsh J, Levine M 1992 Blood 79: 1–17.)

Low-molecular-weight heparins are given subcutaneously. They have a longer elimination half-life than unfractionated heparin, and this is independent of dose (first-order kinetics), so the effects are more predictable and dosing less frequent (once or twice a day). LMWHs do not prolong the APTT. Unlike unfractionated heparin, the effect of a standard dose is sufficiently predictable that monitoring is not required routinely. LMWHs are eliminated mainly by renal excretion, and unfractionated heparin is preferred in renal failure, but with this exception LMWHs are at least as safe and effective as unfractionated heparin and are more convenient to use, because patients can be taught to inject themselves at home and there is generally no need for blood tests and dose adjustment.

Unwanted effects

Haemorrhage. The main hazard is haemorrhage, which is treated by stopping therapy and, if necessary, giving **protamine sulfate.** This heparin antagonist is a strongly basic protein that forms an inactive complex with heparin; it is given intravenously. The dose is estimated from the dose of heparin that has been administered recently, and it is important not to give too much, as this can itself cause bleeding. If necessary, an in vitro neutralisation test is performed on a sample of blood from the patient to provide a more precise indication of the required dose.

Thrombosis. This is an uncommon but serious adverse effect of heparin and, as with warfarin necrosis (see below), may be misattributed to the natural history of the disease for which heparin is being administered.

▼ Paradoxically, it is associated with *heparin-induced thrombocytopenia* (HIT). A transitory early decrease in platelet numbers is not uncommon after initiating heparin treatment, and is not clinically important.

[4]This kind of good fortune also favoured Vane and his colleagues in their discovery of PGI_2 (Ch. 17), where they were looking for one kind of biological activity and found another. More specific chemical assays (Ch. 7), for all their strengths, cannot throw up this kind of unexpected discovery.

More serious thrombocytopenia occurring 2–14 days after the start of therapy is uncommon and is referred to as type II HIT. This is caused by IgM or IgG antibodies against complexes of heparin and platelet factor 4. Circulating immune complexes bind to Fc receptors (see Ch. 6) on circulating platelets, thereby activating them and releasing more platelet factor 4 and causing thrombocytopenia. Antibody also binds to platelet factor 4 complexed with glycosaminoglycans on the surface of endothelial cells, leading to immune injury of the vessel wall, thrombosis and disseminated intravascular coagulation. LMWHs are less liable than standard heparin to activate platelets to release platelet factor 4, and they bind less avidly to platelet factor 4. LMWHs are less likely than unfractionated heparin to cause thrombocytopenia and thrombosis by this mechanism. HIT is usually treated with either **danaparoid** or with a direct thrombin inhibitor (see below). Danaparoid is a low-molecular-weight heparinoid consisting of a mixture of heparan, dermatan and chondroitin sulfates, with well-established antithrombotic activity.

Osteoporosis with spontaneous fractures has been reported with long-term (6 months or more) treatment with heparin (usually during pregnancy, when warfarin is contraindicated or problematic—see below). Its explanation is unknown.

Hypoaldosteronism (with consequent hyperkalaemia) is uncommon, but increases with prolonged treatment. It is recommended to check plasma K^+ concentration if treatment is to be continued for > 7 days.

Hypersensitivity reactions are rare with heparin but more common with protamine. (Protamine sensitivity also occurs in patients treated with protamine zinc insulin; Ch. 30. Protamine is extracted from fish roe, and sensitivity to protamine occurs in some people with fish allergy.)

DIRECT THROMBIN INHIBITORS AND RELATED DRUGS

Hirudins are direct thrombin inhibitors derived from the anticoagulant present in saliva from the medicinal leech. Unlike the heparins they do not depend on activation of antithrombin. **Lepirudin** is used clinically. It is a polypeptide related to hirudin that binds irreversibly both to the fibrin-binding and catalytic sites on thrombin and is used for thromboembolic disease in patients with type II HIT. It is administered intravenously, the dose being adjusted depending on the APTT, and can cause bleeding or hypersensitivity reactions (rash or fever). **Bivalirudin**, another hirudin analogue, is used by cardiologists in selected patients undergoing percutaneous coronary interventions. Treatment is initiated with an intravenous bolus followed by an infusion during and up to 4 h after the procedure. It can cause bleeding and hypersensitivity reactions.

Orally active inhibitors. This field has had more than one false dawn, but hope springs eternal as thrombin inhibitors could replace warfarin, a venerable but inconvenient drug that is a common cause of serious adverse effects (see below). **Dabigatran** is a synthetic serine protease inhibitor; **dabigatran etexilate**, a prodrug with a hydrophobic tail, is orally active as a direct thrombin inhibitor and is licensed for prevention of venous thromboembolism following hip or knee replacement. It works rapidly and is administered shortly after surgery and then once daily for up to a month. **Rivaroxaban**, also a synthetic inhibitor, is selective for factor Xa rather than for thrombin, but similar to dabigatran in other respects These drugs are administered in standard doses without laboratory monitoring of their anticoagulant effects. The commonest adverse effects of both drugs are predictable (bleeding, anaemia); rivaroxaban also commonly causes nausea. Other indications for both drugs are being investigated, and if they prove safe and effective for a range of indications, this could transform the clinical management of the large group of patients currently maintained on warfarin (see the clinical box on the clinical use of anticoagulants).

▼ Various other approaches are being explored. These include several naturally occurring anticoagulants (tissue factor pathway inhibitor, thrombomodulin and protein C) synthesised by recombinant technology. A particularly ingenious approach is the development of thrombin agonists that are selective for the anticoagulant properties of thrombin. One such modified thrombin, differing by a single amino acid substitution, has substrate specificity for protein C. It produces anticoagulation in monkeys without prolonging bleeding times, suggesting that it may be less likely than standard anticoagulants to cause bleeding (Bah et al., 2009).

Clinical uses of anticoagulants

Heparin (often as low-molecular-weight heparin) is used acutely. **Warfarin** or a direct thrombin or Xa inhibitor is used for prolonged therapy. Anticoagulants are used to prevent:

- deep vein thrombosis (e.g. perioperatively)
- extension of established deep vein thrombosis
- pulmonary embolus
- thrombosis and embolisation in patients with atrial fibrillation (Ch. 21)
- thrombosis on prosthetic heart valves
- clotting in extracorporeal circulations (e.g. during haemodialysis)
- myocardial infarction in patients with unstable angina.

VITAMIN K ANTAGONISTS: WARFARIN

▼ Oral anticoagulants were discovered as an indirect result of a change in agricultural policy in North America in the 1920s. Sweet clover was substituted for corn in cattle feed, and an epidemic of deaths of cattle from haemorrhage ensued. This turned out to be caused by bishydroxycoumarin in spoiled sweet clover, and it led to the discovery of warfarin (named for the Wisconsin Alumni Research Foundation). One of the first uses to which this was put was as a rat poison, but for more than 50 years it has been the standard anticoagulant for the treatment and prevention of thromboembolic disease.

Warfarin (Fig. 24.3) is the most important oral anticoagulant; alternatives with a similar mechanism of action, for example **phenindione**, are now used only in rare patients who experience idiosyncratic adverse reactions to warfarin. Warfarin and other vitamin K antagonists require frequent blood tests to individualise dose, and are consequently inconvenient as well as having a low margin of safety.

Mechanism of action

Vitamin K antagonists act only in vivo and have no effect on clotting if added to blood in vitro. They interfere with the post-translational γ-carboxylation of glutamic acid residues in clotting factors II, VII, IX and X. They do this by inhibiting *vitamin K epoxide reductase component 1* (VKORC1), thus inhibiting the reduction of vitamin K epoxide to its active hydroquinone form (Fig. 24.5). Inhibition is competitive (reflecting the structural similarity between warfarin and vitamin K; Fig. 24.3). The *VKORC1* gene is polymorphic (see Ch. 11), and different haplotypes have different affinities for warfarin. Genotyping to determine the haplotype, combined with genotyping *CYP2C9* (see below), while not yet routine, can reduce the variability in response

to warfarin by around one-third. The effect of warfarin takes several days to develop because of the time taken for degradation of preformed carboxylated clotting factors. Onset of action thus depends on the elimination half-lives of the relevant factors. Factor VII, with a half-life of 6 h, is affected first, then IX, X and II, with half-lives of 24, 40 and 60 h, respectively.

Administration and pharmacokinetic aspects

Warfarin is absorbed rapidly and completely from the gut after oral administration. It has a small distribution volume, being strongly bound to plasma albumin (see Ch. 8). The peak concentration in the blood occurs within an hour of ingestion, but because of the mechanism of action this does not coincide with the peak pharmacological effect, which occurs about 48 h later. The effect on prothrombin time (PT, see below) of a single dose starts after approximately 12–16 h and lasts 4–5 days. Warfarin is metabolised by CYP2C9, which is polymorphic (see Ch. 11). Partly in consequence of this, its half-life is very variable, being of the order of 40 h in many individuals.

Warfarin crosses the placenta and is not given in the first months of pregnancy because it is teratogenic (see below), nor in the later stages because it can cause intracranial haemorrhage in the baby during delivery. It appears in milk during lactation. This could theoretically be important because newborn infants are naturally deficient in vitamin K. However, infants are routinely prescribed vitamin K to prevent haemorrhagic disease, so warfarin treatment of the mother does not generally pose a risk to the breastfed infant.

The therapeutic use of warfarin requires a careful balance between giving too little, leaving unwanted coagulation unchecked, and giving too much, thereby causing haemorrhage. Therapy is complicated not only because the effect of each dose is maximal some 2 days after its administration, but also because numerous medical and environmental conditions modify sensitivity to warfarin, including interactions with other drugs (see Ch. 56). The effect of warfarin is monitored by measuring PT, which is expressed as an *international normalised ratio* (INR).

▼ The PT is the time taken for clotting of citrated plasma after the addition of Ca^{2+} and standardised reference thromboplastin; it is expressed as the ratio (PT ratio) of the PT of the patient to the PT of a pool of plasma from healthy subjects on no medication. Because of the variability of thromboplastins, different results are obtained in different laboratories. To standardise PT measurements internationally, each thromboplastin is assigned an international sensitivity index (ISI), and the patient's PT is expressed as an INR, where INR = $(\text{PT ratio})^{ISI}$. This kind of normalisation procedure shocks purists but provides similar results when a patient moves from, say, Birmingham to Baltimore, permitting warfarin dose adjustment independent of laboratory. Pragmatic haematologists argue that the proof of the pudding is in the eating!

The dose of warfarin is usually adjusted to give an INR of 2–4, the precise target depending on the clinical situation. The duration of treatment also varies, but for several indications (e.g. to prevent thromboembolism in chronic atrial fibrillation), treatment is long term, with the logistical challenge of providing a worldwide network of anticoagulant clinics and demands on the patient in terms of repeat visits and blood tests.

FACTORS THAT POTENTIATE ORAL ANTICOAGULANTS

Various diseases and drugs potentiate warfarin, increasing the risk of haemorrhage.

Disease

Liver disease interferes with the synthesis of clotting factors; conditions in which there is a high metabolic rate, such as fever and thyrotoxicosis, increase the effect of anticoagulants by increasing degradation of clotting factors.

Drugs (see also Chs 9 and 56)

Many drugs potentiate warfarin.

Agents that inhibit hepatic drug metabolism. Examples include **co-trimoxazole**, **ciprofloxacin**, **metronidazole**, **amiodarone** and many antifungal azoles. Stereoselective effects (warfarin is a racemate, and its isomers are metabolised differently from one another) are described in Chapter 56.

Drugs that inhibit platelet function. **Aspirin** increases the risk of bleeding if given during warfarin therapy, although this combination can be used safely with careful monitoring. Other non-steroidal anti-inflammatory drugs (NSAIDs) also increase the risk of bleeding, partly by their effect on platelet thromboxane synthesis (Ch. 26) and, in the case of some NSAIDs, also by inhibiting warfarin metabolism as above. Some antibiotics, including **moxalactam** and **carbenicillin**, inhibit platelet function.

Drugs that displace warfarin from binding sites on plasma albumin. Some of the NSAIDs and **chloral hydrate** cause a transient increase in the concentration of free warfarin in plasma by competing with it for binding to plasma albumin. This mechanism seldom causes clinically important effects, unless accompanied by inhibition of warfarin metabolism, as with **phenylbutazone** (Ch. 56).

Drugs that inhibit reduction of vitamin K. Such drugs include the *cephalosporins*.

Drugs that decrease the availability of vitamin K. Broad-spectrum antibiotics and some *sulfonamides* (see Ch. 49) depress the intestinal flora that normally synthesise vitamin K_2 (see above); this has little effect unless there is concurrent dietary deficiency.

FACTORS THAT LESSEN THE EFFECT OF ORAL ANTICOAGULANTS

Physiological state/disease

There is a decreased response to warfarin in conditions (e.g. pregnancy) where there is increased coagulation factor synthesis. Similarly, the effect of oral anticoagulants is lessened in hypothyroidism, which is associated with reduced degradation of coagulation factors.

Drugs (see also Chs 9 and 56)

Several drugs reduce the effectiveness of warfarin; this leads to increased doses being used to achieve the target INR. Furthermore, the dose of warfarin must be reduced when the interacting drug is discontinued, to avoid haemorrhage.

Vitamin K. This vitamin is a component of some parenteral feeds and vitamin preparations.

Drugs that induce hepatic P450 enzymes. Enzyme induction (e.g. by **rifampicin**, **carbamazepine**) increases the rate of degradation of warfarin. Induction may wane only slowly after the inducing drug is discontinued, making it difficult to adjust the warfarin dose appropriately.

Drugs that reduce absorption. Drugs that bind warfarin in the gut, for example **colestyramine**, reduce its absorption.

Drugs affecting blood coagulation

Procoagulant drugs: vitamin K

- Reduced vitamin K is a co-factor in the post-translational γ-carboxylation of glutamic acid (Glu) residues in factors II, VII, IX and X. The γ-carboxylated glutamic acid (Gla) residues are essential for the interaction of these factors with Ca^{2}+ and negatively charged phospholipid.

Injectable anticoagulants (e.g. heparin, low-molecular-weight heparins)

- Potentiate antithrombin III, a natural inhibitor that inactivates Xa and thrombin.
- Act both in vivo and in vitro.
- Anticoagulant activity results from a unique pentasaccharide sequence with high affinity for antithrombin III.
- Heparin therapy is monitored via activated partial thromboplastin time (APTT), and dose individualised. Unfractionated heparin (UFH) is used for patients with impaired renal function.
- Low-molecular-weight heparins (LMWHs) have the same effect on factor X as heparin but less effect on thrombin; therapeutic efficacy is similar to heparin but monitoring and dose individualisation are not needed. Patients can administer them subcutaneously at home. They are preferred over UFH except for patients with impaired renal function.

Oral anticoagulants (e.g. warfarin)

- Act on vitamin K epoxide reductase component 1 (VKORC1) to inhibit the reduction of vitamin K epoxide, thus inhibiting the γ-carboxylation of Glu in II, VII, IX and X.
- Act only in vivo, and their effect is delayed until preformed clotting factors are depleted.
- Many factors modify their action; genetic factors (polymorphisms of CYP2C6 and VKORC1) and drug interactions are especially important.
- There is wide variation in response; their effect is monitored by measuring the international normalised ratio (INR) and the dose individualised accordingly.
- Orally active direct thrombin inhibitors (e.g. dabigatran etexilate) or factor Xa inhibitors (e.g. rivaroxaban) are used increasingly and do not require monitoring/dose individualisation.

UNWANTED EFFECTS OF ORAL ANTICOAGULANTS

Haemorrhage (especially into the bowel or the brain) is the main hazard. Depending on the urgency of the situation, treatment may consist of withholding warfarin (for minor problems), administration of vitamin K, or fresh plasma or coagulation factor concentrates (for life-threatening bleeding).

Oral anticoagulants are *teratogenic*, causing disordered bone development which is believed to be related to binding to the vitamin K-dependent protein osteocalcin.

Hepatotoxicity occurs but is uncommon.

Necrosis of soft tissues (e.g. breast or buttock) owing to thrombosis in venules occurs shortly after starting treatment and is attributed to inhibition of biosynthesis of protein C, which has a shorter elimination half-life than do the vitamin K-dependent coagulation factors; this results in a procoagulant state soon after starting treatment. This is a rare but serious adverse effect. Treatment with a heparin is usually started at the same time as warfarin, avoiding this problem except in individuals experiencing HIT as an adverse effect of heparin (see above).

The clinical use of anticoagulants is summarised in the box.

PLATELET ADHESION AND ACTIVATION

Platelets maintain the integrity of the circulation: a low platelet count results in *thrombocytopenic purpura*.[5]

When platelets are activated, they undergo a sequence of reactions that are essential for haemostasis, important for the healing of damaged blood vessels, and play a part in inflammation (see Ch. 17). These reactions, several of which are redundant (in the sense that if one pathway of activation is blocked another is available) and several autocatalytic, include:

- *adhesion* following vascular damage (via von Willebrand factor bridging between subendothelial macromolecules and glycoprotein [GP] Ib receptors on the platelet surface)[6]
- *shape change* (from smooth discs to spiny spheres with protruding pseudopodia)
- *secretion* of the granule contents (including platelet agonists, such as ADP and 5-hydroxytryptamine, and coagulation factors and growth factors, such as platelet-derived growth factor)
- *biosynthesis of labile mediators* such as platelet-activating factor and thromboxane $(TX)A_2$ (see Ch. 17 and Fig. 24.7)
- *aggregation*, which is promoted by various agonists, including collagen, thrombin, ADP, 5-hydroxytryptamine and TXA_2, acting on specific receptors on the platelet surface; activation by agonists leads to expression of GPIIb/IIIa receptors that bind fibrinogen, which links adjacent platelets to form aggregates
- *exposure of acidic phospholipid* on the platelet surface, promoting thrombin formation (and hence further platelet activation via thrombin receptors and fibrin formation via cleavage of fibrinogen; see above).

These processes are essential for haemostasis but may be inappropriately triggered if the artery wall is diseased, most commonly with atherosclerosis, resulting in thrombosis (Fig. 24.7).

ANTIPLATELET DRUGS

Platelets play such a critical role in thromboembolic disease that it is no surprise that antiplatelet drugs are of great therapeutic value. Clinical trials of **aspirin** radically altered clinical practice, and more recently drugs that block ADP receptors and GPIIb/IIIa have also been found to be therapeutically useful. Sites of action of antiplatelet drugs are shown in Figure 24.7.

[5]Purpura means a purple rash caused by multiple spontaneous bleeding points in the skin. When this is caused by reduced circulating platelets, bleeding can occur into other organs, including the gut and brain.

[6]Various platelet membrane glycoproteins act as receptors or binding sites for adhesive proteins such as von Willebrand factor or fibrinogen.

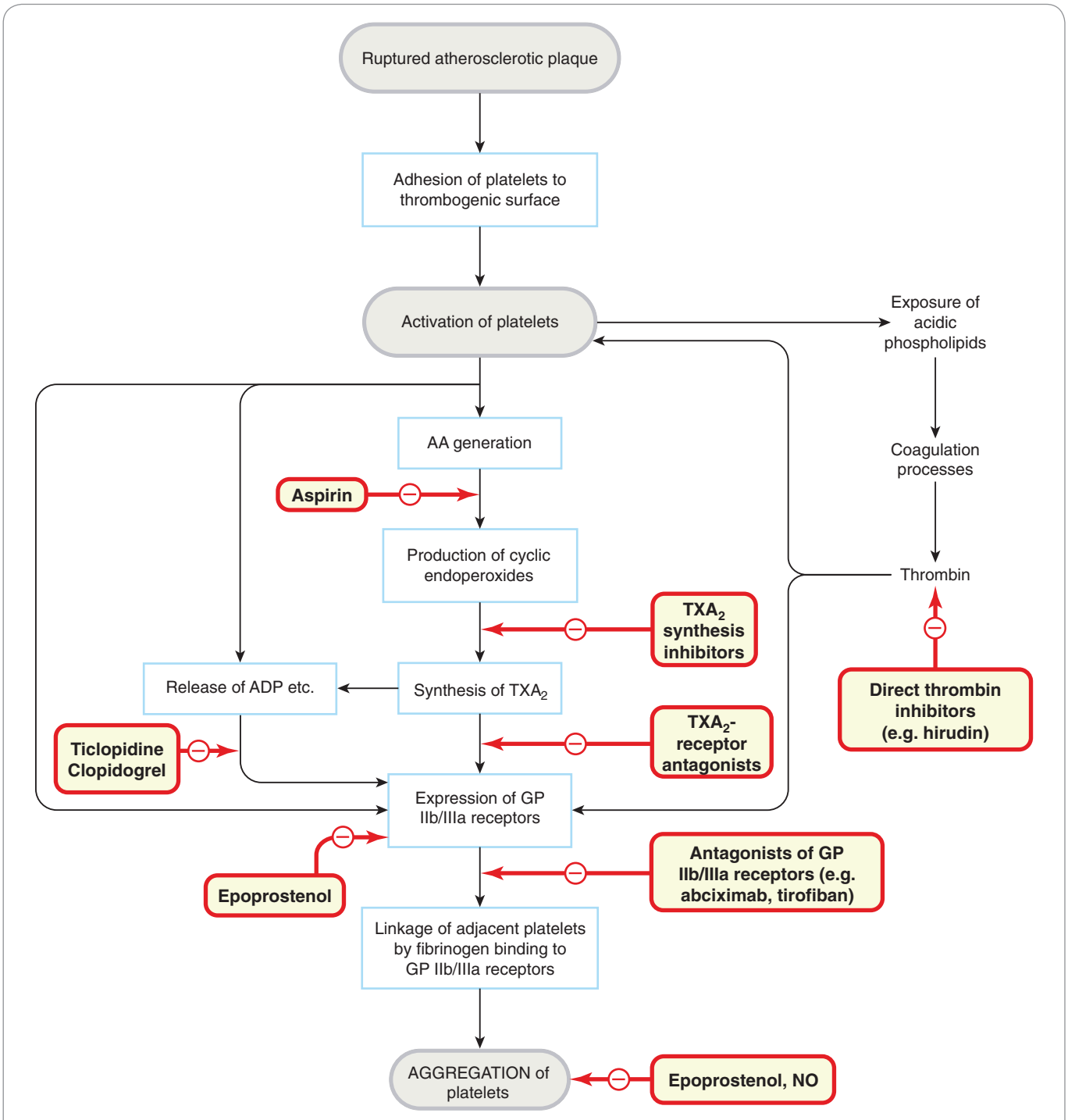

Fig. 24.7 Platelet activation. Events involved in platelet adhesion and aggregation are shown, with the sites of action of drugs and endogenous mediators. AA, arachidonic acid; ADP, adenosine bisphosphate; GP, glycoprotein; NO, nitric oxide; TXA_2, thromboxane A_2.

ASPIRIN

Low-dose **aspirin** (see Ch. 26) profoundly (> 95%) inhibits platelet TXA_2 synthesis, by irreversible acetylation of a serine residue in the active site of cyclo-oxygenase I (COX-I). Oral administration is relatively selective for platelets because of presystemic elimination (Ch. 9). Unlike nucleated cells, platelets cannot synthesise proteins, so after administration of aspirin, TXA_2 synthesis does not recover until the affected cohort of platelets is replaced in 7–10 days. Clinical trials have demonstrated the efficacy of aspirin in several clinical settings (e.g. Fig. 24.8). Adverse effects of aspirin, mainly on the gastrointestinal tract, are, however, clearly dose related, so a low dose (often 75 mg once daily) is usually recommended for thromboprophylaxis. Thromboprophylaxis is reserved for people at high cardiovascular risk (e.g. survivors of myocardial

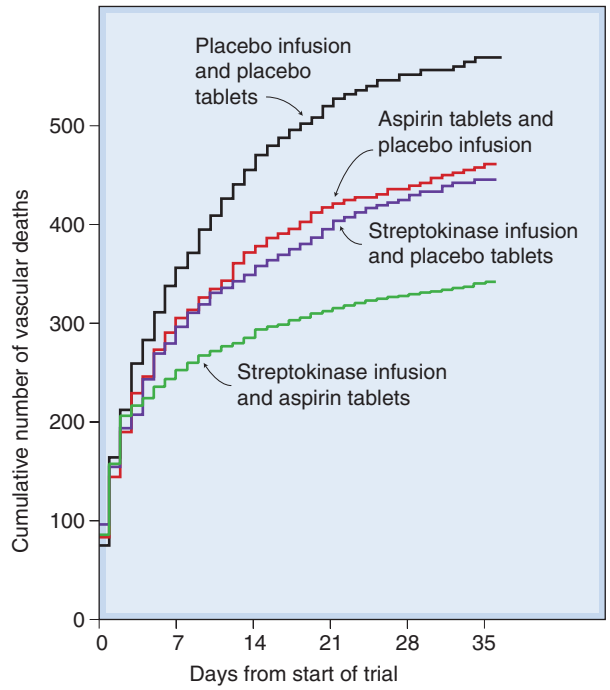

Fig. 24.8 Efficacy of aspirin and streptokinase for myocardial infarction. The curves show cumulative vascular mortality in patients treated with placebo, aspirin alone, streptokinase alone or a combined aspirin–streptokinase regimen. (ISIS-2 trial 1988 Lancet ii: 350–360.)

Platelet function

- Healthy vascular endothelium prevents platelet adhesion.
- Platelets adhere to diseased or damaged areas and become activated, i.e. they change shape, exposing negatively charged phospholipids and glycoprotein (GP) IIb/IIIa receptors, and synthesise and/or release various mediators, for example thromboxane A_2 and ADP, which activate other platelets, causing aggregation.
- Aggregation entails fibrinogen binding to and bridging between GPIIb/IIIa receptors on adjacent platelets.
- Activated platelets constitute a focus for fibrin formation.
- Chemotactic factors and growth factors necessary for repair, but also implicated in atherogenesis, are released during platelet activation.

infarction), in whom the cardiovascular benefit of aspirin usually outweighs the risk of gastrointestinal bleeding.

▼ Treatment failure can occur despite taking aspirin, and there is current interest in the possibility that some patients exhibit a syndrome of 'aspirin resistance', although the mechanism and possible importance of this remains controversial (see Goodman et al., 2008). Other non-steroidal drugs which inhibit platelet TXA_2 synthesis > 95% (e.g. **sulfinpyrazone**, for which there is also supportive clinical trial evidence) may have antithrombotic effects, but where inhibition of platelet TXA_2 synthesis does not reach this threshold there is evidence that such drugs are *proaggregatory*, related to inhibition of COX-2, possibly due to inhibition of antiaggregatory PGI_2.

DIPYRIDAMOLE

Dipyridamole inhibits platelet aggregation by several mechanisms, including inhibition of phosphodiesterase, block of adenosine uptake into red cells (see Ch. 16) and inhibition of TXA_2 synthesis (see Ch. 26). Clinical effectiveness has been uncertain, but one study showed that a modified-release form of dipyridamole reduced the risk of stroke and death in patients with transient ischaemic attacks by around 15%—similar to aspirin (25 mg twice daily).[7] The beneficial effects of aspirin and dipyridamole were additive. The main side effects of dipyridamole are dizziness, headache and gastrointestinal disturbances; unlike aspirin, it does not increase the risk of bleeding.

ADENOSINE (P2Y) RECEPTOR ANTAGONISTS (THIENOPYRIDINES)

Ticlopidine was the first to be introduced, but causes neutropenia and thrombocytopenia and is now little used. The main agent is currently **clopidogrel**; **prasugrel** has been introduced recently.

These drugs inhibit ADP-induced platelet aggregation by irreversible inhibition of $P2Y_{12}$ receptors (Ch. 16) to which they link via a disulfide bond.

Pharmacokinetics and unwanted effects

Clopidogrel is well absorbed when administered by mouth, and in urgent situations is given orally as a loading dose of 300 mg followed by maintenance dosing of 75 mg once daily. It is a prodrug and is converted into its active sulfhydryl metabolite by CYP enzymes in the liver including CYP2C19. Patients with variant alleles of *CYP2C19* (poor metabolisers) are at increased risk of therapeutic failure. There is a potential for interaction with other drugs, such as **omeprazole** (Ch. 29), that are metabolised by CYP2C19 and current labelling recommends against use with proton pump inhibitors for this reason.

Clopidogrel can cause dyspepsia, rash or diarrhoea. The serious blood dyscrasias caused by ticlopidine are very rare with clopidogrel.

Clinical use

Clopidogrel was slightly more effective than aspirin in reducing a composite outcome of ischaemic stroke, myocardial infarction or vascular death in one large trial; it can be used instead of aspirin in patients with symptomatic atheromatous disease, but, because of cost, is usually reserved for patients who are intolerant of aspirin. Clinical trials of adding clopidogrel to aspirin in patients with acute coronary syndromes (see Fig. 24.9) and (in a megatrial of over 45 000 patients) in patients with acute myocardial infarction (COMMIT Collaborative Group, 2005) demonstrated that combined treatment reduces mortality. Treatment with clopidogrel for this indication is given for 4 weeks. Prasugrel is more effective than clopidogrel in acute coronary syndromes, but more often causes serious bleeding (Wiviott et al., 2007). Pretreatment with

[7]This dose regimen of aspirin is unconventional, being somewhat lower than the 75 mg once daily commonly used in thromboprophylaxis.

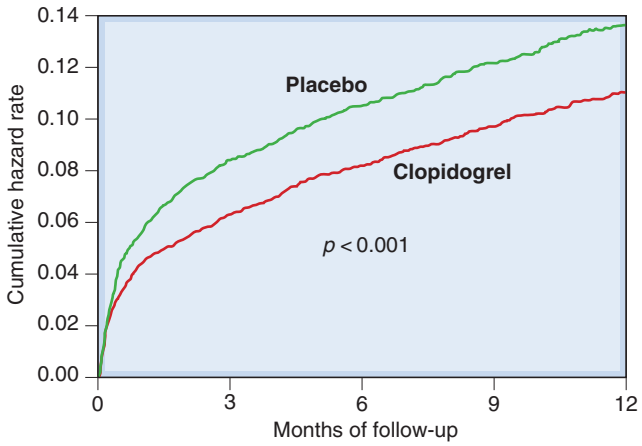

Fig. 24.9 Effect of adding clopidogrel to aspirin. The curves show cumulative hazard rates for major vascular events in patients with acute coronary syndromes treated either with placebo + aspirin or clopidogrel + aspirin. (Modified from CURE Investigators 2001 N Engl J Med 345: 494–502.)

clopidogrel and aspirin followed by longer-term therapy is also effective in patients with ischaemic heart disease undergoing percutaneous coronary interventions.

GLYCOPROTEIN IIB/IIIA RECEPTOR ANTAGONISTS

Antagonists of the GPIIb/IIIa receptor have the theoretical attraction that they inhibit all pathways of platelet activation (because these all converge on activation of GPIIb/IIIa receptors). A hybrid murine–human monoclonal antibody Fab fragment directed against the GPIIb/IIIa receptor, which rejoices in the catchy little name of **abciximab**,[8] is licensed for use in high-risk patients undergoing coronary angioplasty, as an adjunct to **heparin** and **aspirin**. It reduces the risk of restenosis at the expense of an increased risk of bleeding. Immunogenicity limits its use to a single administration.

Tirofiban is a synthetic non-peptide and **eptifibatide** is a cyclic peptide based on the Arg–Gly–Asp ('RGD') sequence that is common to ligands for GPIIb/IIIa receptors. Neither is absorbed if administered by mouth. Given intravenously as an adjunct to aspirin and a heparin preparation, they reduce early events in acute coronary syndrome, but long-term oral therapy with GPIIb/IIIa receptor antagonists is not effective and may be harmful. Unsurprisingly, they increase the risk of bleeding.

OTHER ANTIPLATELET DRUGS

Epoprostenol (PGI_2), an agonist at prostanoid IP receptors (see Ch. 17), causes vasodilatation as well as inhibiting platelet aggregation. It is added to blood entering the dialysis circuit in order to prevent thrombosis during haemodialysis, especially in patients in whom heparin is contraindicated. It is also used in severe pulmonary hypertension (Ch. 22) and circulatory shock. It is unstable under physiological conditions and has a half-life of around 3 min, so it is administered by an intravenous infusion pump. Adverse effects related to its vasodilator action include flushing, headache and hypotension.

The clinical use of antiplatelet drugs is summarised in the clinical box below.

Antiplatelet drugs

- **Aspirin** inhibits cyclo-oxygenase irreversibly. Low doses very effectively (> 95%) inhibit platelet thromboxane (TX) A_2 synthesis and reduce the risk of thrombosis.
- **Clopidogrel** is a prodrug. Given by mouth, it irreversibly inhibits $P2Y_{12}$ receptors and thereby inhibits platelet responses to ADP. Its clinical effect is additive with aspirin. **Prasugrel** is similar.
- Antagonists of GPIIb/IIIa receptors include a monoclonal antibody (**abciximab**) and several synthetic molecules (e.g. **tirofiban**). They inhibit diverse agonists, for example ADP and TXA_2, because different pathways of activation converge on GPIIb/IIIa receptors. They are administered intravenously for short-term treatment.
- **Dipyridamole** inhibits phosphodiesterase and adenosine uptake. It is used in addition to aspirin in some patients with stroke or transient ischaemic attack.
- **Epoprostenol** (synthetic PGI_2) is chemically unstable. Given as an intravenous infusion, it acts on I prostanoid (IP) receptors on vascular smooth muscle and platelets (Ch. 17), stimulating adenylyl cyclase and thereby causing vasodilatation and inhibiting aggregation caused by any pathway (e.g. ADP or TXA_2).

Clinical uses of antiplatelet drugs

The main drug is **aspirin**. Other drugs with distinct actions (e.g. **dipyridamole**, **clopidogrel**) can have additive effects, or be used in patients who are intolerant of aspirin. Uses of antiplatelet drugs relate mainly to arterial thrombosis and include:

- *acute myocardial infarction*
- high risk of myocardial infarction, including a history of *myocardial infarction*, *angina or intermittent claudication* (see Ch. 22)
- following *coronary artery bypass grafting*
- *unstable coronary syndromes* (**clopidogrel** is added to **aspirin**)
- following coronary artery *angioplasty* and/or *stenting* (intravenous glycoprotein IIb/IIIa antagonists, e.g. **abciximab**, are used in some patients in addition to aspirin)
- *transient cerebral ischaemic attack* ('ministrokes') or *thrombotic stroke*, to prevent recurrence (**dipyridamole** can be added to **aspirin**)
- *atrial fibrillation*, if oral anticoagulation is contraindicated.

Other antiplatelet drugs such as **epoprostenol** (PGI_2; see Ch. 17) have specialised clinical applications (e.g. in *haemodialysis* or *haemofiltration*, Ch. 28, or in *pulmonary hypertension*, Ch. 22).

[8]The convention for naming monoclonals is: -momab = **mo**use **m**onoclonal **a**nti**b**ody; -umab = human; -zumab = humanised; -ximab = chimeric—a kind of medieval mouse–man nightmare.

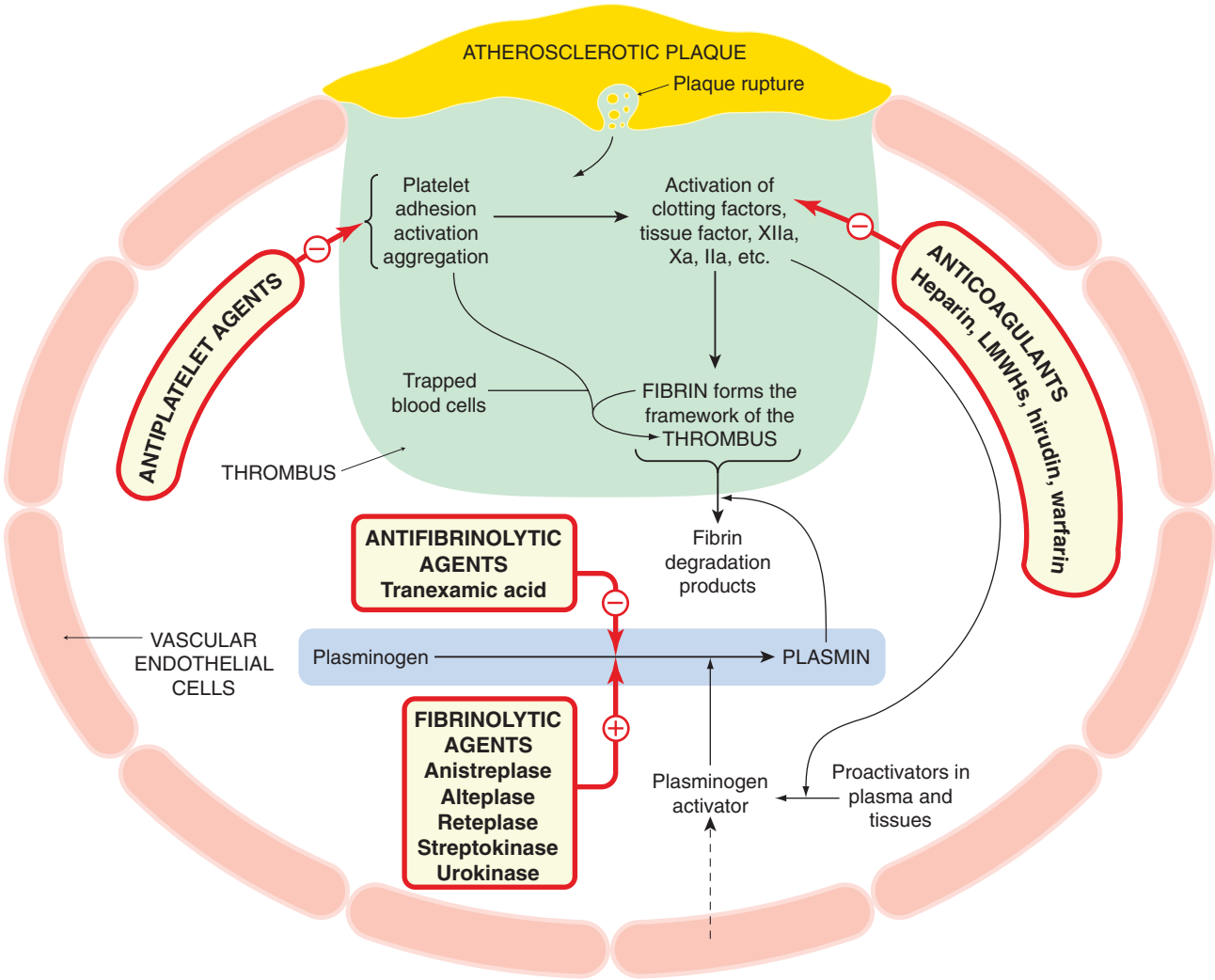

Fig. 24.10 Fibrinolytic system. The schematic shows interactions with coagulation and platelet pathways and sites of action of drugs that modify these systems. LMHs, low-molecular-weight heparins. For more details of platelet activation and the coagulation cascade, refer to Figures 24.1, 24.2 and 24.7.

FIBRINOLYSIS (THROMBOLYSIS)

When the coagulation system is activated, the fibrinolytic system is also set in motion via several endogenous *plasminogen activators*, including tissue plasminogen activator (tPA), urokinase-type plasminogen activator, kallikrein and neutrophil elastase. tPA is inhibited by a structurally related lipoprotein, *lipoprotein(a)*, increased concentrations of which constitute an independent risk factor for myocardial infarction (Ch. 23). Plasminogen is deposited on the fibrin strands within a thrombus. Plasminogen activators are serine proteases and are unstable in circulating blood. They diffuse into thrombus and cleave plasminogen, a zymogen present in plasma, to release plasmin (see Fig. 24.10).

▼ Plasmin is trypsin-like, acting on Arg–Lys bonds, and thus digests not only fibrin but fibrinogen; factors II, V and VIII; and many other proteins. It is formed locally and acts on the fibrin meshwork, generating fibrin degradation products and lysing the clot. Its action is localised to the clot, because plasminogen activators are effective mainly on plasminogen adsorbed to fibrin; any plasmin that escapes into the circulation is inactivated by plasmin inhibitors, including PAI-1 (see above and Ch. 22), which protect us from digesting ourselves from within.

Drugs affect this system by increasing or inhibiting fibrinolysis (*fibrinolytic* and *antifibrinolytic* drugs, respectively).

FIBRINOLYTIC DRUGS

Figure 24.10 summarises the interaction of the fibrinolytic system with the coagulation cascade and platelet activation, and the action of drugs that modify this. Several fibrinolytic (thrombolytic) drugs are used clinically, principally to reopen the occluded arteries in patients with acute myocardial infarction[9] or stroke, less commonly in patients

[9]Fibrinolytic drugs are now less widely used in acute myocardial infarction since many units throughout the world provide an emergency angioplasty service (the blocked artery is identified angiographically, opened with a balloon catheter and, if necessary, kept open by means of a stent, Ch. 21). The important thing is to open up the thrombosed artery as swiftly as possible. If facilities are available to do this surgically, this is at least as good as using a lytic drug.

with life-threatening venous thrombosis or pulmonary embolism.

Streptokinase is a protein extracted from cultures of streptococci. It activates plasminogen. Infused intravenously, it reduces mortality in acute myocardial infarction, and this beneficial effect is additive with aspirin (Fig. 24.8). Its action is blocked by antibodies, which appear 4 days or more after the initial dose: its use should not be repeated after this time has elapsed.

Alteplase and **duteplase** are, respectively, single- and double-chain recombinant tPA. They are more active on fibrin-bound plasminogen than on plasma plasminogen, and are therefore said to be 'clot selective'. Recombinant tPA is not antigenic, and can be used in patients likely to have antibodies to streptokinase. Because of their short half-lives, they must be given as intravenous infusions. **Reteplase** is similar but has a longer elimination half-life, allowing for bolus administration and making for simplicity of administration. It is available for clinical use in myocardial infarction.

UNWANTED EFFECTS AND CONTRAINDICATIONS

The main hazard of all fibrinolytic agents is bleeding, including gastrointestinal haemorrhage and haemorrhagic stroke. If serious, this can be treated with **tranexamic acid** (see below), fresh plasma or coagulation factors. Streptokinase can cause allergic reactions and low-grade fever. Streptokinase causes a burst of plasmin formation, generating kinins (see Ch. 17), and can cause hypotension by this mechanism.

Contraindications to the use of these agents are active internal bleeding, haemorrhagic cerebrovascular disease, bleeding diatheses, pregnancy, uncontrolled hypertension, invasive procedures in which haemostasis is important, and recent trauma—including vigorous cardiopulmonary resuscitation.

WHICH FIBRINOLYTIC AGENT IS BEST?

Much has been written as to which drug is best, but an authoritative review (Collins et al., 1997) concluded that:

> … the choice of fibrinolytic drug makes little difference to the overall probability of stroke-free survival, because the regimens that dissolve coronary thrombi more rapidly produce greater risks of cerebral haemorrhage … It is … important that any uncertainties about which fibrinolytic regimen or dose of aspirin to use do not engender uncertainty about whether to use fibrinolytic and antiplatelet therapies routinely.

CLINICAL USE

Several large placebo-controlled studies in patients with myocardial infarction have shown convincingly that fibrinolytic drugs reduce mortality if given within 12 h of the onset of symptoms, and that the sooner they are administered the better is the result. Similar considerations apply to their use in thrombotic stroke. Scanning to exclude haemorrhagic stroke is advisable, though not always practicable in an emergency situation. Other uses of fibrinolytic agents are listed in the clinical box.

Fibrinolysis and drugs modifying fibrinolysis

- A fibrinolytic cascade is initiated concomitantly with the coagulation cascade, resulting in the formation within the coagulum of plasmin, which digests fibrin.
- Various agents promote the formation of plasmin from its precursor plasminogen, for example **streptokinase**, and tissue plasminogen activators (tPAs) such as **alteplase**, **duteplase** and **reteplase**. Most are infused; reteplase can be given as a bolus injection.
- Some drugs (e.g. **tranexamic acid**) inhibit fibrinolysis.

Clinical uses of fibrinolytic drugs

The main drugs are **streptokinase** and tissue plasminogen activators (tPAs), for example **alteplase**.

- The main use is in *acute myocardial infarction*, with ST segment elevation on the ECG within 12 h of onset (the earlier the better!).
- Other uses include:
  - *acute thrombotic stroke* within 3 h of onset (tPA), in selected patients
  - clearing *thrombosed shunts* and *cannulae*
  - *acute arterial thromboembolism*
  - life-threatening *deep vein thrombosis* and *pulmonary embolism* (streptokinase, given promptly).

ANTIFIBRINOLYTIC AND HAEMOSTATIC DRUGS

Tranexamic acid inhibits plasminogen activation and thus prevents fibrinolysis. It can be given orally or by intravenous injection. It is used to treat various conditions in which there is bleeding or risk of bleeding, such as haemorrhage following prostatectomy or dental extraction, in menorrhagia (excessive menstrual blood loss) and for life-threatening bleeding following thrombolytic drug administration. It is also used in patients with the rare disorder of hereditary angio-oedema.

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25 Haemopoietic system and treatment of anaemia

OVERVIEW

This chapter summarises the different kinds of anaemia, caused by nutrient deficiencies, bone marrow depression or increased red cell destruction, and covers the main haematinic agents used to treat them. We describe haemopoietic growth factors for red and white blood cells, and conclude by mentioning two drugs (*hydroxycarbamide* and *eculizumab*) used in treating, respectively, sickle cell anaemia and paroxysmal nocturnal haemoglobinuria.

INTRODUCTION

In this chapter we briefly review the haemopoietic system and different types of anaemia due to deficiency of nutrients, depression of the bone marrow or increased destruction of red cells (haemolytic anaemias). Nutritional deficiencies of *iron*, *vitamin B_{12}* or *folic acid* are common and important and most of the chapter is devoted to these haematinic agents (i.e. nutrients needed for healthy haemopoiesis and related drugs). Treatment of many forms of bone marrow depression is mainly supportive, but *haemopoietic growth factors* (especially *epoetins*—preparations of the natural hormone erythropoietin) have a place, especially in patients with chronic renal failure, and are covered briefly, as are other haemopoietic factors, known as *colony-stimulating factors* (CSFs), which are used to increase numbers of circulating white blood cells. Treatment of haemolytic anaemias is again mainly supportive, but we mention two drugs (**hydroxycarbamide** and **eculizumab**) that provide mechanistic insights as well as clinical benefit in two specific haemolytic disorders.

THE HAEMOPOIETIC SYSTEM

The main components of the haemopoietic system are the blood, bone marrow, lymph nodes and thymus, with the spleen, liver and kidneys as important accessory organs. Blood consists of formed elements (red and white blood cells and platelets) and plasma. This chapter deals mainly with red cells, which have the principal function of carrying oxygen. Their oxygen-carrying power depends on their haemoglobin content. The most important site of formation of red blood cells in adults is the bone marrow, whereas the spleen acts as their graveyard. Red cell loss in healthy adults is precisely balanced by production of new cells. The liver stores vitamin B_{12} and is involved in the process of breakdown of the haemoglobin liberated when red blood cells are destroyed. The kidney manufactures *erythropoietin*, a hormone that stimulates red cell production. Cells from various organs synthesise and release CSFs, which regulate the production of leukocytes and platelets. Drugs used in the chemotherapy of leukemias are described in Chapter 55.

TYPES OF ANAEMIA

Anaemia is characterised by a reduced concentration of haemoglobin in the blood. It may cause fatigue but, especially if it is chronic, is often surprisingly asymptomatic. The commonest cause is blood loss resulting from menstruation, drug treatment (e.g. with **aspirin** or other non-steroidal anti-inflammatory drugs; Ch. 26) or pathological processes such as colonic carcinoma or (especially in developing countries) parasitic infestation (Ch. 54). Pregnancy and child bearing are other important physiological drains on iron reserves. There are several different types of anaemia and several different diagnostic levels. Determining indices of red cell size and haemoglobin content and microscopical examination of a stained blood smear allow characterisation into:

- *hypochromic, microcytic anaemia* (small red cells with low haemoglobin; caused by chronic blood loss giving rise to iron deficiency)
- *macrocytic anaemia* (large red cells, few in number)
- *normochromic normocytic anaemia* (fewer normal-sized red cells, each with a normal haemoglobin content)
- mixed pictures.

Further evaluation may include determination of concentrations of ferritin, iron, vitamin B_{12} and folic acid in serum, and microscopic examination of smears of bone marrow. This leads to more precise diagnostic groupings of anaemias into:

- Deficiency of nutrients necessary for haemopoiesis, most importantly:
  - iron
  - folic acid and vitamin B_{12}
  - pyridoxine and vitamin C.
- Depression of the bone marrow, caused by:
  - drug toxicity (e.g. anticancer drugs, **clozapine**)
  - radiation therapy
  - diseases of the bone marrow of unknown origin (e.g. idiopathic aplastic anaemia, leukaemias)
  - reduced production of, or responsiveness to, erythropoietin (e.g. chronic renal failure, rheumatoid arthritis, AIDS).
- Excessive destruction of red blood cells (i.e. haemolytic anaemia); this has many causes, including *haemoglobinopathies* (such as sickle cell anaemia), adverse reactions to drugs and inappropriate immune reactions.

HAEMATINIC AGENTS

It is important to note that the use of haematinic agents is often only an adjunct to treatment of the underlying cause of the anaemia—for example, surgery for colon cancer (a common cause of iron deficiency) or anthelminthic drugs for patients with hookworm (a frequent cause of anaemia

Table 25.1 The distribution of iron in the body of a healthy 70 kg man

Protein	Tissue	Iron content (mg)
Haemoglobin	Erythrocytes	2600
Myoglobin	Muscle	400
Enzymes (cytochromes, catalase, guanylyl cyclase, etc.)	Liver and other tissues	25
Transferrin	Plasma and extracellular fluid	8
Ferritin and haemosiderin	Liver Spleen Bone marrow	410 48 300

Data from Jacobs A, Worwood M 1982 Chapter 5. In: Hardisty R M, Weatherall D J [eds] Blood and its disorders. Blackwell Scientific, Oxford.

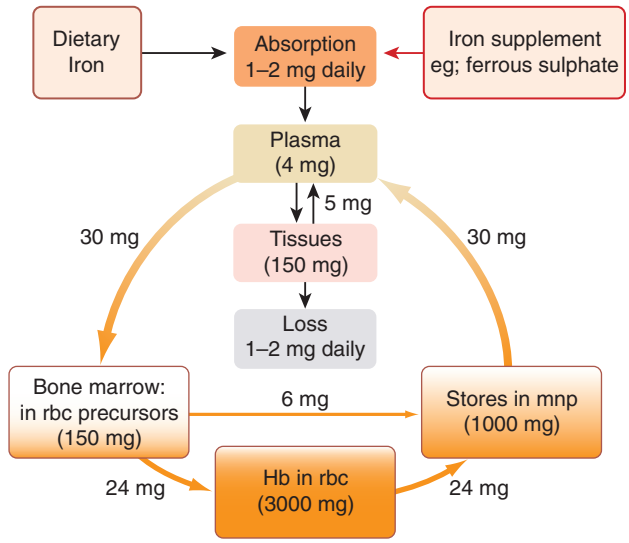

Fig. 25.1 Distribution and turnover of iron in the body. The quantities by the arrows indicate the usual amounts transferred each day. The transfer of 6 mg from red cell precursors to phagocytes represents aborted cells that fail to develop into functional red blood cells. Hb, haemoglobin; mnp, mononuclear phagocytes (mainly in liver, spleen and bone marrow); rbc, red blood cells.

in parts of Africa and Asia; Ch. 54). Sometimes treatment consists of stopping an offending drug, for example a non-steroidal anti-inflammatory drug that causes blood loss from the stomach (Ch. 26).

IRON

Iron is a transition metal with two important properties relevant to its biological role:

1. Ability to exist in several oxidation states.
2. Ability to form stable coordination complexes.

The body of a 70 kg man contains about 4 g of iron, 65% of which circulates in the blood as haemoglobin. About one-half of the remainder is stored in the liver, spleen and bone marrow, chiefly as *ferritin* and *haemosiderin*. The iron in these molecules is available for haemoglobin synthesis. The rest, which is not available for haemoglobin synthesis, is present in myoglobin, cytochromes and various enzymes.

The distribution and turnover of iron in an average adult man are shown in Table 25.1 and Figure 25.1. The corresponding values in a woman would be about 55% of the values in Table 25.1. Because most of the iron in the body is either part of—or destined to be part of—haemoglobin, the most obvious clinical result of iron deficiency is anaemia, and the only indication for therapy with iron is for treatment or prophylaxis of iron deficiency anaemia.

Haemoglobin is made up of four protein chain subunits (globins), each of which contains one haem moiety. Haem consists of a tetrapyrrole porphyrin ring containing ferrous (Fe^{2+}) iron. Each haem group can carry one oxygen molecule, which is bound reversibly to Fe^{2+} and to a histidine residue in the globin chain. This reversible binding is the basis of oxygen transport.

IRON TURNOVER AND BALANCE

Both the normal physiological turnover of iron and pharmacokinetic factors affecting iron when it is given therapeutically will be dealt with here. The normal daily requirement for iron is approximately 5 mg for men, and 15 mg for growing children and for menstruating women. A pregnant woman needs between 2 and 10 times this amount because of the demands of the fetus and increased requirements of the mother.[1] The average diet in Western Europe provides 15–20 mg of iron daily, mostly in meat. Iron in meat is generally present as haem, and about 20–40% of haem iron is available for absorption.

▼ Humans are adapted to absorb haem iron. It is thought that one reason why modern humans have problems in maintaining iron balance (there are an estimated 500 million people with iron deficiency in the world) is that the change from hunting to grain cultivation 10 000 years ago led to cereals, which have a relatively small amount of utilisable iron, replacing meat in the diet. Non-haem iron in food is mainly in the ferric state, and this needs to be converted to ferrous iron for absorption. Ferric iron, and to a lesser extent ferrous iron, has low solubility at the neutral pH of the intestine; however, in the stomach iron dissolves and binds to a mucoprotein carrier. In the presence of ascorbic acid, fructose and various amino acids, iron is detached from the carrier, forming soluble low-molecular-weight complexes that enable it to remain in soluble form in the intestine. Ascorbic acid stimulates iron absorption partly by forming soluble iron–ascorbate chelates and partly by reducing ferric iron to the more soluble ferrous form. **Tetracycline** forms an insoluble iron chelate, impairing absorption of both substances.

The amount of iron in the diet and the various factors affecting its availability are thus important determinants in absorption, but the regulation of iron absorption is a function of the intestinal mucosa, influenced by the body's iron stores. Because there is no mechanism whereby iron excretion is regulated, the absorptive mechanism has a central role in iron balance as it is the sole mechanism by which body iron is controlled.

The site of iron absorption is the duodenum and upper jejunum, and absorption is a two-stage process involving

[1]Each pregnancy 'costs' the mother 680 mg of iron, equivalent to 1300 ml of blood, owing to the demands of the fetus, plus requirements of the expanded blood volume and blood loss at delivery.

first a rapid uptake across the brush border and then transfer into the plasma from the interior of the epithelial cells. The second stage, which is rate limiting, is energy dependent. Haem iron in the diet is absorbed as intact haem, and the iron is released in the mucosal cell by the action of haem oxidase. Non-haem iron is absorbed in the ferrous state. Within the cell, ferrous iron is oxidised to ferric iron, which is bound to an intracellular carrier, a transferrin-like protein; the iron is then either held in storage in the mucosal cell as *ferritin* (if body stores of iron are high) or passed on to the plasma (if iron stores are low).

▼ Iron is carried in the plasma bound to *transferrin*, a β-globulin with two binding sites for ferric iron, which is normally only 30% saturated. Plasma contains 4 mg of iron at any one time, but the daily turnover is about 30 mg (Fig. 25.1). Most of the iron that enters the plasma is derived from mononuclear phagocytes, following the degradation of time-expired erythrocytes. Intestinal absorption and mobilisation of iron from storage depots contribute only small amounts. Most of the iron that leaves the plasma each day is used for haemoglobin synthesis by red cell precursors (erythroblasts). These have receptors that bind transferrin, releasing it again when its cargo of iron has been captured.

Iron is stored in two forms: soluble ferritin and insoluble *haemosiderin*. Ferritin is present in all cells, the mononuclear phagocytes of liver, spleen and bone marrow containing especially high concentrations. It is also present in plasma. The precursor of ferritin, *apoferritin*, is a protein of molecular weight 450 000, composed of 24 identical polypeptide subunits that enclose a cavity in which up to 4500 iron atoms can be stored. Apoferritin takes up ferrous iron, oxidises it and deposits the ferric iron in its core. In this form, it constitutes ferritin, the primary storage form of iron, from which the iron is most readily available. The lifespan of this iron-laden protein is only a few days. Haemosiderin is a degraded form of ferritin in which the iron cores of several ferritin molecules have aggregated, following partial disintegration of the outer protein shells.

The ferritin in plasma has virtually no iron associated with it. It is in equilibrium with the storage ferritin in cells, and its concentration in plasma provides an estimate of total body iron stores.

The body has no means of actively excreting iron. Small amounts leave the body through desquamation (peeling off) of mucosal cells containing ferritin, and even smaller amounts leave in the bile, sweat and urine. A total of about 1 mg is lost daily. Iron balance is therefore critically dependent on the active absorption mechanism in the intestinal mucosa. This absorption is influenced by the iron stores in the body, but the precise mechanism of this control is still a matter of debate; the amount of ferritin in the intestinal mucosa may be important, as may the balance between ferritin and the transferrin-like carrier molecule in these cells. The daily movement of iron in the body is illustrated in Figure 25.1. Since red cells contain approximately 0.6 mg iron per ml of blood, loss of only a few ml of blood per day substantially increases dietary iron requirement.

ADMINISTRATION OF IRON

Iron is usually given orally, e.g. as **ferrous sulfate**. Other salts for oral administration are **ferrous succinate**, **gluconate** or **fumarate**.

Parenteral iron (e.g. **iron dextran**, **iron sucrose**) may be necessary in individuals who are not able to absorb oral iron because of malabsorption syndromes, or as a result of surgical procedures or inflammatory conditions involving the gastrointestinal tract. It is also used for patients who do not tolerate oral preparations, and patients with chronic renal failure or with chemotherapy-induced anaemia who are receiving treatment with erythropoietin (see below). Iron-dextran can be given by deep intramuscular injection or slow intravenous infusion; iron-sucrose is given by slow intravenous infusion. A small initial dose is given because of the risk of anaphylactoid reaction.

Clinical uses of iron salts

To treat iron deficiency anaemia, which can be caused by:

- *chronic blood loss* (e.g. with menorrhagia, hookworm, colon cancer)
- *increased demand* (e.g. in pregnancy and early infancy)
- *inadequate dietary intake* (uncommon in developed countries)
- *inadequate absorption* (e.g. following gastrectomy).

Unwanted effects

The unwanted effects of oral iron administration are dose related and include nausea, abdominal cramps and diarrhoea. Parenteral iron can cause anaphylactoid reactions (Ch. 57). Iron is an important nutrient for several pathogens and there is concern that excessive iron could worsen the clinical course of infection. Iron treatment is usually avoided during infection for this reason.

Acute iron toxicity, usually seen in young children who have swallowed attractively coloured iron tablets in mistake for sweets, can result in severe necrotising gastritis with vomiting, haemorrhage and diarrhoea, followed by circulatory collapse.

Iron overload

Chronic iron toxicity or iron overload occurs in chronic haemolytic anaemias requiring frequent blood transfusions, such as the *thalassaemias* (a large group of genetic disorders of globin chain synthesis) and *haemochromatosis* (a genetic iron storage disease with increased iron absorption, resulting in damage to liver, islets of Langerhans, joints and skin[2]).

The treatment of acute and chronic iron toxicity involves the use of iron chelators such as **desferrioxamine**. This is not absorbed from the gut but is nonetheless given intragastrically following acute overdose (to bind iron in the bowel lumen and prevent its absorption) as well as intramuscularly and, if necessary, intravenously. In severe poisoning, it is given by slow intravenous infusion. Desferrioxamine forms a complex with ferric iron and, unlike unbound iron, this is excreted in the urine. **Deferiprone**, an orally absorbed iron chelator, is an alternative treatment for iron overload in patients with thalassaemia major who are unable to take desferrioxamine. Agranulocytosis and other blood dyscrasias are serious potential adverse effects. **Defasirox**, an oral iron chelator, is used for selected patients with thalassaemia.

FOLIC ACID AND VITAMIN B_{12}

Vitamin B_{12} and folic acid are essential constituents of the human diet, being necessary for DNA synthesis and consequently for cell proliferation. Their biochemical actions are interdependent (see below), and treatment of vitamin B_{12} deficiency with folic acid corrects some, but not all, of the features of vitamin B_{12} deficiency. Deficiency of either vitamin B_{12} or folic acid affects tissues with a rapid cell turnover, particularly bone marrow, but vitamin B_{12}

[2]'Bronze diabetes'—where chronic iron overload is treated by repeated venesection, one of the few modern uses of this once near-universal 'remedy'.

Iron

- Iron is important for the synthesis of haemoglobin, myoglobin, cytochromes and other enzymes.
- Ferric iron (Fe^{3}+) must be converted to ferrous iron (Fe^{2}+) for absorption in the gastrointestinal tract.
- Absorption involves active transport into mucosal cells in the jejunum and upper ileum, from where it can be transported into the plasma and/or stored intracellularly as ferritin.
- Total body iron is controlled exclusively by absorption; in iron deficiency, more is transported into plasma than is stored as ferritin in jejunal mucosa.
- Iron loss occurs mainly by sloughing of ferritin-containing mucosal cells.
- Iron in plasma is bound to transferrin, and most is used for erythropoiesis. Some is stored as ferritin in other tissues. Iron from time-expired erythrocytes enters the plasma for reuse.
- The main therapeutic preparation is ferrous sulfate; iron-sucrose can be given as an intravenous infusion.
- Unwanted effects include gastrointestinal disturbances. Severe toxic effects occur if large doses are ingested; these can be countered by desferrioxamine, an iron chelator.

deficiency also causes important neuronal disorders, which are not corrected (or may even be made worse) by treatment with folic acid. Deficiency of either vitamin causes *megaloblastic haemopoiesis*, in which there is disordered erythroblast differentiation and defective erythropoiesis in the bone marrow. Large abnormal erythrocyte precursors appear in the marrow, each with a high RNA:DNA ratio as a result of decreased DNA synthesis. The circulating erythrocytes (macrocytes) are large fragile cells, often distorted in shape. Mild leukopenia and thrombocytopenia usually accompany the anaemia, and the nuclei of polymorphonuclear leukocytes are abnormal (hypersegmented). Neurological disorders caused by deficiency of vitamin B_{12} include peripheral neuropathy and dementia, as well as subacute combined[3] degeneration of the spinal cord. Folic acid deficiency is caused by dietary deficiency, especially in settings of increased demand (e.g. during pregnancy—especially important because of the link between folate deficiency and neural tube defects in the baby [see Ch. 57] or because of chronic haemolysis in patients with haemoglobinopathies such as *sickle cell anaemia*—see below). Vitamin B_{12} deficiency, however, is usually due to decreased absorption (see below).

FOLIC ACID

Some aspects of folate structure and metabolism are dealt with in Chapters 50 and 55, because several important antibacterial and anticancer drugs are antimetabolites that interfere with folate synthesis in microorganisms or tumour cells. Liver and green vegetables are rich sources of folate. In healthy non-pregnant adults, the daily requirement is about 0.2 mg daily, but this is increased during pregnancy.

[3]'Combined' because the lateral as well as the dorsal columns are involved, giving rise to motor as well as sensory symptoms.

Mechanism of action

Reduction of folic acid, catalysed by *dihydrofolate reductase* in two stages yields *dihydrofolate* (FH_2) and *tetrahydrofolate* (FH_4), co-factors which transfer methyl groups (1-carbon transfers) in several important metabolic pathways. FH_4 is essential for DNA synthesis because of its role as co-factor in the synthesis of purines and pyrimidines. It is also necessary for reactions involved in amino acid metabolism.

FH_4 is especially important for the conversion of deoxyuridylate monophosphate (DUMP) to deoxythymidylate monophosphate (DTMP). This reaction is rate limiting in mammalian DNA synthesis and is catalysed by thymidylate synthetase, with FH_4 acting as methyl donor.

Pharmacokinetic aspects

Therapeutically, folic acid is given orally and is absorbed in the ileum. Methyl-FH_4 is the form in which folate is usually carried in blood and which enters cells. It is functionally inactive until it is demethylated in a vitamin B_{12}-dependent reaction (see below). Folate is taken up into hepatocytes and bone marrow cells by active transport. Within the cells, folic acid is reduced and formylated before being converted to the active polyglutamate form. **Folinic acid**, a synthetic FH_4, is converted much more rapidly to the polyglutamate form.

Unwanted effects

Unwanted effects do not occur even with large doses of folic acid—except possibly in the presence of vitamin B_{12} deficiency, when administration of folic acid may improve the anaemia while exacerbating the neurological lesion. It is therefore important to determine whether a megaloblastic anaemia is caused by folate or vitamin B_{12} deficiency and treat accordingly.

Clinical uses of folic acid and vitamin B_{12} (hydroxocobalamin)

Folic acid

- Treatment of megaloblastic anaemia resulting from folate deficiency, which can be caused by:
  - *poor diet* (common in alcoholic individuals)
  - *malabsorption syndromes*
  - drugs (e.g. **phenytoin**).
- Treatment or prevention of toxicity from methotrexate, a folate antagonist (see Ch. 51).
- Prophylactically in individuals at hazard from developing folate deficiency, for example:
  - *pregnant women* and *before conception* (especially if there is a risk of birth defects)
  - *premature infants*
  - patients with *severe chronic haemolytic anaemias*, including haemoglobinopathies (e.g. sickle cell anaemia).

Vitamin B_{12} (hydroxocobalamin)

- Treatment of *pernicious anaemia* and other causes of vitamin B_{12} deficiency.
- Prophylactically after surgical operations that remove the site of production of intrinsic factor (the stomach) or of vitamin B_{12} absorption (the terminal ileum).

VITAMIN B_{12}

Vitamin B_{12} is a complex cobalamin. The vitamin B_{12} preparation used therapeutically is **hydroxocobalamin**. The principal dietary sources are meat (particularly liver, where it is stored), eggs and dairy products. For activity, cobalamins must be converted to *methylcobalamin* (methyl-B_{12}) or *5′-deoxyadenosylcobalamin* (ado-B_{12}). The average European diet contains 5–25 µg of vitamin B_{12} per day, and the daily requirement is 2–3 µg. Absorption requires *intrinsic factor* (a glycoprotein secreted by gastric parietal cells). Vitamin B_{12}, complexed with intrinsic factor, is absorbed by active transport in the terminal ileum. Healthy stomach secretes a large excess of intrinsic factor, but in patients with pernicious anaemia (an autoimmune disorder where the lining of the stomach atrophies), or following total gastrectomy, the supply of intrinsic factor is inadequate to maintain vitamin B_{12} absorption in the long term. Surgical removal of the terminal ileum, for example to treat Crohn's disease (see Ch. 29), can also impair B_{12} absorption.

Vitamin B_{12} is carried in the plasma by binding proteins called *transcobalamins*. It is stored in the liver, the total amount in the body being about 4 mg. This store is so large compared with the daily requirement, that if vitamin B_{12} absorption stops suddenly—as after a total gastrectomy—it takes 2–4 years for evidence of deficiency to become manifest.

Mechanism of action

Vitamin B_{12} is required for two main biochemical reactions in humans.

The conversion of methyl-FH_4 to FH_4. The role of vitamin B_{12} in folate coenzyme synthesis is illustrated in Figure 25.2. It is through these mechanisms that the metabolic activities of vitamin B_{12} and folic acid are linked and implicated in the synthesis of DNA. It is also through this pathway that folate/vitamin B_{12} treatment can lower plasma homocysteine concentration. Because increased homocysteine concentrations may have undesirable vascular effects (Ch. 23, Table 23.1), this has potential therapeutic and public health implications. The reaction involves conversion of both methyl-FH_4 to FH_4 and homocysteine to methionine. The enzyme that accomplishes this (*homocysteine–methionine methyltransferase*) requires vitamin B_{12} as co-factor and methyl-FH_4 as methyl donor. The methyl group from methyl-FH_4 is transferred first to B_{12}, and then to homocysteine to form methionine (Fig. 25.2). Vitamin B_{12} deficiency thus traps folate in the inactive methyl-FH_4 form, thereby depleting the folate polyglutamate coenzymes needed for DNA synthesis (see above). Vitamin B_{12}-dependent methionine synthesis also affects the synthesis of folate polyglutamate coenzymes by an additional mechanism. The preferred substrate for polyglutamate synthesis is formyl-FH_4, and the conversion of FH_4 to formyl-FH_4 requires a formate donor such as methionine.

Isomerisation of methylmalonyl-CoA to succinyl-CoA. This isomerisation reaction is part of a route by which propionate is converted to succinate. Through this pathway, cholesterol, odd-chain fatty acids, some amino acids and thymine can be used for gluconeogenesis or for energy production via the tricarboxylic acid cycle. Coenzyme B_{12} (ado-B_{12}) is an essential co-factor, so methylmalonyl-CoA accumulates in vitamin B_{12} deficiency. This distorts the pattern of fatty acid synthesis in neural tissue and may be the basis of neuropathy in vitamin B_{12} deficiency.

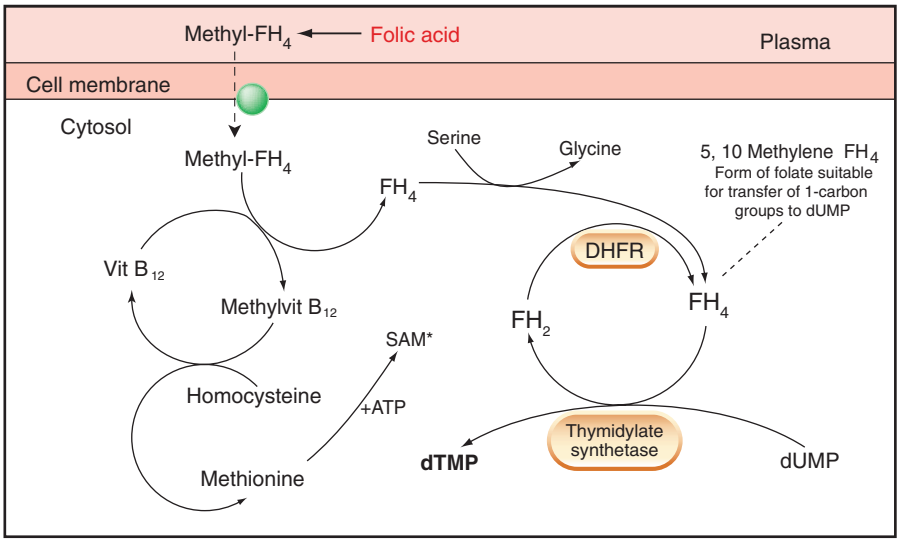

Fig. 25.2 Simplified diagram of the role of folate and vitamin B_{12} in the reactions necessary for the eventual synthesis of thymidylate. Methyl-FH_4 enters cells from the plasma by carrier. The methyl group is transferred to homocysteine to form methionine via vitamin B_{12}, which is bound to a methyltransferase (not shown). Methionine reacts with ATP to form S-adenosyl methionine (SAM*) which is a universal methyl donor for several reactions including methylation of cytosine in DNA molecules. FH_4 functions as a carrier of a one-carbon unit, providing the methyl group necessary for the conversion of 2′deoxyuridylate monophosphate (DUMP) to 2′ deoxythymidylate (DTMP) by thymidylate synthetase. During the transfer of the one-carbon unit, FH_4 is oxidised to FH_2, which must be reduced by dihydrofolate reductase (DHFR) to FH_4 (before it can act again). The thymidylate synthetase action is rate-limiting in DNA synthesis. Note that in all the actions of folates it is the polyglutamate form that is most active. DHFR, dihydrofolate reductase; DTMP, thymidylate; DUMP, deoxyuridylate monophosphate.

Vitamin B_{12} and folic acid

Both vitamin B_{12} and folic acid are needed for DNA synthesis. Deficiencies particularly affect erythropoiesis, causing macrocytic megaloblastic anaemia.

Folic acid

- There is active uptake of folic acid into cells and reduction to tetrahydrofolate (FH_4) by dihydrofolate reductase; extra glutamates are then added.
- Folate polyglutamate is a co-factor (a carrier of 1-carbon units) in the synthesis of purines and pyrimidines (especially thymidylate).

Vitamin B_{12} (hydroxocobalamin)

- Vitamin B_{12} needs intrinsic factor (a glycoprotein) secreted by gastric parietal cells for absorption in the terminal ileum.
- It is stored in the liver.
- It is required for:
  - conversion of methyl-FH_4 (inactive form of FH_4) to active formyl-FH_4, which, after polyglutamation, is a co-factor in the synthesis of purines and pyrimidines (see above)
  - isomerisation of methylmalonyl-CoA to succinyl-CoA.
- Deficiency occurs most often in pernicious anaemia, which results from malabsorption caused by lack of intrinsic factor from the stomach. It causes neurological disease as well as anaemia.
- Vitamin B_{12} is given by injection to treat pernicious anaemia.

Administration of vitamin B_{12}

When vitamin B_{12} is used therapeutically (as **hydroxocobalamin**), it is usually given by injection[4] because, as explained above, vitamin B_{12} deficiency commonly results from malabsorption. Patients with pernicious anaemia require life-long therapy. Hydroxocobalamin does not cause unwanted effects.

HAEMOPOIETIC GROWTH FACTORS

Every 60 seconds, a human being must generate about 120 million granulocytes and 150 million erythrocytes, as well as numerous mononuclear cells and platelets. The cells responsible for this remarkable productivity are derived from a relatively small number of self-renewing, pluripotent stem cells laid down during embryogenesis. Maintenance of haemopoiesis necessitates a balance between self-renewal of the stem cells on the one hand, and differentiation into the various types of blood cell on the other. The factors involved in controlling this balance are the *haemopoietic growth factors*, which direct the division and maturation of the progeny of these cells down eight possible lines of development (Fig. 25.3). These cytokine growth factors are highly potent glycoproteins, acting at concentrations of 10^{-12} to 10^{-10} mol/l. They are present in plasma at very low concentrations under basal conditions, but on stimulation their concentrations can increase within hours by 1000-fold or more. *Erythropoietin* regulates the red cell line, and the signal for its production is blood loss and/or low tissue oxygen tension. *Colony-stimulating factors* (CSFs) regulate the myeloid divisions of the white cell line, and the main stimulus for their production is infection (see also Ch. 6).

Recombinant erythropoietin (**epoetin**),[5] and recombinant granulocyte CSF (**filgrastim**, **lenograstim**, **pegfilgrastim**) are used clinically (see below); *thrombopoietin* is available in recombinant form but there are concerns about effects on tumour progression (it activates a cell surface protein that is an oncogene product). Some of the other haemopoietic growth factors (e.g. interleukin-3, interleukin-5 and various other cytokines) are covered in Chapter 6.

ERYTHROPOIETIN

Erythropoietin is produced in juxtatubular cells in the kidney and also in macrophages; it stimulates committed erythroid progenitor cells to proliferate and generate erythrocytes (Fig. 25.3). Recombinant human erythropoietins are used to treat symptomatic anaemia caused by erythropoietin deficiency. **Darbepoetin**, a hyperglycosylated form of epoetin, has a longer half-life and can be administered less frequently; **methoxy polyethylene glycol-epoetin beta** is another preparation with long half-life. Epoetin and darbopoietin are given intravenously or subcutaneously, the response being greatest after subcutaneous injection and fastest after intravenous injection.

Epoetins are reaching the end of their periods of patent protection and the first 'biosimilar' products have recently been licensed; unlicensed uses include its use in sport (e.g. 'blood-doping' in cyclists)—see Chapter 58.

Unwanted effects

Transient influenza-like symptoms are common. Hypertension is also common and can cause encephalopathy with headache, disorientation and sometimes convulsions. Iron deficiency can be induced because more iron is required for the enhanced erythropoiesis. Blood viscosity increases as the haematocrit (i.e. the fraction of the blood that is occupied by red blood cells) rises, increasing the risk of thrombosis, especially during dialysis. There have been rare reports of a devastating chronic condition known as pure red cell aplasia, connected with development of antibodies directed against erythropoietin.

Clinical use

Iron or folate deficiency must be corrected before starting treatment. Parenteral iron preparations are often needed (see above). Haemoglobin must be monitored and maintained within the range 10–12 g/dl to avoid the unwanted effects described above. The clinical use of epoetin is given in the box below.

[4] At least in Anglo-Saxon countries; in France, very large doses of vitamin B_{12} are given by mouth to achieve sufficient absorption for therapeutic efficacy despite the absence of intrinsic factor. Either method is a great improvement on eating the prodigious quantities of raw liver required by Minot and Murphy's 'liver diet' of 1925!

[5] The first therapeutic agent to be produced by recombinant technology, by Amgen in 1989—a huge commercial success, heralding the emergence of the new biotechnology industry.

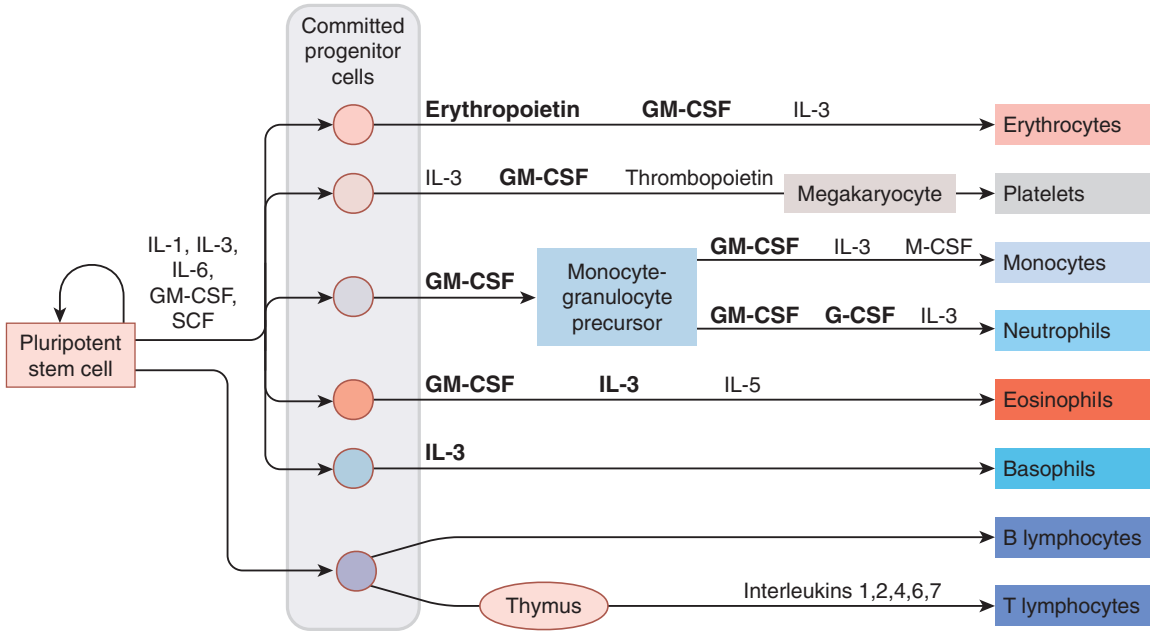

Fig. 25.3 Haemopoietic growth factors in blood cell differentiation. Various preparations of the factors shown in bold are in clinical use (see text). Most T cells generated in the thymus die by apoptosis; those that emerge are either CD4 or CD8 T cells. The colours used for the mature blood cells reflect how they appear in common staining preparations (and after which some are named). CSF, colony-stimulating factor; G-CSF, granulocyte CSF; GM-CSF, granulocyte–macrophage CSF; IL-1, interleukin-1; IL-3, interleukin-3 or multi-CSF; M-CSF, macrophage CSF; SCF, stem cell factor. (See also Ch. 6.)

COLONY-STIMULATING FACTORS

The CSFs are cytokines that stimulate the formation of maturing colonies of leukocytes, observable in tissue culture. They not only stimulate particular committed progenitor cells to proliferate (Fig. 25.3) but also cause irreversible differentiation. The responding precursor cells have membrane receptors for specific CSFs and may express receptors for more than one factor, thus permitting collaborative interactions between factors.

Granulocyte CSF is produced mainly by monocytes, fibroblasts and endothelial cells, and controls primarily the development of neutrophils, increasing their proliferation and maturation, stimulating their release from bone marrow storage pools and enhancing their function. Recombinant forms (**filgrastim**, which is not glycosylated, and glycosylated **lenograstim**) are used therapeutically. **Pegfilgrastim** is a derivative of filgrastim conjugated with polyethylene glycol ('pegylated'), which has the effect of increasing its duration of action.

Thrombopoietin, made in liver and kidney, stimulates proliferation and maturation of megakaryocytes to form platelets. Recombinant thrombopoietin is not used clinically.

Administration and unwanted effects

Filgrastim and lenograstim are given either subcutaneously or by intravenous infusion. Pegfilgrastim is administered subcutaneously. Gastrointestinal effects, fever, bone pain, myalgia and rash are recognised adverse effects; less common effects include pulmonary infiltrates and enlargement of liver or spleen.

Haemopoietic growth factors

Erythropoietin

- Regulates red cell production.
- Is given intravenously, subcutaneously, intraperitoneally.
- Can cause transient flu-like symptoms, hypertension, iron deficiency and increased blood viscosity.
- Is available, as epoetin, to treat patients with anaemia caused by chronic renal failure.

Granulocyte colony-stimulating factor

- Stimulates neutrophil progenitors.
- Is available as **filgrastim**, **pegfilgrastim** or **lenograstim**; it is given parenterally.

Clinical uses of epoetin

- Anaemia of chronic *renal failure*.
- Anaemia during *chemotherapy* for cancer.
- Prevention of the anaemia that occurs in *premature infants* (unpreserved formulations are used because benzyl alcohol, used as a preservative, has been associated with a fatal toxic syndrome in neonates).
- To increase the yield of autologous blood before *blood donation*.
- Anaemia of *AIDS* (exacerbated by **zidovudine**).
- Anaemia of *chronic inflammatory conditions* such as rheumatoid arthritis (investigational).

Clinical uses of the colony-stimulating factors

Colony-stimulating factors are used in specialist centres:

- To reduce the severity/duration of neutropenia induced by cytotoxic drugs during:
  - intensive *chemotherapy* necessitating autologous *bone marrow rescue*
  - following *bone marrow transplant*.
- To harvest *progenitor cells*.
- To expand the number of harvested progenitor cells ex vivo before reinfusing them.
- For persistent neutropenia in *advanced HIV infection*.
- In *aplastic anaemia*.

HAEMOLYTIC ANAEMIA

Anaemia associated with increased red cell destruction can arise from genetic causes (e.g. sickle cell disease, thalassaemia) or a variety of non-genetic causes such as autoimmunity, infections and adverse drug reactions.

▼ Adult haemoglobin (haemoglobin A) contains two α- and two β-globin chains. The cause of sickle cell anaemia is a mutation in the gene that codes the β-globin chain, resulting in a single amino acid substitution. The abnormal haemoglobin (haemoglobin S) can polymerise when deoxygenated, changing the physical properties of the red cells (which deform to a sickle shape, hence the name) and damaging the cell membranes. This can block the microcirculation, causing painful crises, and haemolysis can reduce the availability of nitric oxide (Ch. 20). Polymerisation is markedly reduced when other forms of haemoglobin are present. Almost 100% of the haemoglobin is in the form haemoglobin A in healthy adults of African (or European) origin, but in some ethnic groups (from Saudi Arabia), fetal haemoglobin (haemoglobin F, which contains two α- and two γ-globin chains) persists into adulthood. Such individuals, if they inherit the sickle cell gene, suffer a milder form of the illness. Since all adults possess the gene to make γ-globin, a means to turn it back on again might ameliorate the course of sickle cell disease.

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare and previously untreatable form of haemolytic anaemia caused by clonal expansion of haemopoietic stem cells with somatic mutations in an X-linked gene *PIG-A*. The mutation prevents formation of glycophosphatidylinositol (GPI) which anchors many proteins to the cell surface. Haemolysis is a feature of PNH because of the absence of a GPI-linked protein, CD59, which blocks the formation of the terminal complement complex (the membrane attack complex) on the cell surface. In addition to anaemia, patients with PNH suffer from other features including thrombosis, attacks of abdominal pain and pulmonary hypertension (Ch. 22).

Eculizumab, now licensed for clinical use, is a humanised monoclonal antibody which blocks the terminal complement protein C5 (Ch. 17). In a double-blind, randomised, controlled trial in 87 patients, treatment with eculizumab dramatically reduced haemolysis and transfusion requirement during 6 months of treatment (Fig. 25.4). Patients must be innoculated against meningococcal infection before treatment. It is administered by intravenous infusion weekly for 4 weeks and then approximately every 2 weeks. Serious adverse effects include infection, notably meningococcal infection, but are uncommon. The commonest adverse effects are headache and back pain.

In most forms of haemolytic anaemia, treatment is symptomatic (e.g. analgesia for painful crises in patients with sickle cell disease) and supportive (e.g. attention to fluid balance, oxygen therapy, blood transfusion when essential, treatment of iron overload, provision of adequate folate to support increased red cell turnover and, in some cases, antibiotics and immunisation). Acute haemolytic anaemia associated with autoantibodies may respond to treatment with glucocorticoids (Ch. 32).

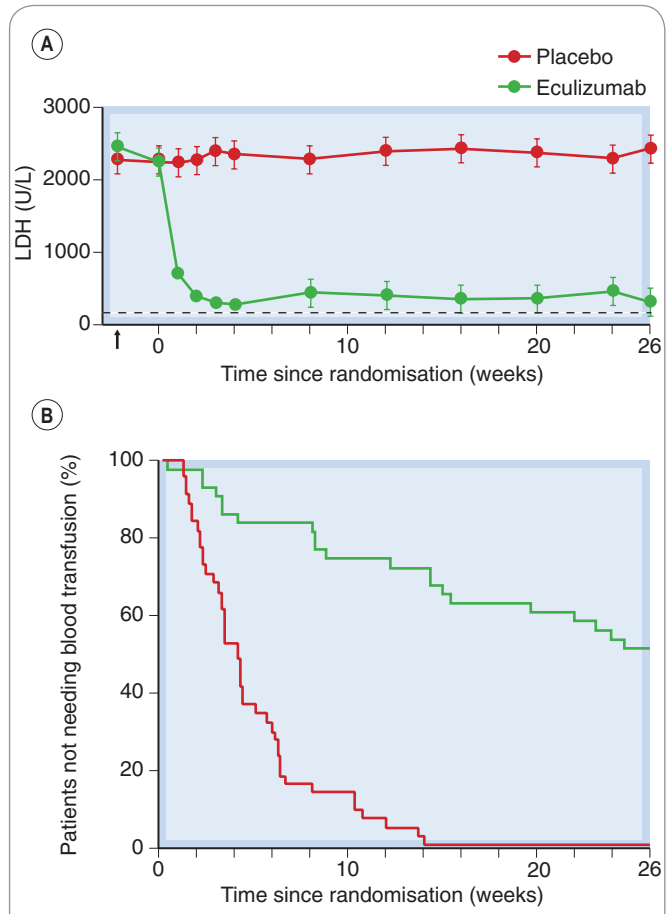

Fig 25.4 Effect of eculizumab in patients with paroxysmal nocturnal haemoglobinuria (PNH). [**A**] Effect on plasma lactate dehydrogenase (LDH) activity, a measure of haemolysis. The horizontal dotted line shows the upper limit of normal. The arrow shows the baseline level at screening (n = 44 in placebo group, n = 43 in eculizumab group, $P < 0.001$). [**B**] Kaplan–Meier curves for the time to first transfusion during treatment in the same patients shown in [**A**] ($P < 0.001$). (Redrawn from Hillmen et al. 2006 NEJM 355: 1233–43.)

HYDROXYCARBAMIDE

Hydroxycarbamide (previously known as hydroxyurea) is a cytotoxic drug that has been used for decades to lower the red cell and platelet counts in patients with *polycythaemia rubra vera* (a myeloproliferative disorder affecting especially the red cell lineage) or to treat chronic myeloid leukemia. It shifts haemoglobin production from haemoglobin S to haemoglobin F (for further details see Platt, 2008). In one randomised, controlled trial in 499 adults with sickle cell anaemia, hydroxycarbamide ameliorated the clinical course with fewer painful crises and less need for transfusion. There were no serious adverse

effects, but long-term safety is uncertain (Charache et al., 1995).

Mechanism of action

Hydroxycarbamide inhibits DNA synthesis by inhibiting *ribonucleotide reductase* and is S-phase specific (Ch. 5). Consequently, it is relatively selective for rapidly dividing red cells and reduces the production of red cells containing haemoglobin S while favouring production of red cells containing a high concentration of haemoglobin F (rapidly dividing F cells). Hydroxycarbamide metabolism gives rise to nitric oxide, which may contribute to its beneficial effect in sickle cell disease. Some of its beneficial effect in reducing painful crises could relate to anti-inflammatory effects secondary to its cytotoxic action.

Administration and unwanted effects

Hydroxycarbamide is administered by mouth once daily in rather lower starting dose than is used for treating malignant disease; reduced doses are used in patients with impaired renal function. The blood count and haemoglobin F are monitored and the dose adjusted accordingly. Once stabilised, treatment may be continued indefinitely.

Myelosuppression, nausea and rashes are the commonest adverse effects. Secondary leukaemias have occurred during treatment of myeloproliferative disorders, but it is unknown if this is drug related rather than part of the natural history of these disorders. Animal studies demonstrated teratogenicity, and potential adverse effects on spermatogenesis.

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