46 Antidepressant drugs

OVERVIEW

Depression is an extremely common psychiatric condition, about which a variety of neurochemical theories exist, and for which a corresponding variety of different types of drug are used in treatment. It is a field in which therapeutic empiricism has led the way, with mechanistic understanding tending to lag behind, part of the difficulty being that animal models cannot address the mood changes that define the human condition. In this chapter, we discuss the current understanding of the nature of the disorder, and describe the major drugs that are used to treat it.

THE NATURE OF DEPRESSION

Depression is the most common of the *affective disorders* (defined as disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Worldwide, depression is a major cause of disability and premature death. In addition to the significant suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer. Depression is a heterogeneous disorder with patients presenting with one or more core symptoms and depression is often associated with other psychiatric conditions including anxiety, eating disorders and drug addiction.

The symptoms of depression include emotional and biological components. Emotional symptoms include:

- low mood, excessive rumination of negative thought, misery, apathy and pessimism
- low self-esteem: feelings of guilt, inadequacy and ugliness
- indecisiveness, loss of motivation
- anhedonia, loss of reward.

Biological symptoms include:

- retardation of thought and action
- loss of libido
- sleep disturbance and loss of appetite.

There are two distinct types of depressive syndrome, namely *unipolar depression*, in which the mood changes are always in the same direction, and *bipolar affective disorder*, in which depression alternates with mania. Mania is in most respects exactly the opposite, with excessive exuberance, enthusiasm and self-confidence, accompanied by impulsive actions, these signs often being combined with irritability, impatience and aggression, and sometimes with grandiose delusions of the Napoleonic kind. As with depression, the mood and actions are inappropriate to the circumstances.

Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life events, and usually accompanied by symptoms of anxiety and agitation; this type is sometimes termed *reactive depression*. Other cases (about 25%, sometimes termed *endogenous depression*) show a familial pattern, unrelated to obvious external stresses, and with a somewhat different symptomatology. This distinction is made clinically, but there is little evidence that antidepressant drugs show significant selectivity between these conditions.

Bipolar depression, which usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. It can be difficult to differentiate between mild bipolar depression and unipolar depression. Also, bipolar manic episodes can be confused with episodes of psychosis (see Ch. 45). There is a strong hereditary tendency, but no specific susceptibility genes have been identified either by genetic linkage studies of affected families, or by comparison of affected and non-affected individuals.

Depression cannot be attributed to altered neuronal activity within a single brain region. Brain imaging studies have indicated that the prefrontal cortex, amygdala and hippocampus may all be involved in different components of these disorders.

THEORIES OF DEPRESSION

THE MONOAMINE THEORY

The main biochemical theory of depression is the monoamine hypothesis, first proposed by Schildkraut in 1965, which states that depression is caused by a functional deficit of the monoamine transmitters, noradrenaline and 5-hydroxytryptamine (5-HT) at certain sites in the brain, while mania results from a functional excess. For reviews of the evolving status of the theory, see Maes & Meltzer (1995) and Manji et al. (2001).

The monoamine hypothesis grew originally out of associations between the clinical effects of various drugs that cause or alleviate symptoms of depression and their known neurochemical effects on monoaminergic transmission in the brain. This pharmacological evidence, which is summarised in Table 46.1, gives general support to the monoamine hypothesis, although there are several anomalies. Attempts to obtain more direct evidence, by studying monoamine metabolism in depressed patients or by measuring changes in the number of monoamine receptors in postmortem brain tissue, have tended to give inconsistent and equivocal results, and the interpretation of these studies is often problematic, because the changes described are not specific to depression. Similarly, investigation by functional tests of the activity of known monoaminergic pathways (e.g. those controlling pituitary hormone release) in depressed patients have also given equivocal results.

The pharmacological evidence does not enable a clear distinction to be drawn between the noradrenaline and

Table 46.1 Pharmacological evidence supporting the monoamine hypothesis of depression

Drug(s)	Principal action	Effect in depressed patients
Tricyclic antidepressants	Block noradrenaline and 5-HT reuptake	Mood ↑
Monoamine oxidase (MAO) inhibitors	Increase stores of noradrenaline and 5-HT	Mood ↑
Reserpine	Inhibits noradrenaline and 5-HT storage	Mood ↓
α-Methyltyrosine	Inhibits noradrenaline synthesis	Mood ↓ (calming of manic patients)
Methyldopa	Inhibits noradrenaline synthesis	Mood ↓
Electroconvulsive therapy	? Increases central nervous system responses to noradrenaline and 5-HT	Mood ↑
Tryptophan (5-hydroxytryptophan)	Increases 5-HT synthesis	Mood ? ↑ in some studies
Tryptophan depletion	Decreases brain 5-HT synthesis	Induces relapse in SSRI-treated patients

5-HT, 5-hydroxytryptamine; SSRI, selective serotonin reuptake inhibitor.

5-HT theories of depression. Clinically, it seems that inhibitors of noradrenaline reuptake and of 5-HT reuptake are equally effective as antidepressants (see below), although individual patients may respond better to one or the other.

Other evidence in support of the monoamine theory is that agents known to block noradrenaline or 5-HT synthesis consistently reverse the therapeutic effects of antidepressant drugs that act selectively on these two transmitter systems (see Table 46.1).

Any theory of depression has to take account of the fact that the direct neurochemical effects of antidepressant drugs appear very rapidly (minutes to hours), whereas their antidepressant effects take weeks to develop. A similar situation exists in relation to antipsychotic drugs (Ch. 45) and some anxiolytic drugs (Ch. 43), suggesting that the secondary, adaptive changes in the brain, rather than the primary drug effect, are responsible for the clinical improvement. Rather than thinking of the monoamine deficiency as causing direct changes in the activity of putative 'happy' or 'sad' neurons in the brain, we should think of the monoamines as regulators of longer-term trophic effects, whose time course is paralleled by mood changes.

With improved neuroimaging methods for studying neurotransmitter function in the living human brain, as described in Chapter 36, our understanding of the causes of depression and how drugs can alleviate depression should improve.

NEUROENDOCRINE MECHANISMS

Various attempts have been made to test for a functional deficit of monoamine pathways in depression. Hypothalamic neurons controlling pituitary function receive noradrenergic and 5-HT inputs, which control the discharge of these cells. Hypothalamic cells release corticotrophin-releasing hormone (CRH), which stimulates pituitary cells to secrete adrenocorticotrophic hormone (ACTH), leading in turn to cortisol secretion. The plasma cortisol concentration is usually high in depressed patients, and it fails to respond with the normal fall when a synthetic steroid, such as **dexamethasone**, is given. This formed the basis of a clinical test, the *dexamethasone suppression test* (also used in the diagnosis of Cushing's syndrome; see Ch. 32). Other hormones in plasma are also affected, for example growth hormone concentration is reduced and prolactin is increased. While these changes are consistent with deficiencies in monoamine transmission, they are not specific to depressive syndromes.

Corticotrophin-releasing hormone is widely distributed in the brain and has behavioural effects that are distinct from its endocrine functions. Injected into the brain of experimental animals, CRH mimics some effects of depression in humans, such as diminished activity, loss of appetite and increased signs of anxiety. Furthermore, CRH concentrations in the brain and cerebrospinal fluid of depressed patients are increased. Therefore CRH hyperfunction, as well as monoamine hypofunction, may be associated with depression (see Holsboer, 1999). Raised CRH levels are associated with stress and, in many cases, depression is preceded by periods of chronic stress.

TROPHIC EFFECTS AND NEUROPLASTICITY

It has been suggested that lowered levels of brain-derived neurotrophic factor (BDNF) or malfunction of its receptor, TrkB, plays a significant role in the pathology of this condition. Depressive behaviour is often associated with a reduction in BDNF expression and treatment with antidepressants elevates BDNF levels.

Changes in glutamatergic neurotransmission may also be involved in depression. Sufferers from depression have been shown to have elevated cortical levels of glutamate. Antidepressant treatment may reduce glutamate release and depress NMDA receptor function. The effects of antidepressants on activity-induced long-term potentiation (LTP; see Ch. 37) at hippocampal glutamatergic synapses is complex—both depression and facilitation have been observed and may occur with acute antidepressant administration, thus calling into question the relevance to the therapeutic response.

Another view (see Charney & Manji, 2004; Duman, 2004; Racagni & Popoli, 2008) is that major depression is associated with neuronal loss in the hippocampus and prefrontal cortex, and that antidepressant therapies of different kinds act by inhibiting or actually reversing this loss by stimulat-

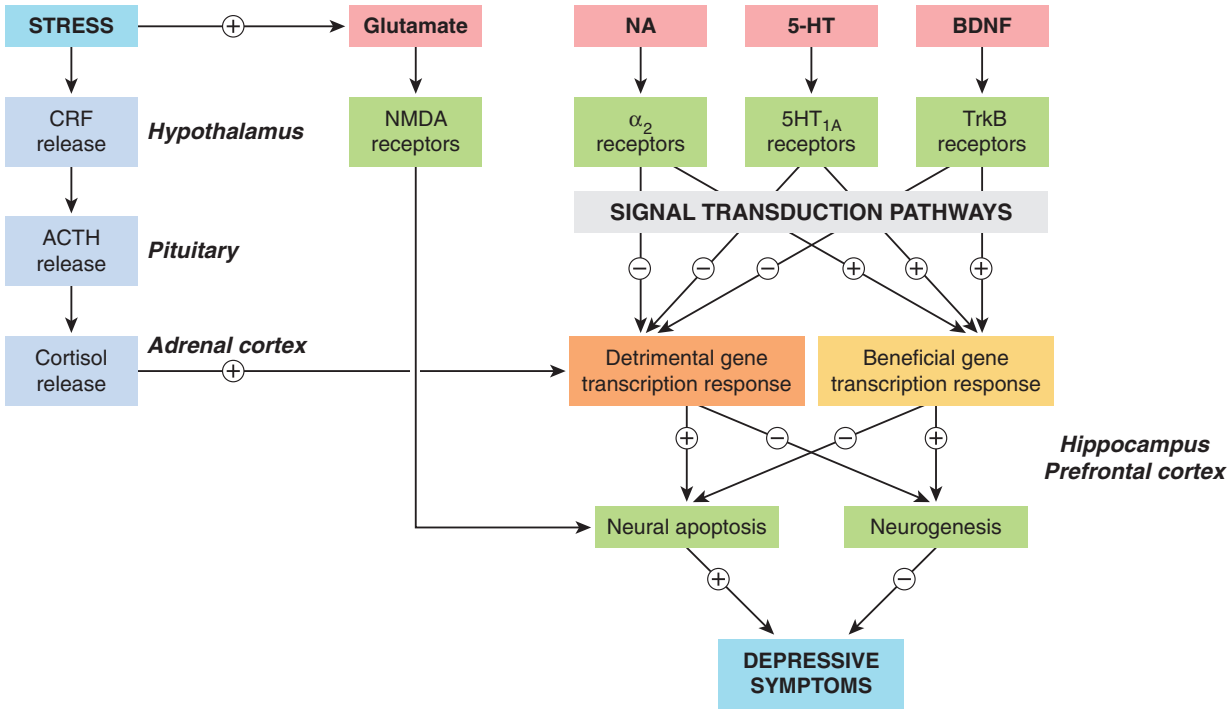

Fig. 46.1 Simplified diagram showing mechanisms believed to be involved in the pathophysiology of depression. The main prodepressive pathways involve the hypothalamic–pituitary–adrenal axis, which is activated by stress and in turn enhances the excitotoxic action of glutamate, mediated by NMDA receptors (see Ch. 37), and switches on the expression of genes that promote neural apoptosis in the hippocampus and prefrontal cortex. The antidepressive pathways involve the monoamines noradrenaline (NA) and 5-hydroxytryptamine (5-HT), which act on G-protein-coupled receptors, and the brain-derived neurotrophic factor (BDNF), which acts on a kinase-linked receptor (TrkB), switching on genes that protect neurons against apoptosis and also promote neurogenesis. For further detail, see Charney & Manji (2004). ACTH, adrenocorticotrophic hormone; CRF, corticotrophin-releasing factor.

ing neurogenesis.[1] This surprising idea is supported by various lines of evidence:

- Brain imaging and postmortem studies show ventricular enlargement as well as shrinkage of the hippocampus and prefrontal cortex of depressed patients, with loss of neurons and glia. Functional imaging reveals reduced neuronal activity in these regions.
- In animals, the same effect is produced by chronic stress of various kinds, or by administration of glucocorticoids, mimicking the increased cortisol secretion in human depression. Excessive glucocorticoid secretion in humans (Cushing's syndrome; see Ch. 32) often causes depression.
- In experimental animals, antidepressant drugs, or other treatments such as electroconvulsions (see later section on Brain Stimulation Therapies), promote neurogenesis in these regions, and (as in humans) restore functional activity. Preventing hippocampal neurogenesis prevents the behavioural effects of antidepressants in rats (Santarelli et al., 2003).
- 5-HT and noradrenaline, whose actions are enhanced by many antidepressants, promote neurogenesis probably through activation of 5-HT_{1A} receptors and α_2 adrenoceptors, respectively. This effect may be mediated by BDNF.
- Exercise has also been shown to promote neurogenesis in animals and to be effective in some patients with mild to moderate depression.

Figure 46.1 summarises the possible mechanisms involved. It should be stressed that these hypotheses are far from proven, but the diagram emphasises the way in which the field has moved on since the formulation of the monoamine hypothesis, suggesting a range of possible targets for the next generation of antidepressant drugs.[2]

[1]Neurogenesis (see Ch. 39)—the formation of new neurons from stem cell precursors—occurs to a significant degree in the adult hippocampus, and possibly elsewhere in the brain, contradicting the old dogma that it occurs only during brain development.

[2]Cynics may feel that these mechanisms, in which glutamate, neurotrophic factors, monoamines and steroids all interact to control neuronal death, survival and plasticity, are being invoked just as enthusiastically to account for almost every neurological and psychiatric disorder that you can think of, from stroke and Parkinson's disease to schizophrenia. 'Are we missing something,' they may feel, 'or are all these diseases basically the same? If so, why are their effects so different? Is this just a scientific bandwagon, or does this mechanistic convergence point to some fundamental principles of neural organisation?' We do not have the answers, of course, but it is a field worth watching.

Monoamine theory of depression

- The monoamine theory, first proposed in 1965, suggests that depression results from functionally deficient monoaminergic (noradrenaline and/or 5-hydroxytryptamine) transmission in the central nervous system.
- The theory is based on the ability of known antidepressant drugs (tricyclic antidepressants and monoamine oxidase inhibitors) to facilitate monoaminergic transmission, and of drugs such as **reserpine** to cause depression.
- Biochemical studies on depressed patients do not clearly support the monoamine hypothesis in its simple form.
- An abnormally weak response of plasma cortisol to exogenous steroid (dexamethasone suppression test) is common in depression and may reflect defective monoamine transmission in the hypothalamus.
- Recent evidence suggests that depression may be associated with neurodegeneration and reduced neurogenesis in the hippocampus.
- Although the monoamine hypothesis in its simple form is insufficient as an explanation of depression, pharmacological manipulation of monoamine transmission remains the most successful therapeutic approach.
- Current approaches focus on other mediators (e.g. corticotrophin-releasing hormone), signal transduction pathways, growth factors, etc., but theories remain imprecise.

Types of antidepressant drugs

- Main types are:
  - monoamine uptake inhibitors (tricyclic antidepressants, selective serotonin reuptake inhibitors, newer inhibitors of noradrenaline and 5-HT reuptake)
  - monoamine receptor antagonists
  - monoamine oxidase (MAO) inhibitors.
- Monoamine uptake inhibitors act by inhibiting uptake of noradrenaline and/or 5-HT by monoaminergic nerve terminals.
- α_2 Adrenoceptor antagonists can indirectly elevate 5-HT release.
- MAO inhibitors inhibit one or both forms of brain MAO, thus increasing the cytosolic stores of noradrenaline and 5-HT in nerve terminals. Inhibition of type A MAO correlates with antidepressant activity. Most are non-selective; **moclobemide** is specific for MAO-A.
- All types of antidepressant drug appear to take at least 2 weeks to produce any beneficial effects, even though their pharmacological effects are produced immediately, indicating that secondary adaptive changes are important.
- The most consistent adaptive change seen with different types of antidepressant drugs is downregulation of β- and α_2 adrenoceptors, as well as 5-HT_2 receptors. How this is related to therapeutic effect is not clear.
- Recent evidence suggests that antidepressants may act by increasing neurogenesis in the hippocampus and other brain areas.

ANTIDEPRESSANT DRUGS

TYPES OF ANTIDEPRESSANT DRUG

Antidepressant drugs fall into the following categories.

Inhibitors of monoamine uptake

- Selective serotonin (5-HT) reuptake inhibitors (SSRIs) (e.g. **fluoxetine**, **fluvoxamine**, **paroxetine**, **sertraline**, **citalopram**, **escitalopram**).
- Classical tricyclic antidepressants (TCAs) (e.g. **imipramine**, **desipramine**, **amitriptyline**, **nortriptyline**, **clomipramine**). These vary in their ability to inhibit noradrenaline and 5-HT reuptake.
- Newer, mixed 5-HT and noradrenaline reuptake inhibitors (e.g. **venlafaxine** [somewhat selective for 5-HT, although less so than SSRIs], **desvenlafaxine**, **duloxetine**, **milnacipran**).
- Noradrenaline reuptake inhibitors (e.g. **bupropion**, **reboxetine**, **atomoxetine**).
- The herbal preparation St John's wort, whose main active ingredient is hyperforin: it has similar clinical efficacy to most of the prescribed antidepressants. It is a weak monoamine uptake inhibitor but also has other actions.[3]

Monoamine receptor antagonists

- Drugs such as **mirtazapine**, **trazodone**, **mianserin** are non-selective and inhibit a range of amine receptors including α_2 adrenoceptors and 5-HT_2 receptors. They may also have weak effects on monoamine uptake.

Monoamine oxidase inhibitors (MAOIs)

- Irreversible, non-competitive inhibitors (e.g. **phenelzine**, **tranylcypromine**), which are non-selective with respect to the MAO-A and -B subtypes.
- Reversible, MAO-A-selective inhibitors (e.g. **moclobemide**).

Table 46.2 summarises the main features of these types of drug. Recent updates (Bosker et al., 2004; Pacher & Kecsemeti, 2004; Stahl, 2008) provide more detail. Mention should also be made of electroconvulsive therapy (ECT), electromagnetic therapy, deep brain stimulation and vagus stimulation, which are effective and usually act more rapidly than antidepressant drugs (see later section).

[3]Although relatively free of acute side effects, hyperforin activates cytochrome P450, resulting in loss of efficacy, with serious consequences, of several important drugs, including ciclosporin, oral contraceptives, some anti-HIV and anticancer drugs, and oral anticoagulants—underlining the principle that herbal remedies need to be used with the same degree of informed caution as any other drug.

Table 46.2 Types of antidepressant drugs and their characteristics

Type and examples	Action(s)	Unwanted effects	Risk of overdose	Pharmacokinetics	Notes
Monoamine uptake inhibitors					
(1) SSRIs	All highly selective for 5-HT	Nausea, diarrhoea, agitation, insomnia, anorgasmia Inhibit metabolism of other drugs, so risk of interactions	Low risk in overdose but must not be used in combination with MAO inhibitors	—	—
Fluoxetine	As above	As above	As above	Long $t_{1/2}$ (24–96 h)	—
Fluvoxamine	As above	As above	As above	$t_{1/2}$ 18–24 h	Less nausea than with other SSRIs
Paroxetine	As above	As above	As above	$t_{1/2}$ 18–24 h	Withdrawal reaction
Citalopram	As above	As above	As above	$t_{1/2}$ 24–36 h	—
Escitalopram	As above	As above	As above	$t_{1/2}$ 24–36 h	Active *S* isomer of citalopram Fewer side effects
Sertraline	As above	As above	As above	$t_{1/2}$ 24–36 h	—
(2) Classical TCA group	Inhibition of NA and 5-HT reuptake	Sedation Anticholinergic effects (dry mouth, constipation, blurred vision, urinary retention, etc.) Postural hypotension Seizures Impotence Interaction with CNS depressants (especially alcohol, MAO inhibitors)	Ventricular dysrhythmias High risk in combination with CNS depressants	—	'First-generation' antidepressants, still very widely used, although newer compounds generally have fewer side effects and lower risk with overdose
Imipramine	Non-selective Converted to desipramine	As above	As above	$t_{1/2}$ 4–18 h	—
Desipramine	NA selective	As above	As above	$t_{1/2}$ 12–24 h	—
Amitriptyline	Non-selective	As above	As above	$t_{1/2}$ 12–24 h; converted to nortriptyline	Widely used, also for neuropathic pain (Ch. 41)
Nortriptyline	NA selective (slight)	As above	As above	Long $t_{1/2}$ (24–96 h)	Long duration, less sedative
Clomipramine	Non-selective	As above	As above	$t_{1/2}$ 18–24 h	Also used for anxiety disorders
(3) Other 5-HT/NA uptake inhibitors					
Venlafaxine	Weak non-selective NA/5-HT uptake inhibitor Also non-selective receptor-blocking effects	As SSRIs Withdrawal effects common and troublesome if doses are missed	Safe in overdose	Short $t_{1/2}$ (~5 h) Converted to desvenlafaxine which inhibits NA uptake	Claimed to act more rapidly than other antidepressants, and to work better in 'treatment-resistant' patients Usually classed as non-selective NA/5-HT uptake blocker, although in vitro data show selectivity for 5-HT

Duloxetine	Potent non-selective NA/5-HT uptake inhibitor No action on monoamine receptors	Fewer side effects than venlafaxine Sedation, dizziness, nausea Sexual dysfunction	See SSRIs above	$t_{1/2}$ ~14 h	Also used to treat urinary incontinence (see Ch. 28) and for anxiety disorders
Milnacipran	NA-selective (slight)	Fewer than TCAs	See SSRIs above	$t_{1/2}$ ~8 h	Unlike SSRIs, does not depress sexual function
St John's wort (active principle: hyperforin)	Weak non-selective NA/5-HT uptake inhibitor Also non-selective receptor-blocking effects	Few side effects reported Risk of drug interactions due to enhanced drug metabolism (e.g. loss of efficacy of ciclosporin, antidiabetic drugs, etc.)		$t_{1/2}$ ~12 h	Freely available as crude herbal preparation Similar efficacy to other antidepressants, with fewer acute side effects but risk of serious drug interactions
NA-selective inhibitors					
Bupropion	Selective inhibitor of NA over 5-HT uptake but also inhibits dopamine uptake Converted to active metabolites (e.g. radafaxine)	Headache, dry mouth, agitation, insomnia	Seizures at high doses	$t_{1/2}$ ~12 h Plasma half-life ~20 h	Used mainly in depression associated with anxiety Slow-release formulation used to treat nicotine dependence (Ch. 48)
Maprotiline	Selective NA uptake inhibitor	As TCAs; no significant advantages	As TCAs	Long $t_{1/2}$ ~40 h	No significant advantages over TCAs
Reboxetine	Selective NA uptake inhibitor	Dizziness Insomnia Anticholinergic effects	Safe in overdose (low risk of cardiac dysrhythmia)	$t_{1/2}$ ~12 h	Less effective than TCAs The related drug atomoxetine now used mainly to treat ADHD (Ch. 47)
(4) Monoamine receptor antagonists					
Mirtazapine	Blocks α_2, 5-HT_{2C} and 5-HT_3 receptors	Dry mouth Sedation Weight gain	No serious drug interactions	$t_{1/2}$ 20–40 h	Claimed to have faster onset of action than other antidepressants
Trazodone	Blocks 5-HT_{2A} and 5-HT_{2C} receptors as well as H_1 receptors Weak 5-HT uptake inhibitor (enhances NA/5-HT release)	Sedation Hypotension Cardiac dysrhythmias	Safe in overdose	$t_{1/2}$ 6–12 h	Nefazodone is similar

Table 46.2 (cont'd) Types of antidepressant drugs and their characteristics

Type and examples	Action(s)	Unwanted effects	Risk of overdose	Pharmacokinetics	Notes
Mianserin	Blocks α_1, α_2, 5-HT_{2A} and H_1 receptors	Milder antimuscarinic and cardiovascular effects than TCAs Agranulocytosis, aplastic anaemia	—	$t_{1/2}$ 10–35 h	Blood count advised in early stages of use
MAO inhibitors					
	Inhibit MAO-A and/or MAO-B Earlier compounds have long duration of action due to covalent binding to enzyme				
Phenelzine	Non-selective	'Cheese reaction' to tyramine-containing foods (see text) Anticholinergic side effects Hypotension Insomnia Weight gain Liver damage (rare)	Many interactions (TCAs, opioids, sympathomimetic drugs)—risk of severe hypertension due to cheese reaction	$t_{1/2}$ 1–2 h Long duration of action due to irreversible binding	—
Tranylcypromine	Non-selective	As phenelzine	As phenelzine	$t_{1/2}$ 1–2 h Long duration of action due to irreversible binding	—
Isocarboxazid	Non-selective	As phenelzine	As phenelzine	Long $t_{1/2}$ ~36 h	—
Moclobemide	MAO-A selective Short acting	Nausea, insomnia, agitation	Interactions less severe than with other MAO inhibitors; no cheese reactions reported	$t_{1/2}$ 1–2 h	Safer alternative to earlier MAO inhibitors

5-HT, 5-hydroxytryptamine; ADHD, attention-deficit hyperactivity disorder; CNS, central nervous system; MAO, monoamine oxidase; NA, noradrenaline; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

Table 46.3 Animal models used to study depression

Model	Description
Forced swim test (Porsolt test)	Classical model for antidepressant efficacy. Rodents are placed in an inescapable container of water on two occasions. On the second test, acute antidepressant drugs increase the escape behaviour Provides good assessment of efficacy for monoaminergic antidepressant drugs. Effects are seen after acute treatment unlike the delayed effects seen in humans
Modified swim test	Same basic test as above but separates swimming versus climbing behaviour to dissociate between serotonergic and catecholaminergic activity
Tail suspension test	Primarily used for mice. The animal is suspended from the tail and the time to an immobile posture recorded
Learned helplessness	Rodents are exposed to repeated inescapable foot shock resulting in a failure to subsequently escape when able to Antidepressant drugs increase the number of escapes after conditioning. Acute effects with antidepressants are observed but not all animals develop the response
Olfactory bulbectomy	Removal of the olfactory bulbs in rats causes behavioural and neurochemical changes that reflect symptoms observed in depressed subjects. Responds to chronic antidepressant treatment
Maternal deprivation	Pups are removed from the dam for brief periods early postnatal which changes the maternal care of the offspring. The offspring go on to develop a phenotype that expresses behavioural, neurochemical and biochemical changes that reflect aspects of depression. Not all animals develop these changes
Chronic mild stress	Animals are subjected to a sequence of stressors over a period of ~14 days. The stressors differ each day forming a period of unpredictable chronic stress. The animals develop a range of behavioural, neurochemical and biochemical changes that reflect symptoms seen in depression. Responds to chronic antidepressant treatment

TESTING OF ANTIDEPRESSANT DRUGS

ANIMAL MODELS

Progress in unravelling the neurochemical mechanisms is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. There is no known animal condition corresponding to the inherited condition of depression in humans, but various procedures have been described that produce in animals behavioural states (withdrawal from social interaction, loss of appetite, reduced motor activity, etc.) typical of human depression (see Table 46.3 and review by Cryan & Slattery, 2007). The use of genetically modified mice to mimic various aspects of the disorder may provide interesting models (see Gardier, 2009). However, the similarity of these animal models to human depression is questionable.

TESTS ON HUMANS

Clinically, the effect of antidepressant drugs is usually measured by a subjective rating scale such as the 17-item Hamilton Rating Scale. Clinical depression takes many forms, and the symptoms vary between patients and over time. Quantitation is therefore difficult, and the many clinical trials of antidepressants have generally shown rather weak effects, after allowance for quite large placebo responses. There is also a high degree of individual variation, with 30–40% of patients failing to show any improvement, possibly due to genetic factors (see later section on Clinical Effectiveness).

MECHANISM OF ACTION OF ANTIDEPRESSANT DRUGS

CHRONIC ADAPTIVE CHANGES

Given the discrepancy between the fast onset of the neurochemical effects of antidepressant drugs and the slow onset of their antidepressant effects, efforts have been made to determine whether the therapeutic benefits arise from slow adaptive changes induced by chronic exposure to these drugs (Racagni & Popoli, 2008).

This approach led to the discovery that certain monoamine receptors, in particular β_1- and α_2 adrenoceptors, are consistently downregulated following chronic antidepressant treatment and, in some cases, by electroconvulsive therapy too. This can be demonstrated in experimental animals as a reduction in the number of binding sites, as well as by a reduction in the functional response to agonists (e.g. stimulation of cAMP formation by β-adrenoceptor agonists). Receptor downregulation probably also occurs in humans, because endocrine responses to **clonidine**, an α_2 adrenoceptor agonist, are reduced by long-term antidepressant treatment. However, the relevance of these findings to the antidepressant response is unclear. Loss of β-adrenoceptors as a factor in alleviating depression does not fit comfortably with theory, because β-adrenoceptor antagonists are not antidepressant.

On acute administration, one would expect inhibition of 5-HT uptake (e.g. by SSRIs) to increase the level of 5-HT in the synapse by inhibiting reuptake into the nerve terminals. However, the increase in synaptic 5-HT levels has been observed to be less than expected. This is because increased activation of 5-HT_{1A} receptors on the soma and dendrites of 5-HT-containing raphe neurons (Fig. 46.2A) inhibits these neurons and thus reduces 5-HT release, thus cancelling out to some extent the effect of inhibiting reuptake into the terminals. On prolonged drug treatment, the elevated level of 5-HT in the somatodendritic region desensitises the 5-HT_{1A} receptors, reducing their inhibitory effect on 5-HT release from the nerve terminals.

The need to desensitise somatodendritic 5-HT_{1A} receptors could thus explain the slow onset of antidepressant action of 5-HT uptake inhibitors. Rather than reduce

receptor function by desensitisation, it should be possible to produce the same effect simply by blocking the receptors with an antagonist. **Pindolol**, a non-selective β-adrenoceptor blocker, which also has affinity for 5-HT_{1A} receptors, has been used in conjunction with 5-HT uptake inhibitors to speed up the onset of antidepressant action (see Ballasteros & Callado, 2004). However, drugs with combined 5-HT_{1A} antagonism and SSRI properties have been developed but have not been found to be effective in man, perhaps because they block both 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors, the latter effect occluding the beneficial effect of the former.

NORADRENERGIC CONTROL OF 5-HT RELEASE

Block of presynaptic α_2 autoreceptors on noradrenergic nerve terminals throughout the CNS will reduce the negative feedback from released noradrenaline and thus

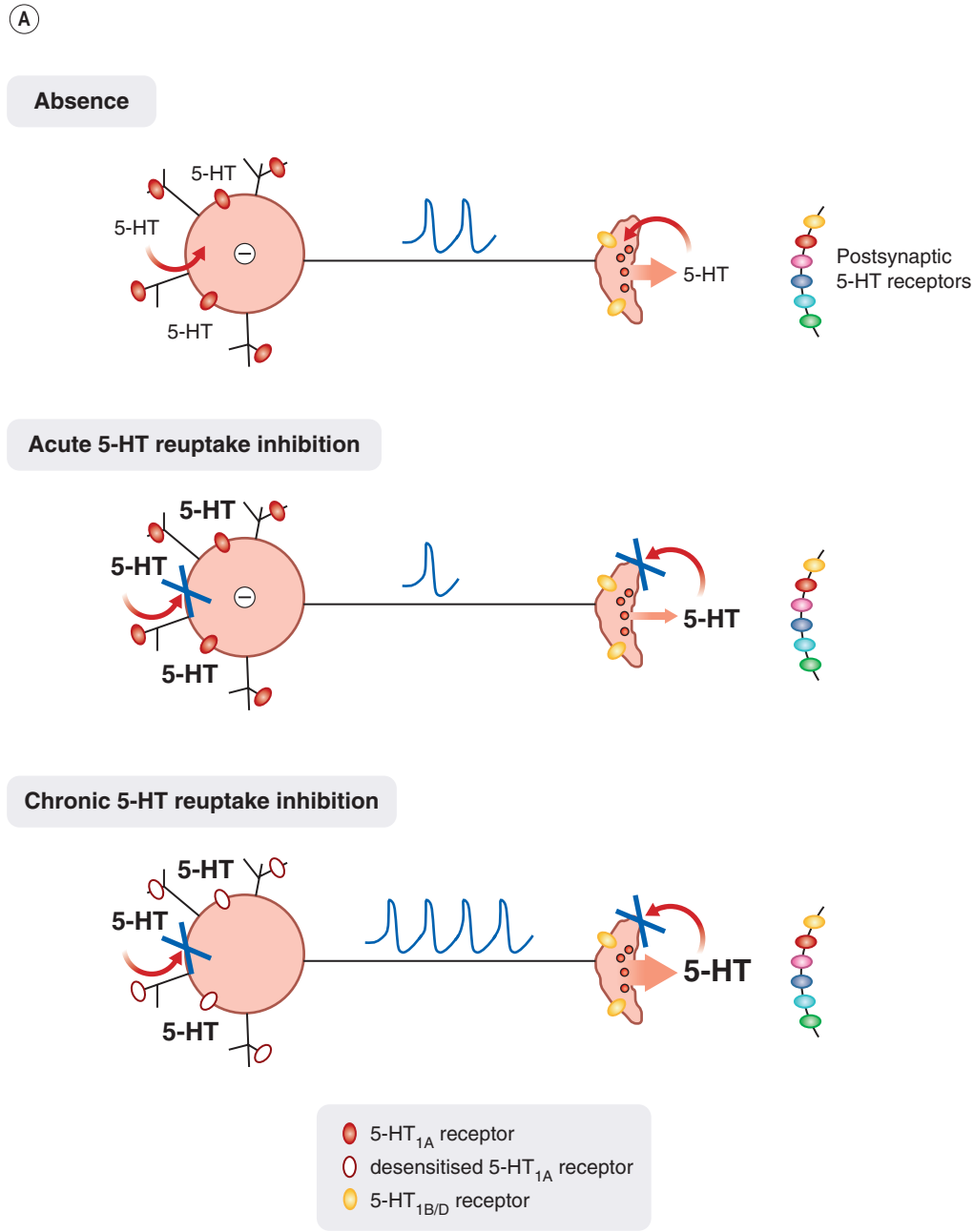

Fig. 46.2 Control of 5-HT release. [**A**] 5-HT release is controlled by the inhibitory action of 5-HT on somatodendritic 5-HT_{1A} receptors. Acute inhibition of 5-HT reuptake results in increased extracellular levels of 5-HT but this increases somatodendritic 5-HT_{1A} receptor-mediated inhibition, hence synaptic 5-HT levels do not rise as much as expected. 5-HT_{1A} receptors eventually desensitise, resulting in reduced inhibition and thus greater 5-HT release. [**B**] 5-HT release is controlled by both an excitatory action of noradrenaline (NA) on somatodendritic α_1-adrenoceptors and an inhibitory action on α_2 adrenoceptors on serotonergic nerve terminals. Block of α_2 adrenoceptors located on noradrenergic neurons (not shown) enhances noradrenaline release resulting in further excitation of serotonergic neurons, while block of α_2 adrenoceptors on serotonergic neurons removes presynaptic inhibition and thus 5-HT release is enhanced. **(Cont'd on next page)**

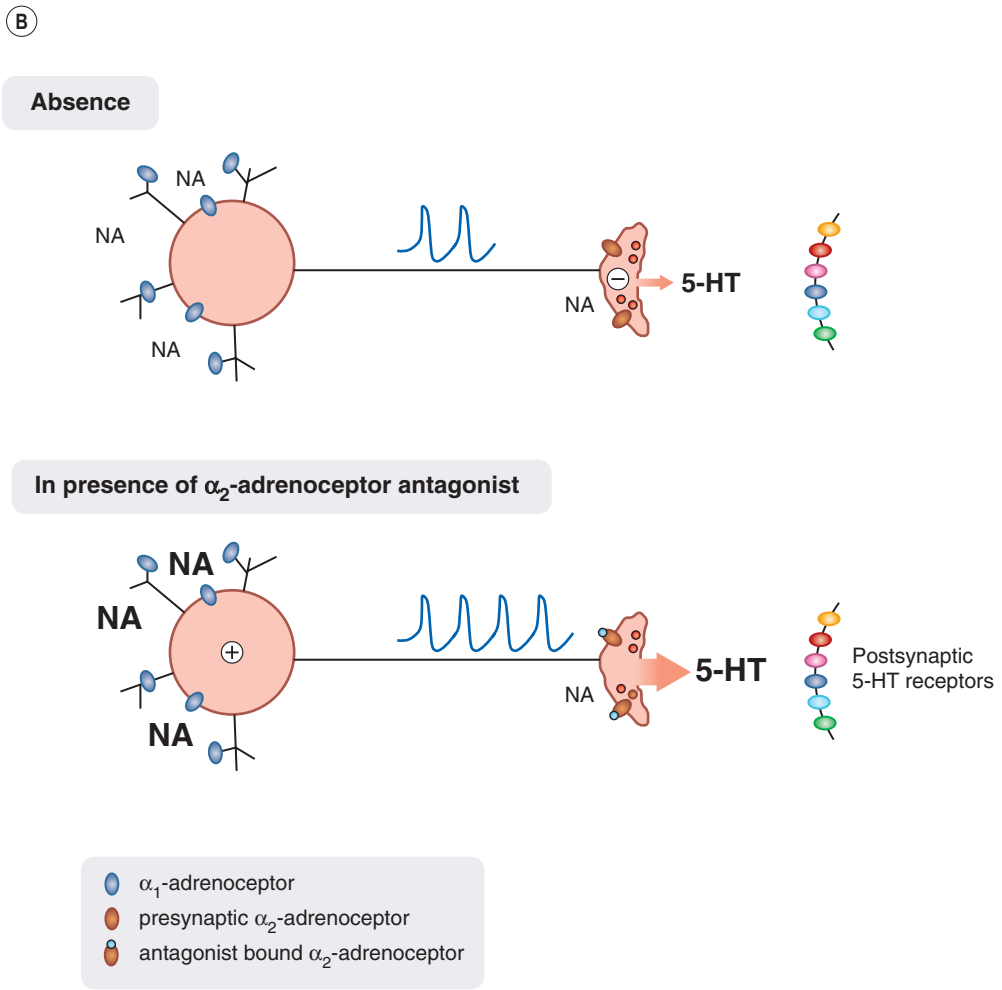

Fig. 46.2 cont'd

enhance further noradrenaline release (see Chs 14 and 36). In addition, α_2 adrenoceptor antagonists can indirectly enhance 5-HT release. This can occur in several ways (see Fig. 46.2B):

- block of inhibitory α_2 heteroreceptors on 5-HT-containing nerve terminals
- block of α_2 autoreceptors on noradrenergic nerve terminals innervating the cell bodies of 5-HT-containing neurons in the dorsal raphe. The enhanced noradrenaline release will activate excitatory postsynaptic α_1 receptors on the 5-HT-containing neurons, enhancing action potential firing and thus subsequently increasing 5-HT release.

The effect of α_2 adrenoceptor antagonists on synaptic noradrenaline and 5-HT levels would be rapid in onset and so these changes must somehow induce other, slower adaptive responses that give rise to the slowly developing antidepressant effects.

GENE EXPRESSION AND NEUROGENESIS

More recently, interest has centred on intracellular signalling pathways, changes in gene expression and neurogenesis. Much attention has been focused on how antidepressants may activate the transcription factor, CREB, a cAMP response element binding protein (see Ch. 48). The role of other transcription factors such as those of the Fos family and NF-κB have been less extensively studied. As described above, several antidepressant drugs appear to promote neurogenesis in the hippocampus, a mechanism that could account for the slow development of the therapeutic effect. The role of raised synaptic noradrenaline and 5-HT levels in inducing changes in gene expression and neurogenesis, and the mechanisms involved, await further elucidation.

MONOAMINE UPTAKE INHIBITORS

SELECTIVE 5-HYDROXYTRYPTAMINE UPTAKE INHIBITORS

Drugs of this type (often termed *selective serotonin reuptake inhibitors* or SSRIs) include **fluoxetine**, **fluvoxamine**, **paroxetine**, **citalopram**, **escitalopram** and **sertraline** (see Table 46.2). They are the most commonly prescribed group of antidepressants. As well as showing selectivity with respect to 5-HT over noradrenaline uptake (Fig. 46.3), they are less likely than TCAs to cause anticholinergic side effects and are less dangerous in overdose. In contrast to MAOIs, they do not cause 'cheese reactions'. They are as effective as TCAs and MAOIs in treating depression of moderate degree, but probably less effective than TCAs in treating severe depression. They are also used to treat anxiety disorders (see Ch. 43).

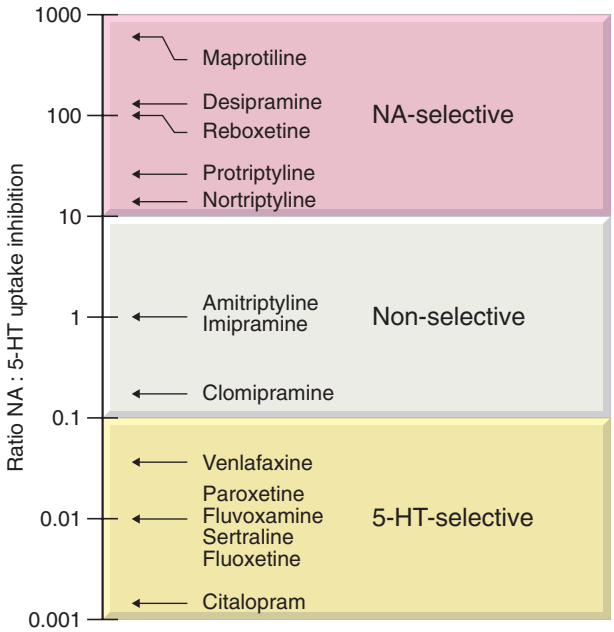

Fig. 46.3 Selectivity of inhibition of noradrenaline and 5-hydroxytryptamine uptake by various antidepressants.

Individual patients may respond more favourably to one SSRI than another. This may reflect other, pharmacological properties of each individual drug as none is devoid of other actions. Fluoxetine has 5-HT_{2C} antagonist activity, a property it shares with other non-SSRI antidepressants such as **mirtazapine**. This may also contribute to its therapeutic effect in the treatment of anorexia and bulimia. Sertraline is a weak inhibitor of dopamine uptake. Escitalopram is the *S* isomer of racemic citalopram. It lacks the antihistamine and CYP2D6 inhibitory properties of the *R* isomer.

Pharmacokinetic aspects

The SSRIs are well absorbed, and most have plasma half-lives of 18–24 h (fluoxetine is longer acting: 24–96 h). The delay of 2–4 weeks before the therapeutic effect develops is similar to that seen with other antidepressants. Paroxetine and fluoxetine are not used in combination with TCAs, whose hepatic metabolism they inhibit through an interaction with CYP2D6, for fear of increasing TCA toxicity.

Unwanted effects

Common side effects include nausea, anorexia, insomnia, loss of libido and failure of orgasm.[4] Some of these unwanted effects result from the enhanced stimulation of postsynaptic 5-HT receptors as a result of the drugs increasing the levels of extracellular 5-HT. This can be either stimulation of the wrong type of 5-HT receptor (e.g. 5-HT_2, 5-HT_3 and 5-HT_4 receptors) or stimulation of the same receptor that gives therapeutic benefit (e.g. postsynaptic 5-HT_{1A} receptors) but in the wrong brain region (i.e. enhanced stimulation of 5-HT receptors can result in both therapeutic and adverse responses).

[4]Thus, conversely, SSRIs can be used to treat premature ejaculation.

Selective serotonin reuptake inhibitors (SSRIs)

- Examples include **fluoxetine**, **fluvoxamine**, **paroxetine**, **sertraline**, **citalopram**, **escitalopram**.
- Antidepressant actions are similar in efficacy and time course to TCAs.
- Acute toxicity (especially cardiotoxicity) is less than that of MAOIs or TCAs, so overdose risk is reduced.
- Side effects include nausea, insomnia and sexual dysfunction. SSRIs are less sedating and have fewer antimuscarinic side effects than the older TCAs.
- No food reactions, but dangerous 'serotonin reaction' (hyperthermia, muscle rigidity, cardiovascular collapse) can occur if given with MAOIs.
- There is concern about the use of SSRIs in children and adolescents, due to reports of an increase in suicidal thoughts on starting treatment.
- Also used for some other psychiatric indications, e.g. anxiety.

In combination with MAOIs, SSRIs can cause a 'serotonin syndrome' characterised by tremor, hyperthermia and cardiovascular collapse, from which deaths have occurred.

There have been reports of increased aggression, and occasionally violence, in patients treated with fluoxetine, but these have not been confirmed by controlled studies. The use of SSRIs is not recommended for treating depression in children under 18, in whom efficacy is doubtful and adverse effects, including excitement, insomnia and aggression in the first few weeks of treatment, may occur. The possibility of increased suicidal ideation is a concern in this age group (see below).

Despite the apparent advantages of 5-HT uptake inhibitors over TCAs in terms of side effects, the combined results of many trials show little overall difference in terms of patient acceptability (Song et al., 1993; Cipriani et al., 2009).

5-HT uptake inhibitors are used in a variety of other psychiatric disorders, as well as in depression, including anxiety disorders and obsessive compulsive disorder (see Ch. 43).

TRICYCLIC ANTIDEPRESSANT DRUGS

Tricyclic antidepressants (TCAs; **imipramine**, **desipramine**, **amitriptyline**, **nortriptyline**, **clomipramine**) are still widely used. They are, however, far from ideal in practice, and it was the need for drugs that act more quickly and reliably, produce fewer side effects and are less hazardous in overdose that led to the introduction of newer 5-HT reuptake inhibitors and other antidepressants.

TCAs are closely related in structure to the phenothiazines (Ch. 45) and were initially synthesised (in 1949) as potential antipsychotic drugs. Several are tertiary amines, with two methyl groups attached to the basic nitrogen atom. They are quite rapidly demethylated in vivo (Fig. 46.4) to the corresponding secondary amines (e.g. imipramine to desipramine, amitriptyline to nortriptyline), which are themselves active and may be administered as drugs in their own right. Other tricyclic derivatives with

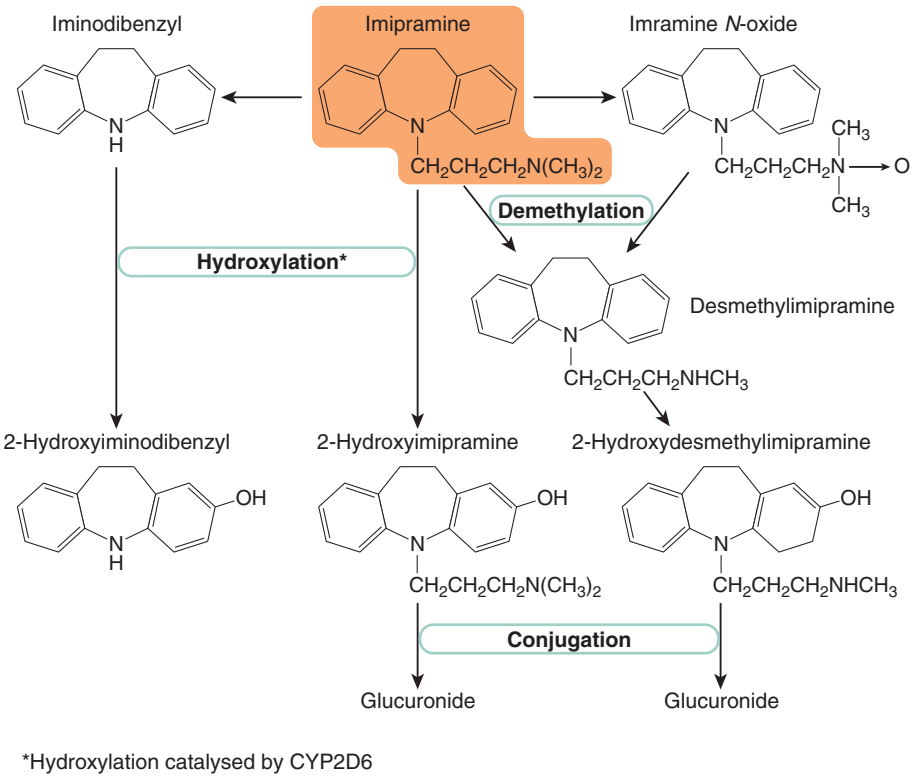

Fig. 46.4 Metabolism of imipramine, which is typical of that of other tricyclic antidepressants. The hydroxylating enzyme, CYP2D6, is subject to genetic polymorphism, which may account for individual variation in response to tricyclic antidepressants (see Ch. 11).

slightly modified bridge structures include **doxepin**. The pharmacological differences between these drugs are not very great and relate mainly to their side effects, which are discussed below.

TCAs are also used to treat neuropathic pain (see Ch. 41).

Mechanism of action

As discussed above, the main immediate effect of TCAs is to block the uptake of amines by nerve terminals, by competition for the binding site of the amine transporter (Ch. 14). Most TCAs inhibit noradrenaline and 5-HT uptake (Fig. 46.3) but have much less effect on dopamine uptake. It has been suggested that improvement of emotional symptoms reflects mainly an enhancement of 5-HT-mediated transmission, whereas relief of biological symptoms results from facilitation of noradrenergic transmission. Interpretation is made difficult by the fact that the major metabolites of TCAs have considerable pharmacological activity (in some cases greater than that of the parent drug) and often differ from the parent drug in respect of their noradrenaline/5-HT selectivity (Table 46.4).

In addition to their effects on amine uptake, most TCAs affect other receptors, including muscarinic acetylcholine receptors, histamine receptors and 5-HT receptors. The antimuscarinic effects of TCAs do not contribute to their antidepressant effects but are responsible for various side effects.

Unwanted effects

In non-depressed human subjects, TCAs cause sedation, confusion and motor incoordination. These effects occur also in depressed patients in the first few days of treatment, but tend to wear off in 1–2 weeks as the antidepressant effect develops.

Tricyclic antidepressants produce a number of troublesome side effects, mainly due to interference with autonomic control.

Atropine-like effects include dry mouth, blurred vision, constipation and urinary retention. These effects are strong with amitriptyline and much weaker with desipramine. Postural hypotension occurs with TCAs. This may seem anomalous for drugs that enhance noradrenergic transmission, and possibly results from an effect on adrenergic transmission in the medullary vasomotor centre. The other

Table 46.4 Inhibition of neuronal noradrenaline and 5-HT uptake by tricyclic antidepressants and their metabolites

Drug/metabolite	NA uptake	5-HT uptake
Imipramine	+++	++
Desmethylimipramine (DMI)	++++	+
Hydroxy-DMI	+++	–
Clomipramine (CMI)	++	+++
Desmethyl-CMI	+++	+
Amitriptyline (AMI)	++	++
Nortriptyline (desmethyl-AMI)	+++	++
Hydroxynortriptyline	++	++

common side effect is sedation, and the long duration of action means that daytime performance is often affected by drowsiness and difficulty in concentrating.

TCAs, particularly in overdose, may cause ventricular dysrhythmias associated with prolongation of the QT interval (see Ch. 21). Usual therapeutic doses of TCAs increase, slightly but significantly, the risk of sudden cardiac death.

Interactions with other drugs

TCAs are particularly likely to cause adverse effects when given in conjunction with other drugs (see Ch. 56). They rely on hepatic metabolism by microsomal CYP enzymes for elimination, and this may be inhibited by competing drugs (e.g. antipsychotic drugs and some steroids).

TCAs potentiate the effects of alcohol and anaesthetic agents, for reasons that are not well understood, and deaths have occurred as a result of this, when severe respiratory depression has followed a bout of drinking. TCAs also interfere with the action of various antihypertensive drugs (see Ch. 22), with potentially dangerous consequences, so their use in hypertensive patients requires close monitoring.

Acute toxicity

TCAs are dangerous in overdose, and were at one time commonly used for suicide attempts, which was an important factor prompting the introduction of safer antidepressants. The main effects are on the central nervous system and the heart. The initial effect of TCA overdosage is to cause excitement and delirium, which may be accompanied by convulsions. This is followed by coma and respiratory depression lasting for some days. Atropine-like effects are pronounced, including dry mouth and skin, mydriasis and inhibition of gut and bladder. Anticholinesterase drugs have been used to counter atropine-like effects but are no longer recommended. Cardiac dysrhythmias (see above) are common, and sudden death (rare) may occur from ventricular fibrillation.

Pharmacokinetic aspects

TCAs are all rapidly absorbed when given orally and bind strongly to plasma albumin, most being 90–95% bound at therapeutic plasma concentrations. They also bind to extravascular tissues, which accounts for their generally very large distribution volumes (usually 10–50 l/kg; see Ch. 8) and low rates of elimination. Extravascular sequestration, together with strong binding to plasma albumin, means that haemodialysis is ineffective as a means of increasing drug elimination.

TCAs are metabolised in the liver by two main routes, N-*demethylation*, and *ring hydroxylation* (Fig. 46.4). Both the desmethyl and the hydroxylated metabolites commonly retain biological activity (see Table 46.4). During prolonged treatment with TCAs, the plasma concentration of these metabolites is usually comparable to that of the parent drug, although there is wide variation between individuals. Inactivation of the drugs occurs by glucuronide conjugation of the hydroxylated metabolites, the glucuronides being excreted in the urine.

The overall half-times for elimination of TCAs are generally long, ranging from 10–20 h for imipramine and desipramine to about 80 h for protriptyline. They are even longer in elderly patients. Therefore, gradual accumulation is possible, leading to slowly developing side effects. The relationship between plasma concentrations and the therapeutic effect is not simple. Indeed, a study on nortriptyline (Fig. 46.5) showed that too high a plasma concentration actually reduces the antidepressant effect, and there is a narrow 'therapeutic window'.

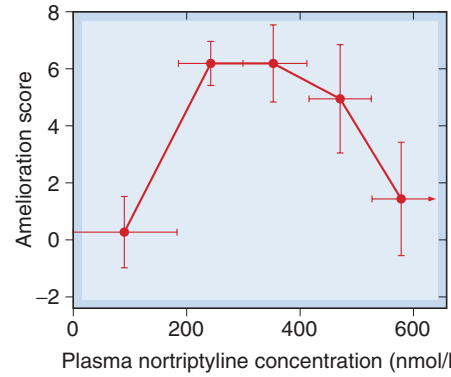

Fig. 46.5 'Therapeutic window' for nortriptyline. The antidepressant effect, determined from subjective rating scales, is optimal at plasma concentrations between 200 nmol/l and 400 nmol/l, and declines at higher levels.

Tricyclic antidepressants

- Tricyclic antidepressants are chemically related to phenothiazines, and some have similar non-selective receptor-blocking actions.
- Important examples are **imipramine**, **amitriptyline** and **clomipramine**.
- Most are long acting, and they are often converted to active metabolites.
- Important side effects: sedation (H_1 block); postural hypotension (α-adrenoceptor block); dry mouth, blurred vision, constipation (muscarinic block); occasionally mania and convulsions. Risk of ventricular dysrhythmias.
- Dangerous in acute overdose: confusion and mania, cardiac dysrhythmias.
- Liable to interact with other drugs (e.g. alcohol, anaesthetics, hypotensive drugs and non-steroidal anti-inflammatory drugs; should not be given with monoamine oxidase inhibitors).
- Also used to treat neuropathic pain.

OTHER NON-SELECTIVE MONOAMINE UPTAKE INHIBITORS

Other relatively non-selective monoamine uptake inhibitors (often referred to as serotonin/noradrenaline reuptake inhibitors, or 'SNRIs') include **venlafaxine**, **desvenlafaxine**, **duloxetine** and **milnacipran** (see Table 46.2). These have become extensively used antidepressant drugs due to their perceived greater therapeutic efficacy and low side effect profiles.

Milnacipran has some selectivity for noradrenaline uptake over 5-HT uptake. As the dose of venlafaxine is increased, its efficacy also increases, which has been

Other monoamine uptake inhibitors

- **Venlafaxine** is a 5-HT uptake inhibitor, but less selective for 5-HT versus noradrenaline than SSRIs. It is metabolised to **desvenlafaxine** which is also antidepressant.
- **Duloxetine** inhibits NA and 5-HT uptake.
- **Bupropion** is a noradrenaline and dopamine uptake inhibitor.
- Generally similar to tricyclic antidepressants but lack major receptor-blocking actions, so fewer side effects.
- Less risk of cardiac effects, so safer in overdose than tricyclic antidepressants.
- Can be used to treat other disorders:
  - venlafaxine, desvenlafaxine and duloxetine—anxiety disorders
  - duloxetine and **milnacipran**—neuropathic pain and fibromyalgia
  - duloxetine—urinary incontinence.

interpreted as demonstrating that its weak action to inhibit noradrenaline reuptake may add to its 5-HT uptake inhibition that occurs at lower doses, the combination providing additional therapeutic benefit. They are all active orally; venlafaxine is available in a slow-release formulation that reduces the incidence of nausea. Venlafaxine, desvenlafaxine and duloxetine are effective in some anxiety disorders (see Ch. 43). Desvenlafaxine may be useful in treating some perimenopausal symptoms such as hot flushes and insomnia. Duloxetine and milnacipran are used in the treatment of neuropathic pain and fibromyalgia (see Ch. 41). Duloxetine is also used to treat urinary incontinence.

Venlafaxine and duloxetine are metabolised by CYP2D6. Venlafaxine is converted to **desvenlafaxine** which shows greater inhibition of noradrenaline reuptake. Unwanted effects of these drugs—largely due to enhanced activation of adrenoceptors—include headache, insomnia, sexual dysfunction, dry mouth, dizziness, sweating and decreased appetite. The most common symptoms in overdose are CNS depression, serotonin toxicity, seizure and cardiac conduction abnormalities. Duloxetine has been reported to cause hepatotoxicity and is contraindicated for patients with hepatic impairment.

OTHER NORADRENALINE UPTAKE INHIBITORS

Bupropion inhibits both noradrenaline and dopamine (but not 5-HT) uptake but, unlike cocaine and amphetamine (see Ch. 47), does not induce euphoria and has so far not been observed to have abuse potential. It is metabolised to active metabolites. It is also used to treat nicotine dependence (see Ch. 48). **Reboxetine** and **atomoxetine** are highly selective inhibitors of noradrenaline uptake but their efficacy in depression is less than that of TCAs. Atomoxetine is approved for the treatment of attention-deficit hyperactivity disorder (see Ch. 47).

MONOAMINE RECEPTOR ANTAGONISTS

Mirtazapine blocks not only α_2 adrenoreceptors but also other receptors, including 5-HT_{2C} receptors, which may contribute to its antidepressant actions. Block of α_2 adrenoceptors will not only increase noradrenaline release but will also enhance 5-HT release (see Fig 46.2B); however, by simultaneously blocking 5-HT_{2A} and 5-HT_3 receptors it will reduce unwanted effects mediated through these receptors (e.g. sexual dysfunction and nausea) but leave intact stimulation of postsynaptic 5-HT_{1A} receptors. It also blocks histamine H_1 receptors which may cause sedation. **Trazodone** combines 5-HT_{2A} and 5-HT_{2C} receptor antagonism with 5-HT reuptake inhibition.

Mianserin, another α_2 adrenoceptor antagonist that also blocks H_1, 5-HT_{2A} and α_1 adrenoreceptors, can cause bone marrow depression, requiring regular blood counts, so its use has declined in recent years.

Table 46.5 Substrates and inhibitors for type A and type B monoamine oxidase

	Type A	Type B
Preferred substrates	Noradrenaline 5-Hydroxytryptamine	Phenylethylamine Benzylamine
Non-specific substrates	Dopamine Tyramine	Dopamine Tyramine
Specific inhibitors	Clorgyline Moclobemide	Selegiline
Non-specific inhibitors	Pargyline Tranylcypromine Isocarboxazid	Pargyline Tranylcypromine Isocarboxazid

MONOAMINE OXIDASE INHIBITORS

Monoamine oxidase inhibitors (MAOIs) were among the first drugs to be introduced clinically as antidepressants but were largely superseded by other types of antidepressants, whose clinical efficacies were considered better and whose side effects are generally less than those of MAOIs. The main examples are **phenelzine**, **tranylcypromine** and **iproniazid**. These drugs cause irreversible inhibition of the enzyme and do not distinguish between the two main isozymes. The discovery of reversible inhibitors that show isozyme selectivity has rekindled interest in this class of drug. Although several studies have shown a reduction in platelet MAO activity in certain groups of depressed patients, there is no clear evidence that abnormal MAO activity is involved in the pathogenesis of depression.

Monoamine oxidase (see Ch. 14) is found in nearly all tissues, and exists in two similar molecular forms coded by separate genes (see Table 46.5). MAO-A has a substrate preference for 5-HT and is the main target for the antidepressant MAOIs. MAO-B has a substrate preference for phenylethylamine and dopamine. Type B is selectively inhibited by **selegiline**, which is used in the treatment of Parkinson's disease (see Ch. 39). Disruption of the MAO-A gene in mice causes increased brain accumulation of 5-HT and, to a lesser extent, noradrenaline, along with aggressive behaviour (Shih et al., 1999). A family has been reported with an inherited mutation leading to loss of MAO-A activity, whose members showed mental retardation and violent behaviour patterns. Most antidepressant

MAOIs act on both forms of MAO, but clinical studies with subtype-specific inhibitors have shown clearly that antidepressant activity, as well as the main side effects of MAOIs, is associated with MAO-A inhibition. MAO is located intracellularly, mostly associated with mitochondria, and has two main functions:

1. Within nerve terminals, MAO regulates the free intraneuronal concentration of noradrenaline or 5-HT, and hence the releasable stores of these transmitters (see Ch. 14). It is not involved in the inactivation of released transmitter.
2. MAO is important in the inactivation of endogenous and ingested amines such as tyramine that would otherwise produce unwanted effects.

Chemical aspects

Monoamine oxidase inhibitors are substrate analogues with a phenylethylamine-like structure, and most contain a reactive group (e.g. hydrazine, propargylamine, cyclopropylamine) that enables the inhibitor to bind covalently to the enzyme, resulting in a non-competitive and long-lasting inhibition. Recovery of MAO activity after inhibition takes several weeks with most drugs, but is quicker after **tranylcypromine**, which forms a less stable bond with the enzyme. **Moclobemide** acts as a reversible competitive inhibitor.

Monoamine oxidase inhibitors are not particularly specific in their actions, and inhibit a variety of other enzymes as well as MAO, including many enzymes involved in the metabolism of other drugs. This is responsible for some of the many clinically important drug interactions associated with MAOIs.

Pharmacological effects

Monoamine oxidase inhibitors cause a rapid and sustained increase in the 5-HT, noradrenaline and dopamine content of the brain, 5-HT being affected most and dopamine least. Similar changes occur in peripheral tissues such as heart, liver and intestine, and increases in the plasma concentrations of these amines are also detectable. Although these increases in tissue amine content are largely due to accumulation within neurons, transmitter release in response to nerve activity is not increased. In contrast to the effect of TCAs, MAOIs do not increase the response of peripheral organs, such as the heart and blood vessels, to sympathetic nerve stimulation. The main effect of MAOIs is to increase the cytoplasmic concentration of monoamines in nerve terminals, without greatly affecting the vesicular stores that form the pool that is releasable by nerve stimulation. The increased cytoplasmic pool results in an increased rate of spontaneous leakage of monoamines, and also an increased release by indirectly acting sympathomimetic amines such as amphetamine and tyramine (see Ch. 14 and Fig. 14.8). Inhibition of MAO increases the proportion that escapes and thus enhances the response. Tyramine thus causes a much greater rise in blood pressure in MAOI-treated animals than in controls. This mechanism is important in relation to the cheese reaction produced by MAOIs in humans (see later section).

In normal human subjects, MAOIs cause an immediate increase in motor activity, and euphoria and excitement develop over the course of a few days. This is in contrast to TCAs, which cause only sedation and confusion when given to non-depressed subjects. The effects of MAOIs on amine metabolism develop rapidly, and the effect of a single dose lasts for several days. There is a clear discrepancy, as with SSRIs and TCAs, between the rapid biochemical response and the delayed antidepressant effect.

Unwanted effects and toxicity

Many of the unwanted effects of MAOIs result directly from MAO inhibition, but some are produced by other mechanisms.

Hypotension is a common side effect; indeed, pargyline was at one time used as an antihypertensive drug. One possible explanation for this effect—the opposite of what might have been expected—is that amines such as dopamine or octopamine accumulate within peripheral sympathetic nerve terminals and displace noradrenaline from the storage vesicles, thus reducing noradrenaline release associated with sympathetic activity.

Excessive central stimulation may cause tremors, excitement, insomnia and, in overdose, convulsions.

Increased appetite, leading to weight gain, can be so extreme as to require the drug to be discontinued.

Atropine-like side effects (dry mouth, blurred vision, urinary retention, etc.) are common with MAOIs, although they are less of a problem than with TCAs.

MAOIs of the hydrazine type (e.g. phenelzine and iproniazid) produce, very rarely (less than 1 in 10 000), severe hepatotoxicity, which seems to be due to the hydrazine moiety of the molecule. Their use in patients with liver disease is therefore unwise.

Interaction with other drugs and foods

Interaction with other drugs and foods is the most serious problem with MAOIs and is the main factor that caused their clinical use to decline. The special advantage claimed for the new reversible MAOIs, such as moclobemide, is that these interactions are reduced.

The cheese reaction is a direct consequence of MAO inhibition and occurs when normally innocuous amines (mainly tyramine) produced during fermentation are ingested. Tyramine is normally metabolised by MAO in the gut wall and liver, and little dietary tyramine reaches the systemic circulation. MAO inhibition allows tyramine to be absorbed, and also enhances its sympathomimetic effect, as discussed above. The result is acute hypertension, giving rise to a severe throbbing headache and occasionally even to intracranial haemorrhage. Although many foods contain some tyramine, it appears that at least 10 mg of tyramine needs to be ingested to produce such a response, and the main danger is from ripe cheeses and from concentrated yeast products such as Marmite. Administration of indirectly acting sympathomimetic amines (e.g. **ephedrine**—a nasal decongestant—or amphetamine—a drug of abuse) also causes severe hypertension in patients receiving MAOIs; directly acting agents such as noradrenaline (used, for example, in conjunction with local anaesthetics; see Ch. 42) are not hazardous. Moclobemide, a specific MAO-A inhibitor, does not cause the cheese reaction, probably because tyramine can still be metabolised by MAO-B.

Hypertensive episodes have been reported in patients given TCAs and MAOIs simultaneously. The probable explanation is that inhibition of noradrenaline reuptake further enhances the cardiovascular response to dietary tyramine, thus accentuating the cheese reaction. This combination of drugs can also produce excitement and hyperactivity.

Monoamine oxidase inhibitors can interact with **pethidine** (see Ch. 41) to cause severe hyperpyrexia, with restlessness, coma and hypotension. The mechanism is uncertain, but it is likely that an abnormal pethidine metabolite is produced because of inhibition of demethylation.

Other antidepressant drugs

- **Mirtazapine** blocks α_2 adrenoceptors and 5-HT_{2C} receptors, enhancing noradrenaline and 5-HT release.
- Mirtazapine may act more rapidly than other antidepressants, and causes less nausea and sexual dysfunction than SSRIs.
- **Trazodone** blocks 5-HT_{2A} and 5-HT_{2C} receptors and blocks 5-HT reuptake
- **Mianserin** blocks H_1, 5-HT_{2A} and α_1 receptors. Use is declining because of risk of bone marrow depression. Regular blood counts are advisable.
- Cardiovascular side effects of these drugs are fewer than those of tricyclic antidepressants

Monoamine oxidase inhibitors (MAOIs)

- Main examples are **phenelzine**, **tranylcypromine**, **isocarboxazid** (irreversible, long-acting, non-selective between MAO-A and B) and **moclobemide** (reversible, short-acting, MAO-A selective).
- Long-acting MAOIs:
  - main side effects: postural hypotension (sympathetic block); atropine-like effects (as with TCAs); weight gain; CNS stimulation, causing restlessness, insomnia, hepatotoxicity and neurotoxicity (rare)
  - acute overdose causes CNS stimulation, sometimes convulsions
  - 'cheese reaction', i.e. severe hypertensive response to tyramine-containing foods (e.g. cheese, beer, wine, well-hung game, yeast or soy extracts). Such reactions can occur up to 2 weeks after treatment is discontinued.
- Interaction with other amines (e.g. **ephedrine** in over-the-counter decongestants, **clomipramine** and other TCAs) and some other drugs (e.g. **pethidine**) are also potentially lethal.
- Moclobemide is used for major depression and social phobia. Cheese reaction and other drug interactions are less severe and shorter lasting than with irreversible MAOIs.
- MAOIs are used much less than other antidepressants because of their adverse effects and serious interactions.
- They are indicated for major depression in patients who have not responded to other drugs.

MISCELLANEOUS AGENTS

Methylfolate, given as a dietary supplement, may be effective in depressed individuals who have lowered folate levels.

Oestrogen which is known to elevate mood in perimenopausal women may also be of value for the treatment of postpartum depression. Its effectiveness in treating other forms of depression is unclear. In addition to its well documented hormonal actions in the body (see Ch. 34), it also has actions on monoaminergic, GABAergic and glutamatergic systems in the brain (see Chs 37 and 38).

FUTURE ANTIDEPRESSANT DRUGS

After a fallow period, there are now several promising new drugs in development (see Lodge & Li, 2008; Mathew et al., 2008). These can be classified broadly into the following:

- Drugs affecting monamine transmission, including drugs with one or more of the following properties—β_3-adrenoreceptor agonism, D_2 dopamine receptor agonism or antagonism, 5-HT_{1A} receptor agonism or partial agonism and 5-HT_{2A} receptor antagonism as well as dopamine, noradrenaline and 5-HT uptake inhibition.
- Drugs acting on ion channels. Rather surprisingly, agonists, partial agonists and antagonists at nicotinic receptors all appear to have antidepressant properties. The explanation may be that what is required is reduced receptor activation and that agonists induce receptor desensitisation and partial agonists inhibit endogenous acetylcholine. Interest in drugs acting at the NMDA receptor has been stimulated by the observation that a single dose of **ketamine** (see Ch. 40) has been reported to rapidly alleviate depression, an effect that lasts for days. AMPAkines, drugs that potentiate responses at the AMPA receptor (see Ch. 37), show efficacy in animal models. Other putative targets are P2X receptors, 5-HT_3 receptors and various potassium channels.
- Drugs acting at novel receptor targets—such as GRII cortisol receptor antagonists, melanocyte inhibiting factor (MIF-1) analogues, melatonin M_1/M_2 receptor agonists, NK_1 and NK_2 receptor antagonists.

Other avenues of research are into the development of compounds that act on the signal transduction pathways responsible for neurogenesis, neural plasticity and apoptosis.

BRAIN STIMULATION THERAPIES

There are now a number of brain stimulation techniques being used or developed to treat depression. The most established are electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (TMS). Brain stimulation treatments are often used as the therapeutic approach of last resort on patients who have not responded to antidepressant drugs.

ECT involves stimulation through electrodes placed on either side of the head, with the patient lightly anaesthetised, paralysed with a short-acting neuromuscular-blocking drug (e.g. **suxamethonium**; Ch. 13) so as to avoid physical injury, and artificially ventilated. Controlled trials have shown ECT to be at least as effective as antidepressant

Clinical uses of drugs in depression

- Mild depression is often best treated initially with non-drug measures, with antidepressant drugs being used in addition if the response is poor.
- The use of antidepressant drugs is advisable in the treatment of moderate to severe depression.
- The clinical efficacy of antidepressant drugs is limited, and varies between individuals. Clinical trials have produced inconsistent results, because of placebo responses and spontaneous fluctuations in the level of depression.
- Different classes of antidepressant drugs have similar efficacy but different side effects.
- Choice of drug is based on individual aspects including concomitant disease (TCAs in particular have several indications) and treatment (MAOIs and TCAs cause important interactions), suicide risk and previous response to treatment. Other things being equal, an SSRI is preferred as these are usually better tolerated and are less dangerous in overdose.
- Antidepressant drugs take several weeks before taking effect, so decisions on dose increment or switching to another class should not be made precipitately. Use of MAOIs is by specialists.
- An effective regimen should be continued for at least 2 years.
- In urgent situations, specialist consideration should be given to possible use of electroconvulsive therapy.
- Anxiolytic (e.g. benzodiazepine, Ch. 43), or antipsychotic drugs (Ch. 45) are useful adjuncts in some patients.

drugs, with response rates ranging between 60% and 80%; it appears to be an effective treatment for severe suicidal depression and has the advantage of producing a fast-onset response. The main disadvantage of ECT is that it often causes confusion and memory loss lasting for days or weeks. TMS gives electrical stimulation without anaesthesia or convulsion and does not produce cognitive impairment (see Kirkcaldie et al., 1997).

The effect of ECT on experimental animals has been carefully analysed to see if it provides clues as to the mode of action of antidepressant drugs, but the clues it gives are enigmatic. 5-HT synthesis and uptake are unaltered, and noradrenaline uptake is somewhat increased (in contrast to the effect of TCAs). Decreased β-adrenoceptor responsiveness, both biochemical and behavioural, occurs with both ECT and long-term administration of antidepressant drugs, but changes in 5-HT-mediated responses tend to go in opposite directions (see Maes & Meltzer, 1995).

There have been reports that deep brain stimulation, which has also been used in the treatment of Parkinson's disease (see Ch. 39), in which the activity in a specific brain region is altered through surgically implanted electrodes, is effective in patients not responding to other treatments (see Mayberg et al., 2005). The effectiveness of another technique, vagal stimulation, in producing long-term benefit in depression is still unclear (see Grimm & Bajbouj, 2010).

CLINICAL EFFECTIVENESS OF ANTIDEPRESSANT TREATMENTS

The overall clinical efficacy of antidepressants has been established in many well-controlled clinical trials, although the degree of improvement may be limited. In long-term therapy, however, the remission rate can be as low as 30%. Moreover, it is clear that some patients recover spontaneously, and that 30–40% of patients fail to improve with drug treatments. Although antidepressants produce significant benefit in patients with moderate or severe depression, their efficacy in mild cases is unclear. Controlled trials show there is little to choose in terms of overall efficacy between any of the drugs currently in use, although clinical experience suggests that individual patients may, for unknown reasons, respond better to one drug than to another.

Pharmacogenetic factors

▼ The individual variation in response to antidepressants may be partly due to genetic factors, as well as to heterogeneity of the clinical condition. Two genetic factors have received particular attention, namely:

1. polymorphism of the cytochrome P450 gene, especially *CYP2D6* (see Kirchheiner et al., 2004) which is responsible for hydroxylation of TCAs
2. polymorphism of monoamine transporter genes (see Glatt & Reus, 2003).

Up to 10% of Caucasians possess a dysfunctional *CYP2D6* gene, and consequently may be susceptible to side effects of TCAs and various other drugs (see Ch. 11) that are metabolised by this route. The opposite effect, caused by duplication of the gene, is common in Eastern European and East African populations, and may account for a lack of clinical efficacy in some individuals. There is some evidence to suggest that responsiveness to SSRIs is related to polymorphism of one of the serotonin transporter genes (see Gerretsen & Pollock, 2008).

Although genotyping may prove to be a useful approach in the future to individualising antidepressant therapy, its practical realisation is still some way off.

Suicide and antidepressants

▼ Some years ago there were reports that antidepressants increased the risk of 'suicidality' in depressed patients, especially in children and adolescents (see Licinio & Wong, 2005). The term *suicidality* encompasses suicidal thoughts and planning as well as unsuccessful attempts; actual suicide, although one of the major causes of death in young people, is much rarer than suicidality. Clinical trials to determine the relationship between antidepressants and suicidality are difficult, because of the clear association between depression and suicide, and have given variable results, with some studies suggesting that suicidality may be increased during the first few weeks of antidepressant treatment, although not thereafter, and some showing a small increase in the risk of actual suicide (see Cipriani et al., 2005). Recent reviews of published data conclude that although antidepressants, including SSRIs, carry a small risk of inducing suicidal thoughts and suicide attempts in young people, the risk is less in older age groups (Hetrick et al., 2007; Möller et al., 2008; Barbui et al., 2009). There is no evidence to suggest that SSRIs carry any greater risk than other antidepressants. Furthermore, the risk has to be balanced against the beneficial effects of these drugs, not only on depression but also on anxiety, panic and obsessive-compulsive disorders (see Ch. 43).

OTHER CLINICAL USES OF ANTIDEPRESSANT DRUGS

To some extent, the term 'antidepressant drug' is misleading as many of these drugs are now used to treat disorders other than depression. These include:

- neuropathic pain (e.g. **amytriptyline**, **nortryptyline**; Ch. 41)
- anxiety disorders (e.g. SSRIs, **venlafaxine**, **duloxetine**; Ch. 43)
- fibromyalgia (e.g. duloxetine, venlafaxine, SSRIs, TCAs; Ch. 41)
- bipolar depression (e.g. **fluoxetine** in conjunction with **olanzepine**; see below)
- obesity (e.g. **sibutramine**; Ch. 31)
- smoking cessation (e.g. **buproprion**; Ch. 48)
- attention-deficit hyperactivity disorder (e.g. **atomoxetine**; Ch. 47).

DRUG TREATMENT OF BIPOLAR DEPRESSION

A range of drugs are now used to control the mood swings characteristic of manic-depressive (bipolar) illness. The major drugs are:

- **lithium**
- several antiepileptic drugs, e.g. **carbamazepine**, **valproate**, **lamotrogine**
- some atypical antipsychotic drugs, e.g. **olanzapine**, **risperidone**, **quetiapine**, **aripiprazole**.

When used to treat bipolar depression, lithium and antiepileptic agents are often referred to as *mood-stabilising* drugs.

Other agents that may have some beneficial effects in the treatment of bipolar depression are benzodiazepines (to calm, induce sleep and reduce anxiety), **memantine**, **amantadine**, and **ketamine**. The use of antidepressant drugs in bipolar depression is somewhat controversial. It is recommended that they are given in combination with an antimanic agent because, in some patients, they may induce or enhance mania.

Used prophylactically in bipolar depression, drugs prevent the swings of mood and thus can reduce both the depressive and the manic phases of the illness. They are given over long periods, and their beneficial effects take 3–4 weeks to develop. Given in an acute attack, they are effective only in reducing mania, but not the depressive phase (although lithium is sometimes used as an adjunct to antidepressants in severe cases of unipolar depression).

LITHIUM

The psychotropic effect of lithium was discovered in 1949 by Cade, who had predicted that urate salts should prevent the induction by uraemia of a hyperexcitability state in guinea pigs. He found lithium urate to produce an effect, quickly discovered that it was due to lithium rather than urate, and went on to show that lithium produced a rapid improvement in a group of manic patients.

Antiepileptic and atypical antipsychotic drugs (see below) are equally effective in treating acute mania; they act more quickly and are considerably safer, so the clinical use of lithium is mainly confined to prophylactic control of manic-depressive illness. The use of lithium is declining.[5] It is relatively difficult to use, as plasma concentration monitoring is required, and there is the potential for problems in patients with renal impairment and for drug interactions, for example with diuretics (see Ch. 56). Lithium may have beneficial effects in neurodegenerative diseases such as Alzheimer's disease (see Ch. 39).

Pharmacological effects and mechanism of action

Lithium is clinically effective at a plasma concentration of 0.5–1 mmol/l, and above 1.5 mmol/l it produces a variety of toxic effects, so the therapeutic window is narrow. In normal subjects, 1 mmol/l lithium in plasma has no appreciable psychotropic effects. It does, however, produce many detectable biochemical changes, and it is still unclear how these may be related to its therapeutic effect.

Lithium is a monovalent cation that can mimic the role of Na^+ in excitable tissues, being able to permeate the voltage-gated Na^+ channels that are responsible for action potential generation (see Ch. 4). It is, however, not pumped out by the Na^+-K^+-ATPase, and therefore tends to accumulate inside excitable cells, leading to a partial loss of intracellular K^+, and depolarisation of the cell.

The biochemical effects of lithium are complex, and it inhibits many enzymes that participate in signal transduction pathways. Those that are thought to be relevant to its therapeutic actions are as follows:

- Inhibition of inositol monophosphatase, which blocks the phosphatidyl inositol (PI) pathway (see Ch. 3) at the point where inositol phosphate is hydrolysed to free inositol, resulting in depletion of PI. This prevents agonist-stimulated inositol trisphosphate formation through various PI-linked receptors, and therefore blocks many receptor-mediated effects.
- Inhibition of glycogen synthase kinase 3 (GSK3) isoforms, possibly by competing with magnesium for its association with these kinases. GSK3 isoforms phosphorylate a number of key enzymes involved in pathways leading to apoptosis and amyloid formation (see Phiel & Klein, 2001). Lithium can also affect GSK3 isoforms indirectly by interfering with their regulation by Akt, a closely related serine/threonine kinase regulated through PI-mediated signalling and by arrestins (see Ch. 3; Beaulieu et al., 2009).

Lithium also inhibits hormone-induced cAMP production and blocks other cellular responses (e.g. the response of renal tubular cells to antidiuretic hormone, and of the thyroid to thyroid-stimulating hormone; see Chs 28 and 33, respectively). This is not, however, a pronounced effect in the brain.

The cellular selectivity of lithium appears to depend on its selective uptake, reflecting the activity of sodium channels in different cells. This could explain its relatively selective action in the brain and kidney, even though many other tissues use the same second messengers. Notwithstanding such insights, our ignorance of the nature of the disturbance underlying the mood swings in bipolar depression leaves us groping for links between the biochemical and prophylactic effects of lithium.

[5]The decline in lithium use may have been influenced by the imbalance in the marketing of this simple inorganic ion versus other pharmacological agents.

Pharmacokinetic aspects and toxicity

Lithium is given by mouth as the carbonate salt and is excreted by the kidney. About half of an oral dose is excreted within about 12 h—the remainder, which presumably represents lithium taken up by cells, is excreted over the next 1–2 weeks. This very slow phase means that, with regular dosage, lithium accumulates slowly over 2 weeks or more before a steady state is reached. The narrow therapeutic window (approximately 0.5–1.5 mmol/l) means that monitoring of the plasma concentration is essential. Na^+ depletion reduces the rate of excretion by increasing the reabsorption of lithium by the proximal tubule, and thus increases the likelihood of toxicity. Diuretics that act distal to the proximal tubule (Ch. 28) also have this effect, and renal disease also predisposes to lithium toxicity.

The main toxic effects that may occur during treatment are as follows:

- nausea, vomiting and diarrhoea
- tremor
- renal effects: polyuria (with resulting thirst) resulting from inhibition of the action of antidiuretic hormone. At the same time, there is some Na^+ retention associated with increased aldosterone secretion. With prolonged treatment, serious renal tubular damage may occur, making it essential to monitor renal function regularly in lithium-treated patients
- thyroid enlargement, sometimes associated with hypothyroidism
- weight gain
- hair loss.

Acute lithium toxicity results in various neurological effects, progressing from confusion and motor impairment to coma, convulsions and death if the plasma concentration reaches 3–5 mmol/l.

ANTIEPILEPTIC DRUGS

Carbamazepine, **valproate** and **lamotrogine** have fewer side effects than lithium and have proved efficacious in the treatment of bipolar depression.

It is assumed that the mechanisms of action of anticonvulsant drugs in reducing bipolar depression are the same as for their anticonvulsant activity. While each drug has multiple actions (see Table 44.1), the antiepileptic drugs effective in bipolar depression share the property of sodium channel blockade, although there are subtle differences in their effectiveness against the different phases of bipolar depression. **Valproate** and **carbamazepine** are effective in treating acute attacks of mania and in the long-term treatment of the disorder, although carbamazepine may not be as effective in treating the depression phase. Valproate is sometimes given along with other drugs such as lithium. **Lamotrogine** is effective in preventing the recurrence of both mania and depression. **Riluzole**, which was developed to treat amyotrophic lateral sclerosis (Ch. 39), has anticonvulsant activity in animal models. It may be useful in the treatment of bipolar disorders resistant to other agents.

The efficacy of **gabapentin** and **pregabalin** in bipolar depression has been questioned (see Stahl, 2008), but they may be useful as adjunct therapies to treat the chronic pain and anxiety that sufferers from bipolar depression may also experience. **Levetiracetam**, **topiramate** and **zonisamide** are sometimes used in the treatment of bipolar depression but their efficacy still remains to be established.

ATYPICAL ANTIPSYCHOTIC DRUGS

Atypical antipsychotic drugs (e.g. **olanzapine**, **risperidone**, **quetiapine**, **aripiprazole**) are second-generation drugs developed for the treatment of schizophrenia (see Ch. 45). These agents have D_2 dopamine and 5-HT_{2A} receptor antagonist properties as well as actions on other receptors and amine transporters that may contribute to their effectiveness in bipolar depression. All appear to be effective against mania while some may also be effective against bipolar depression. In bipolar depression, atypical antipsychotics are often used in combination with lithium or valproate. Olanzepine is given in combination with the antidepressant **fluoxetine**.

Treatment of bipolar depression

- **Lithium**, an inorganic ion, taken orally as lithium carbonate.
- Mechanism of action is not understood. The main biochemical possibilities are:
  - interference with inositol trisphosphate formation
  - inhibition of kinases.
- Antiepileptic drugs (e.g. **carbamazepine**, **valproate**, **lamotrogine**)
  - better side effect and safety profile.
- Atypical antipsychotic drugs (e.g. **olanzapine**, **risperidone**, **quetiapine**, **aripiprazole**).

Clinical uses of mood-stabilising drugs

- **Lithium** (as the carbonate) is the classical drug. It is used:
  - in prophylaxis and treatment of *mania*, and in the prophylaxis of *bipolar* or *unipolar disorder* (manic depression or recurrent depression).
- Points to note include the following:
  - there is a narrow therapeutic window and long duration of action
  - acute toxic effects include cerebellar effects, nephrogenic *diabetes insipidus* (see Ch. 28) and *renal failure*
  - dose must be adjusted according to the plasma concentration
  - elimination is via the kidney and is reduced by proximal tubular reabsorption. Diuretics increase the activity of the reabsorptive mechanism and hence can precipitate lithium toxicity
  - *thyroid disorders* and mild *cognitive impairment* occur during chronic use.
- **Carbamazepine valproate** and **lamotrogine** (sodium channel blockers with antiepileptic actions; Ch. 44) are used for:
  - the prophylaxis and treatment of manic episodes in patients with *bipolar disorder*
  - the treatment of *bipolar depression* (valproate, lamotrogine).
- **Olanzapine**, **risperidone**, **quetiapine**, **aripiprazole** (atypical antipsychotic drugs) are used to treat *mania*.

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47 CNS stimulants and psychotomimetic drugs

OVERVIEW

In this chapter, we describe drugs that have a predominantly stimulant effect on the central nervous system (CNS); these fall into two broad categories:

1. **psychomotor stimulants**
2. **psychotomimetic (hallucinogenic) drugs**

Drugs in the first category have a marked effect on mental function and behaviour, producing excitement and euphoria, reduced sensation of fatigue, and an increase in motor activity.

Drugs in the second category mainly affect thought patterns and perception, distorting cognition in a complex way.

Table 47.1 summarises the classification of the drugs that are discussed in this chapter.

Several of these drugs have no clinical uses but are used for recreational purposes and as such are recognised as drugs of abuse. This aspect is also discussed in Chapter 48.

PSYCHOMOTOR STIMULANTS

AMPHETAMINES AND RELATED DRUGS

Amphetamine (*speed* or *billy whizz*) and its active dextroisomer **dextroamphetamine** (*dexies*), together with **methamphetamine** (*crystal meth* or *ice*) and **methylphenidate** (better known to many by its trade name *Ritalin*), comprise a group of drugs with very similar chemical and pharmacological properties (see Fig. 47.1).These drugs act by releasing monoamines, primarily dopamine and noradrenaline, from nerve terminals in the brain (see Seiden et al., 1993; Green et al., 2003). They are substrates for neuronal amine uptake transporters and cause release of these mediators (see Chs 14 and 38) thus producing the acute effects described below. With prolonged use, they are neurotoxic, causing degeneration of amine-containing nerve terminals and eventually cell death. This effect is probably due to the accumulation of reactive metabolites of the parent compounds within the nerve terminals. It has been well documented in experimental animals, and is believed to occur also in humans, possibly accounting for long-term adverse psychological effects in habitual users of amphetamine derivatives.

Further information on the pharmacology, uses and dangers of amphetamines can be found in the monograph by Iversen (2006).

Pharmacological effects

The main central effects of amphetamine-like drugs are:

- locomotor stimulation
- euphoria and excitement
- insomnia
- increased stamina
- anorexia.

In addition, amphetamines have peripheral sympathomimetic actions, producing a rise in blood pressure and inhibition of gastrointestinal motility.

In humans, amphetamine causes euphoria; with intravenous injection, this can be so intense as to be described as 'orgasmic'. Subjects become confident, hyperactive and talkative, and sex drive is said to be enhanced. Fatigue, both physical and mental, is reduced, and amphetamine-like drugs cause marked anorexia, but with continued administration this effect wears off and food intake returns to normal. Rats quickly learn to press a lever in order to obtain a dose of amphetamine—an indication that the drug is rewarding.

Many studies have shown improvement of both mental and physical performance in fatigued, although not in well-rested, subjects (the use of amphetamines in sport is described in Ch. 58). Mental performance is improved for simple tedious tasks much more than for difficult tasks—in animal studies using complex behavioural analysis paradigms, amphetamines are said to make the animals busier rather than brighter! Amphetamines have been used to improve the performance of soldiers, military pilots and others who need to remain alert under extremely fatiguing conditions. They have also been in vogue as a means of helping students to concentrate before and during examinations, but the improvement caused by reduction of fatigue can be offset by the mistakes of overconfidence and a decreased ability to deal with large amounts of information.[1]

Adverse effects of amphetamines include feelings of anxiety, irritability and restlessness as the body's energy stores are run down. At high doses, amphetamines may induce panic and paranoia.

In experimental animals, the behavioural effects of amphetamines are produced by the release of catecholamines in the brain. Thus pretreatment with 6-hydroxydopamine, which depletes the brain of both noradrenaline and dopamine, abolishes the effect of amphetamine, as does pretreatment with α-methyltyrosine, an inhibitor of catecholamine biosynthesis (see Ch. 14). Similarly, monoamine oxidase inhibitors (see Ch. 46) potentiate the effects of amphetamine, presumably by blocking metabolism. Interestingly, **reserpine**, which inhibits vesicular storage of catecholamines (see Ch. 14), does not block the behavioural effects of amphetamine.

[1]Pay heed to the awful warning of the medical student who, it is said, having taken copious amounts of dextroamphetamine, left the examination hall in confident mood, having spent 3 hours writing his name over and over again.

Table 47.1 Central nervous system stimulants and ph psychotomimetic drugs

Category	Example(s)	Mode(s) of action	Clinical significance
Psychomotor stimulants	Amphetamine and related compounds (e.g. dexamphetamine, methylamphetamine, methylphenidate)	Release of catecholamines Inhibition of catecholamine uptake	Methylphenidate and dexamphetamine used to treat attention-deficit hyperactivity disorder in children; otherwise very limited clinical use Some agents used occasionally to treat narcolepsy and as appetite suppressants Risk of dependence, sympathomimetic side effects and pulmonary hypertension Mainly important as drugs of abuse
	Cocaine	Inhibition of catecholamine uptake Local anaesthetic	Important as drug of abuse Risk of fetal damage Occasionally used for nasopharyngeal and ophthalmic anaesthesia (see Ch. 42)
	Methylxanthines (e.g. caffeine, theophylline)	Inhibition of phosphodiesterase Antagonism of adenosine A_2 receptors	Clinical uses unrelated to stimulant activity, although caffeine is included in various 'tonics' Theophylline used for action on cardiac and bronchial muscle (see Chs 21, 27) Constituents of beverages
Psychotomimetic drugs (hallucinogens)	LSD	Agonist at 5-HT_{2A} receptors (see Chs 15 & 38)	No clinical use Important as drug of abuse
	MDMA (ecstasy)	Releases 5-HT and blocks reuptake	No clinical use Important as drug of abuse
	Mescaline	Not known Chemically similar to amphetamine	—
	Psilocybin	Chemically related to 5-HT; acts on 5-HT_{2A} receptors	—
	Ketamine	Phencyclidine (PCP) is chemically similar Blocks NMDA receptor-operated ion channels (see Ch. 37)	Dissociative anaesthetic drug of abuse PCP used as a model for schizophrenia
	Δ^9-tetrahydrocannabinol	Activates CB_1 and CB_2 receptors (see Ch. 18)	Has analgesic and antiemetic properties (see Ch. 18)
	Salvinorin A	κ-Opioid receptor agonist	No clinical use

5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide; MDMA, Methylenedioxymethamphetamine.

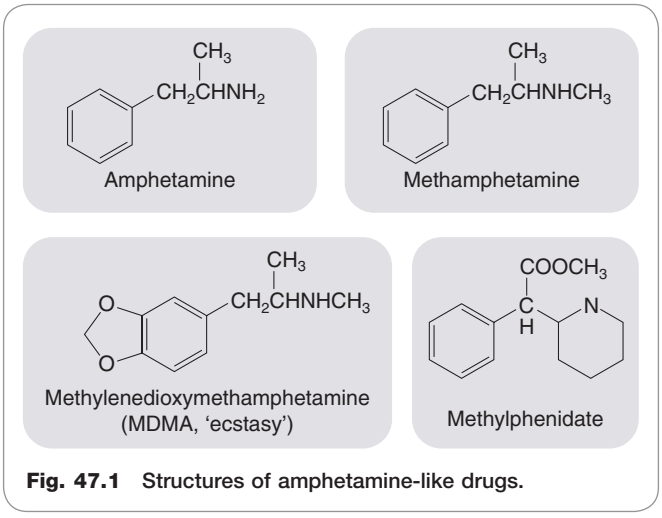

Fig. 47.1 Structures of amphetamine-like drugs.

This is probably because amphetamine releases cytosolic rather than vesicular catecholamines (see Ch. 14). The behavioural effects of amphetamine are due mainly to release of dopamine rather than noradrenaline. The evidence for this is that destruction of the central noradrenergic bundle does not affect locomotor stimulation produced by amphetamine, whereas destruction of the dopamine-containing nucleus accumbens (see Ch. 38) or administration of antipsychotic drugs that antagonise dopamine (see Ch. 45) inhibit both locomotor and rewarding responses.

Chronic use, tolerance and dependence

If amphetamine is taken repeatedly over the course of a few days, which occurs when users seek to maintain the euphoric 'high' that a single dose produces, a state of 'amphetamine psychosis' can develop, which closely resembles an acute schizophrenic attack (see Ch. 45), with hallucinations accompanied by paranoid symptoms and aggressive behaviour. At the same time, repetitive stereotyped behaviour may develop (e.g. polishing shoes or

stringing beads). The close similarity of this condition to schizophrenia, and the effectiveness of antipsychotic drugs in controlling it, is consistent with the dopamine theory of schizophrenia (see Ch. 45). When the drug is stopped after a few days, there is usually a period of deep sleep and on awakening the subject feels lethargic, depressed, anxious (sometimes even suicidal) and hungry. Even a single dose of amphetamine, insufficient to cause psychotic symptoms, usually leaves the subject later feeling tired and depressed. These after-effects may be the result of depletion of the normal stores of dopamine and noradrenaline, but the evidence for this is not clear-cut.

Tolerance develops rapidly to euphoric and anorexic effects of amphetamine, but more slowly to the other effects (locomotor stimulation, stereotyped behaviour and peripheral sympathomimetic action).

Dependence on amphetamine appears to be a consequence of the unpleasant after-effects that it produces and to the insistent memory of euphoria, which leads to a desire for a repeat dose. There is no clear-cut physical withdrawal syndrome such as occurs with opioids. It is estimated that only about 5% of users progress to full dependence, the usual pattern being that the dose is increased as tolerance develops, and then uncontrolled 'binges' occur in which the user takes the drug repeatedly over a period of a day or more, remaining continuously intoxicated. Large doses may be consumed in such binges, with a high risk of acute toxicity, and the demand for the drug displaces all other considerations.

Experimental animals, given unlimited access to amphetamine, take it in such large amounts that they die from the cardiovascular effects within a few days. Given limited amounts, they too develop a binge pattern of dependence.

Pharmacokinetic aspects

Amphetamine is readily absorbed from the gastrointestinal tract, but to increase the intensity of the hit it can be snorted or injected. In crystal form, the free base of methamphetamine can be ignited and smoked in a manner similar to crack cocaine (see below). Amphetamine freely penetrates the blood–brain barrier. It does this more readily than other indirectly acting sympathomimetic amines such as **ephedrine** or **tyramine** (Ch. 14), which probably explains why it produces more marked central effects than those drugs. Amphetamine is mainly excreted unchanged in the urine, and the rate of excretion is increased when the urine is made more acidic (see Ch. 9). The plasma half-life of amphetamine varies from about 5 h to 20–30 h, depending on urine flow and urinary pH.

Clinical use

Attention-deficit hyperactivity disorder (ADHD). The main use of amphetamines is in the treatment of ADHD, particularly in children. **Methylphenidate** is most commonly used, at doses lower than those causing euphoria and other undesired effects. ADHD is a common condition in children—estimated as occurring in up to 9% of youth—whose incessant overactivity and very limited attention span disrupt their education and social development. The efficacy of amphetamines has been confirmed in many controlled trials. Disorders of dopamine pathways are suspected to underlie ADHD symptomatology, but the mechanism of action of amphetamines is unclear.

Other drug treatments for ADHD include the noradrenaline reuptake inhibitor, **atomoxetine**, and α_2 adrenoceptor agonists such as **clonidine** and **guanfacine**. The amine uptake inhibitor, **modafinil**, is not approved for paediatric use but may be effective in adult ADHD.

Narcolepsy

This is a disabling condition, probably a form of epilepsy, in which the patient suddenly and unpredictably falls asleep at frequent intervals during the day. Amphetamine is helpful but not completely effective. Modafinil is also effective in reducing the need for sleep and is becoming increasingly popular as a lifestyle drug (see Ch. 58) with students and young professionals. **Sodium oxybate**, the sodium salt of γ-hydroxybutyrate (see Ch. 37), is a CNS depressant that paradoxically is licensed for the treatment of narcolepsy with cataplexy (abrupt onset of paralysis of variable extent often triggered by emotion, sometimes with 'frozen' posture). The drug is frequently abused and is taxing to take correctly (on retiring and 2–4 hours later—an alarm clock is obligatory!); it is prescribed by specialists in sleep disorders.

Appetite suppression. Amphetamine derivatives proved relatively ineffective in treating obesity in humans, and have been largely abandoned because of their tendency to cause pulmonary hypertension, which can be so severe as to necessitate heart–lung transplantation.

Unwanted effects

The limited clinical usefulness of amphetamine is offset by its many unwanted effects, including hypertension, insomnia, anorexia, tremors, risk of exacerbating schizophrenia and risk of dependence. Cerebral haemorrhage has also been reported after amphetamine use, possibly the result of acutely raised blood pressure. There is evidence that habitual use of amphetamines is associated with long-term psychological effects of many kinds, including psychotic symptoms, anxiety, depression and cognitive impairment. The evidence in man is not conclusive, but taken in conjunction with animal data, it suggests that amphetamines can cause long-term damage.

Amphetamines

- The main effects are:
  - increased motor activity
  - euphoria and excitement
  - insomnia
  - anorexia
  - with prolonged administration, stereotyped and psychotic behaviour.
- Effects are due mainly to release of catecholamines, especially dopamine and noradrenaline.
- Stimulant effect lasts for a few hours and is followed by depression and anxiety.
- Tolerance to the stimulant effects develops rapidly, although peripheral sympathomimetic effects may persist.
- Amphetamines induce strong psychological dependence.
- Amphetamine psychosis, which closely resembles schizophrenia, can develop after prolonged use.
- Amphetamines may be useful in treating narcolepsy, and also (paradoxically) to control hyperkinetic children. They are no longer used as appetite suppressants because of the risk of pulmonary hypertension.
- Their main importance is in drug abuse.

COCAINE

Cocaine (see Streatfeild, 2002) is found in the leaves of the South American shrub, coca. These leaves are used for their stimulant properties by natives of South America, particularly those in mountainous areas, who use it to reduce fatigue during work at high altitude.

Considerable mystical significance was attached to the powers of cocaine to boost the flagging human spirit, and Freud tested it extensively on his patients and his family, publishing an influential monograph in 1884 advocating its use as a psychostimulant.[2] Freud's ophthalmologist colleague, Köller, obtained supplies of the drug and discovered its local anaesthetic action (Ch. 42), but the psychostimulant effects of cocaine have not proved to be clinically useful. On the other hand, they led to it becoming a widespread drug of abuse in Western countries. The mechanisms and treatment of cocaine abuse are discussed in Chapter 48.

Pharmacological effects

Cocaine binds to and inhibits the transporters responsible for the uptake of dopamine and noradrenaline into nerve terminals (see Chs 14 and 38), thereby enhancing the peripheral effects of sympathetic nerve activity and producing a marked psychomotor stimulant effect.

In humans, cocaine produces euphoria, garrulousness, increased motor activity and a magnification of pleasure. Users feel alert, energetic and physically strong and believe they have enhanced mental capabilities. Its effects resemble those of amphetamines, although it has less tendency to produce stereotyped behaviour, delusions, hallucinations and paranoia. With excessive dosage, tremors and convulsions, followed by respiratory and vasomotor depression, may occur. The peripheral sympathomimetic actions lead to tachycardia, vasoconstriction and an increase in blood pressure. Body temperature may increase, owing to the increased motor activity coupled with reduced heat loss.

Experimental animals rapidly learn to press a lever to self-administer cocaine and will consume toxic amounts of the drug if access is not limited. In transgenic mice lacking the D_2 receptor, the enhanced locomotor effects of cocaine are reduced, but surprisingly self-administration of cocaine is increased, in contrast to what is found with other self-administered drugs such as ethanol and morphine (see De Mei et al., 2009).

Chronic use, dependence and tolerance

Cocaine undoubtedly causes strong psychological dependence (see Ch. 48), but there is some debate about whether or not its continued use induces tolerance and physical dependence. Users may increase their intake of the drug but this may reflect a desire for an increased effect rather than the development of tolerance. In experimental animals, sensitisation (the opposite of tolerance) can be observed but the relevance of this to the situation in humans is unclear (see Bradberry, 2007). Like amphetamine, cocaine produces no clear-cut withdrawal syndrome but depression, dysphoria and fatigue may be experienced following the initial stimulant effect. Withdrawal of cocaine after administration for a few days causes a marked deterioration of motor performance and learned behaviour, which are restored by resuming dosage with the drug. Cocaine induces psychological dependence where users crave the drug's euphoric and stimulatory effects. The cellular mechanisms underlying craving and pharmacological approaches to reduce craving are discussed in Chapter 48. The pattern of dependence, evolving from occasional use through escalating dosage to compulsive binges, is similar to that seen with amphetamines.

Pharmacokinetic aspects

Cocaine is readily absorbed by many routes. For many years, illicit supplies have consisted of the hydrochloride salt, which could be given by nasal inhalation or intravenously. The latter route produces an intense and immediate euphoria, whereas nasal inhalation produces a less dramatic sensation and also tends to cause atrophy and necrosis of the nasal mucosa and septum.

Cocaine use increased dramatically when the free-base form ('crack') became available as a street drug. When an aqueous solution of cocaine hydrochloride is heated with sodium bicarbonate, then free-base cocaine, water, CO_2 and NaCl are produced. The free-base cocaine is insoluble in water, precipitates out and can then be rolled into 'rocks' of crack. Free-base cocaine vaporises at around 90°C, much lower than the melting point of cocaine hydrochloride (190°C) which burns rather than vaporises. Thus crack can be smoked, with the uncharged free-base being rapidly absorbed across the large surface area of the alveolae, giving rise to a greater CNS effect than that obtained by snorting cocaine. Indeed, the effect is nearly as rapid as that of intravenous administration, with less inconvenience and social stigma. The social, economic and even political consequences of this small change in formulation have been far-reaching.

The duration of its stimulant effect, about 30 min, is much shorter than that of amphetamine. It is rapidly metabolised in the liver.

A cocaine metabolite is deposited in hair, and analysis of its content along the hair shaft allows the pattern of cocaine consumption to be monitored, a technique that has revealed a much higher incidence of cocaine use than was voluntarily reported. Cocaine exposure in utero can be estimated from analysis of the hair of neonates.

Cocaine is still occasionally used topically as a local anaesthetic, mainly in ophthalmology and minor nose and throat surgery, but has no other clinical uses. It is a valuable pharmacological tool for the study of catecholamine release and reuptake, because of its relatively specific action in blocking noradrenaline and dopamine uptake.

Adverse effects

Toxic effects occur commonly in cocaine abusers. The main acute dangers are serious cardiovascular events (cardiac dysrhythmias, aortic dissection, and myocardial or cerebral infarction or haemorrhage). Progressive myocardial damage can lead to heart failure, even in the absence of a history of acute cardiac effects.

Cocaine can severely impair brain development in utero (see Volpe, 1992). The brain size is significantly reduced in babies exposed to cocaine in pregnancy, and neurological and limb malformations are increased. The incidence of ischaemic and haemorrhagic brain lesions, and of sudden

[2] In the 1860s, a Corsican pharmacist, Mariani, devised cocaine-containing beverages, Vin Mariani and Thé Mariani, which were sold very successfully as tonics. Imitators soon moved in, and Thé Mariani became the forerunner of Coca-Cola. In 1903, cocaine was removed from Coca-Cola because of its growing association with addiction and criminality (see Courtwright, 2001, for a lively account).

Cocaine

- Cocaine acts by inhibiting catecholamine uptake (especially dopamine) by nerve terminals.
- Behavioural effects of cocaine are very similar to those of amphetamines, although psychotomimetic effects are rarer. Duration of action is shorter.
- Cocaine used in pregnancy impairs fetal development and may produce fetal malformations.
- Cocaine produces strong psychological dependence.

infant death, is also higher in cocaine-exposed babies. Interpretation of the data is difficult because many cocaine abusers also take other illicit drugs that may affect fetal development, but the probability is that cocaine is highly detrimental.

Dependence, the main psychological adverse effect of amphetamines and cocaine, has potentially severe effects on quality of life (Ch. 48).

METHYLXANTHINES

Various beverages, particularly tea, coffee and cocoa, contain methylxanthines, to which they owe their mild central stimulant effects. The main compounds responsible are **caffeine** and **theophylline**. The nuts of the cola plant also contain caffeine, which is present in cola-flavoured soft drinks. However, the most important sources, by far, are coffee and tea, which account for more than 90% of caffeine consumption. A cup of instant coffee or strong tea contains 50–70 mg of caffeine, while filter coffee contains about twice as much. Among adults in tea- and coffee-drinking countries, the average daily caffeine consumption is about 200 mg. Further information on the pharmacology and toxicology of caffeine is presented by Fredholm et al. (1999).

Pharmacological effects

Methylxanthines have the following major pharmacological actions:

- CNS stimulation
- diuresis (see Ch. 28)
- stimulation of cardiac muscle (see Ch. 21)
- relaxation of smooth muscle, especially bronchial muscle (see Ch. 27).

The latter two effects resemble those of β-adrenoceptor stimulation (see Chs 14, 21 and 27). This is thought to be because methylxanthines (especially **theophylline**) inhibit phosphodiesterase, which is responsible for the intracellular metabolism of cAMP (Ch. 3). They thus increase intracellular cAMP and produce effects that mimic those of mediators that stimulate adenylyl cyclase. Methylxanthines also antagonise many of the effects of adenosine, acting on both A_1 and A_2 receptors (see Ch. 16). Transgenic mice lacking functional A_2 receptors are abnormally active and aggressive, and fail to show increased motor activity in response to caffeine (Ledent et al., 1997), suggesting that antagonism at A_2 receptors accounts for part, at least, of its CNS stimulant action. Caffeine also sensitises ryanodine receptors (see Ch. 4) but this effect occurs at higher concentrations (> 10 mmol/l) than those achieved by recreational intake of caffeine. The concentration of caffeine reached in plasma and brain after two or three cups of strong coffee—about 100 μmol/l—is sufficient to produce appreciable adenosine receptor block and a small degree of phosphodiesterase inhibition. The diuretic effect probably results from vasodilatation of the afferent glomerular arteriole, causing an increased glomerular filtration rate.

Methylxanthines

- Caffeine and theophylline produce psychomotor stimulant effects.
- Average caffeine consumption from beverages is about 200 mg/day.
- Main psychological effects are reduced fatigue and improved mental performance, without euphoria. Even large doses do not cause stereotyped behaviour or psychotomimetic effects.
- Methylxanthines act mainly by antagonism at A_2 purine receptors, and partly by inhibiting phosphodiesterase, thus producing effects similar to those of β-adrenoceptor agonists.
- Peripheral actions are exerted mainly on heart, smooth muscle and kidney.
- Theophylline is used clinically as a bronchodilator; caffeine is not used clinically.

Caffeine and theophylline have very similar stimulant effects on the CNS. Human subjects experience a reduction of fatigue, with improved concentration and a clearer flow of thought. This is confirmed by objective studies, which have shown that caffeine reduces reaction time and produces an increase in the speed at which simple calculations can be performed (although without much improvement in accuracy). Performance at motor tasks, such as typing and simulated driving, is also improved, particularly in fatigued subjects. Mental tasks, such as syllable learning, association tests and so on, are also facilitated by moderate doses (up to about 200 mg of caffeine, or about two cups of coffee) but impaired by larger doses. Insomnia is common. By comparison with amphetamines, methylxanthines produce less locomotor stimulation and do not induce euphoria, stereotyped behaviour patterns or a psychotic state, but their effects on fatigue and mental function are similar.

Tolerance and habituation develop to a small extent, but much less than with amphetamines, and withdrawal effects are slight. Caffeine is not self-administered by animals, and it cannot be classified as a dependence-producing drug.

Clinical use and unwanted effects

There are few clinical uses for caffeine. It is included with aspirin in some preparations for treating headaches and other aches and pains, and with ergotamine in some antimigraine preparations, the object being to produce a mildly agreeable sense of alertness. Theophylline (formulated as **aminophylline**) is used mainly as a bronchodilator in treating severe asthmatic attacks (see Ch. 27). Caffeine has few unwanted side effects and is safe even in very large doses. In vitro tests show that it has mutagenic activity, and large doses are teratogenic in animals. However, epidemiological studies have shown no evidence of carcinogenic or teratogenic effects of tea or coffee drinking in humans.

OTHER STIMULANTS

Arecoline, a cholinergic agonist, is a mild stimulant contained in the betel nut. Its use is widespread in India, Thailand, Indonesia and other Asian cultures. Arecoline improves learning and memory.

Cathinone and **cathine** are the active ingredients in the khat shrub. Chewing the leaves is popular in parts of Africa such as Ethiopia and Somalia and its use is spreading through immigrant populations in Western countries.

Nitrites such as **amyl nitrite** (see Ch. 21) produce a rush as heart rate increases and blood rushes to the head. Headache, dizziness, nausea and a feeling of light-headedness as well as a slowing of time are experienced. Sexual pleasure may be enhanced.

PSYCHOTOMIMETIC DRUGS

Psychotomimetic drugs (also referred to as *psychedelic* or *hallucinogenic* drugs) affect thought, perception and mood, without causing marked psychomotor stimulation or depression (see Nichols, 2004). Thoughts and perceptions tend to become distorted and dream-like, rather than being merely sharpened or dulled, and the change in mood is likewise more complex than a simple shift in the direction of euphoria or depression. Importantly, psychotomimetic drugs do not cause dependence, even though their psychological effects overlap those of highly addictive major psychostimulants such as cocaine and amphetamines.

Psychotomimetic drugs include the following:

- Drugs that act on 5-hydroxytryptamine (5-HT) receptors and transporters. These include **lysergic acid diethylamide** (LSD), **psilocybin** and **mescaline**, which are agonists at 5-HT_2 receptors (see Chs 15 and 38), and **MDMA** (ecstasy) which acts mainly by inhibiting 5-HT uptake. MDMA also acts on several other receptors and transporters (see Green et al., 2003), and has powerful psychostimulant effects typical of amphetamines, as well as psychotomimetic effects.
- **Ketamine** and **phencyclidine**, antagonists at NMDA-type glutamate receptors.
- **Δ^9-Tetrahydrocannabinol** (THC), the active ingredient in cannabis, produces a mixture of psychotomimetic and depressant effects similar to, but less pronounced than, those of LSD. This drug is discussed in detail in Chapter 18.
- **Salvinorin A,** a κ-opioid receptor agonist.

LSD, PSILOCYBIN AND MESCALINE

LSD is an exceptionally potent psychotomimetic drug capable of producing strong effects in humans in doses less than 1 μg/kg. It is a chemical derivative of lysergic acid, which occurs in the cereal fungus ergot (see Ch. 15), and was first synthesised by Hoffman in 1943. Hoffman deliberately swallowed about 250 μg of LSD (the threshold dose is now known to be around 20 μg) and wrote 30 years later of the experience: 'the faces of those around me appeared as grotesque coloured masks … marked motoric unrest, alternating with paralysis … heavy feeling in the head, limbs and entire body, as if they were filled with lead … clear recognition of my condition, in which state I sometimes observed, in the manner of an independent observer, that I shouted half insanely.' These effects lasted for a few hours, after which Hoffman fell asleep, 'and awoke next morning feeling perfectly well'. Apart from these dramatic psychological effects, LSD has few physiological effects.

Mescaline, which is derived from a Mexican cactus and has been known as a hallucinogenic agent for many centuries, was made famous by Aldous Huxley in *The Doors of Perception*. It is chemically related to amphetamine.

Psilocybin is obtained from fungi (colloquially known as magic mushrooms). The effects of taking psilocybin are similar to those experienced with LSD.

Pharmacological effects

The main effects of these drugs are on mental function, most notably an alteration of perception in such a way that sights and sounds appear distorted and fantastic. Hallucinations—visual, auditory, tactile or olfactory—also occur, and sensory modalities may become confused, so that sounds are perceived as visions. Thought processes tend to become illogical and disconnected, but subjects retain insight into the fact that their disturbance is drug induced, and generally find the experience exhilarating. Occasionally, especially if the user is already anxious, LSD produces a syndrome that is extremely disturbing (the 'bad trip'), in which the hallucinatory experience takes on a menacing quality and may be accompanied by paranoid delusions. Furthermore, 'flashbacks' of the hallucinatory experience have been reported weeks or months later.

LSD acts on various 5-HT-receptor subtypes (see Chs 15 and 38); its psychotomimetic effects are thought to be mediated mainly by its 5-HT_{2A} receptor agonist actions (see Nichols, 2004). It inhibits the firing of 5-HT-containing neurons in the raphe nuclei (see Ch. 38), apparently by acting as an agonist on the inhibitory autoreceptors of these cells. The significance of this response to its psychotomimetic effects is unclear. Psilocybin is dephosphorylated to psilocin which is an agonist at several 5-HT receptors including the 5-HT_{2A} receptor. The mechanism of action of mescaline is less well defined. There are contradictory reports about its activity at 5-HT_{2A} receptors. It has also been reported to act as an inhibitor of monoamine transport.

The main effects of psychotomimetic drugs are subjective, so it is not surprising that animal tests that reliably predict psychotomimetic activity in humans have not been devised.[3]

Dependence and adverse effects

Psychotomimetic agents are largely not self-administered by experimental animals. Indeed, in contrast to most of the drugs that are widely abused by humans, they have aversive rather than reinforcing properties in behavioural tests. Tolerance to their effects develops quite quickly, but there is no physical withdrawal syndrome in animals or humans.

There has been much concern over reports that LSD and other psychotomimetic drugs, as well as causing potentially dangerous bad trips, can lead to more persistent mental disorder (see Abraham & Aldridge, 1993). Unexpected flashbacks can be very disturbing. Also, there are recorded instances in which altered perception and hallucinations have lasted for up to 3 weeks following a single dose of LSD, and of precipitation of attacks in schizophrenic patients.

[3]One of the more bizarre tests involves spiders, whose normal elegantly symmetrical webs become jumbled and erratic if the animals are treated with LSD. It is worth searching the web for 'spiders LSD' to see images.

MDMA (ECSTASY)

MDMA (3,4-methylenedioxymethamphetamine) is widely used as a 'party drug' because of the euphoria, loss of inhibitions and energy surge that it induces. It is a stimulant drug which also has mild hallucinogenic effects. The experience of taking the drug has been likened to taking amphetamine and weak LSD.

Pharmacological effects

Although it is an amphetamine derivative (Fig. 47.1), it affects monoamine function in a different manner from the amphetamines (see Green et al., 2003; Morton, 2005; Iversen, 2006). It inhibits monoamine transporters, principally the 5-HT transporter, and also releases 5-HT, the net effect being a large increase in free 5-HT in certain brain regions, followed by depletion. Similar but smaller changes occur in relation to dopamine and noradrenaline. Simplistically, the effects on 5-HT function determine the psychotomimetic effects, while dopamine and noradrenaline changes account for the initial euphoria and later rebound dysphoria. Although not addictive, MDMA carries serious risks, both acute and long term.

Sudden illness and death can occur even after small doses of MDMA. This can be due to several factors:

- Acute hyperthermia (see Fig. 47.2), resulting in damage to skeletal muscle and renal failure. It is still unclear how this effect is produced in humans. It may be mediated centrally through activation of 5-HT or dopamine receptors. It could also reflect an action of MDMA on mitochondrial function. It is exacerbated by energetic dancing and high ambient temperature and certain individuals may be particularly susceptible to this danger.
- Excess water intake and water retention. MDMA causes inappropriate secretion of antidiuretic hormone, leading to thirst, over-hydration and hyponatraemia ('water intoxication'). Symptoms include dizziness and disorientation leading to collapse into coma.

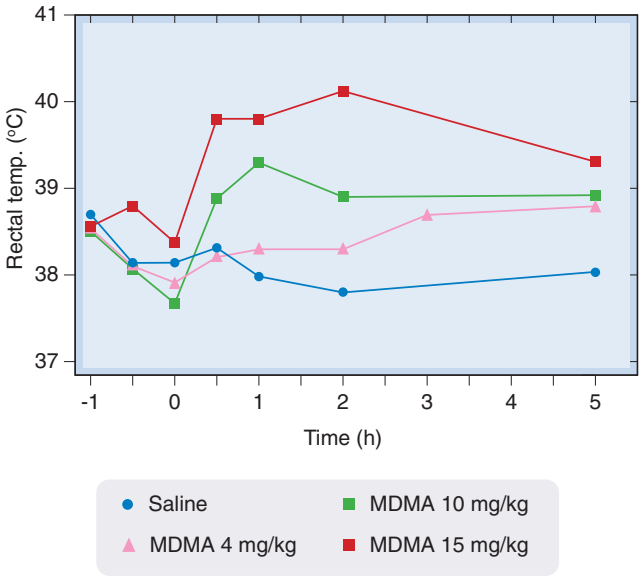

Fig. 47.2 A single injection of MDMA causes a dose-related increase in body temperature in rats. Drug administered at time zero (Reproduced with permission from Green et al., 2004.)

- Heart failure in individuals with an undiagnosed heart condition.

The after-effects of MDMA persist for a few days and comprise depression, anxiety, irritability and increased aggression—the 'mid-week blues'. There is also evidence of long-term deleterious effects on memory and cognitive function in heavy MDMA users. In animal studies, MDMA can cause degeneration of 5-HT and dopamine neurons, but whether this occurs in humans is uncertain (see Morton, 2005).

Illicit 'ecstasy' tablets and powder are sometimes contaminated or entirely substituted with ***para*-methoxyamphetamine** which produces similar behavioural effects but which may be more dangerous to the user. Another related drug is **4-bromo-2,5-dimethoxyphenethylamine** (2CB).

KETAMINE AND PHENCYCLIDINE

Ketamine (*'Special K'*) is a dissociative anaesthetic (Ch. 40) now also used as a recreational drug. An analogue, **phencyclidine** (PCP, *'Angel dust'*), was a popular hallucinogen in the 1970s but its use has declined. These drugs produce a feeling of euphoria. At higher doses they cause hallucinations and a feeling of detachment, disorientation and numbness. PCP was reported to cause psychotic episodes and is used in experimental animals to produce a model of schizophrenia (see Ch. 45 and Morris et al., 2005).

Pharmacological effects

Their main pharmacological effect is block of the NMDA receptor channel (see Ch. 37). This was at one time mistakenly described as 'acting at σ opioid receptors'. Long-term regular use of ketamine can result in severe bladder pain through an as yet unknown mechanism. Combination of ketamine with **depressant** drugs such as **alcohol**, **barbiturates** and **heroin** can result in dangerous overdose.

OTHER PSYCHOTOMIMETIC DRUGS

Salvinorin A is a hallucinogenic agent contained in the American sage plant *Salvia divinorum*, a member of the mint family. It was originally used by the Mazatecs in Mexico; in recent years its use has spread and it has become known as ***herbal ecstasy***. It is a κ-opioid receptor agonist (see Ch. 41).[4] At high doses, delirium may be produced.

DMT (dimethyltryptamine) and **DOM** (2,5-dimethoxy-4-methylamphetamine) are synthetic hallucinogenic drugs that produce effects similar to **LSD**.

Muscarinic receptor antagonists (see Chs 13 and 36), **hyoscine**, **hyoscyamine** and **atropine** are contained in various plants, including henbane and mandrake. Consumption can cause hallucinations, drowsiness and disorientation.

Ibogaine is contained in the root bark of iboga shrubs in Africa, South America and Australia. At high doses, it is hallucinogenic. Users have reported experiencing a reduced desire to take other drugs such as cocaine and heroin leading to ibogaine being investigated as a potential treatment for drug craving (see Ch. 48).

[4]In Phase 1 clinical trials of synthetic κ agonists as potential analgesic agents, the drugs were reported to induce a feeling of dysphoria. Perhaps the 'normal' volunteers in those trials were disturbed by the hallucinations they probably experienced. Interesting then that a naturally occurring κ agonist has now become a recreational drug.

Psychotomimetic drugs

- The main types are:
  - **lysergic acid diethylamide** (LSD), **psilocybin** and **mescaline** (actions related to 5-hydroxytryptamine (5-HT) and catecholamines)
  - **methylenedioxymethamphetamine** (MDMA, 'ecstasy')
  - **ketamine** and **phencyclidine**.
- Their main effect is to cause sensory distortion and hallucinatory experiences.
- LSD is exceptionally potent, producing a long-lasting sense of dissociation and disordered thought, sometimes with frightening hallucinations and delusions, which can lead to violence. Hallucinatory episodes can recur after a long interval.
- LSD and phencyclidine precipitate schizophrenic attacks in susceptible patients, and LSD may cause long-lasting psychopathological changes.
- LSD appears to act as an agonist at 5-HT_{2A} receptors.
- MDMA is an amphetamine analogue that has powerful psychostimulant as well as psychotomimetic effects.
- MDMA can cause an acute hyperthermic reaction as well as excess water intake and retention, sometimes fatal.
- Psychotomimetic drugs do not cause physical dependence and tend to be aversive, rather than reinforcing, in animal models.
- Ketamine and phencyclidine act by blocking the glutamate-activated NMDA receptor channel.

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48 Drug addiction, dependence and abuse

OVERVIEW

In this chapter we consider those drugs that are consumed because people choose to, and not because they are advised to by their doctor. Largely these drugs are taken because they are pleasurable (hedonic). A list of the more frequently used drugs is given in Table 48.1. It includes drugs that are also used for medicinal purposes (e.g. general anaesthetics, benzodiazepines, opioids and some psychostimulants), non-therapeutic drugs that are legal in many countries (e.g. nicotine and ethanol) and many other drugs that are widely used although their manufacture, sale and consumption have been declared illegal in most Western countries.

The reasons why the use of a particular drug is viewed as a problem to society—and hence may be considered 'drug abuse'—are complex and largely outside the scope of this book. The drug and its pharmacological activity are only the starting point. For many, but not all, drugs of abuse, continued use leads to dependence. Here, we briefly review the classes of drug, the biological processes underlying drug dependence and describe in detail the pharmacology of two important drugs that have no place in therapeutics but are consumed in large amounts, namely nicotine and ethanol. Other drugs that are abused are described elsewhere in this book (see Table 48.1). 'Lifestyle' and 'sport' drugs are discussed in Chapter 58.

For further information on various aspects of drug abuse, see Winger et al. (2004), Karch (2006) and Koob & Le Moal (2006).

DRUG USE AND ABUSE

A number of terms are used, sometimes interchangeably and sometimes incorrectly, to describe drug use and the consequences of administration of drugs. Terms that are best avoided are described in Table 48.2. Other, more useful terms are defined in the text below.

A vast and ever increasing array of drugs is used to alter mood and perception. These range from drugs that are also used as medicines, through non-medicinal synthetic drugs to herbal preparations (Table 48.1). The popularity of each varies between different societies across the world, and within societies popularity differs among different groups of individuals.[1] Frequently, users will take more than one drug concomitantly or sequentially. Polydrug use is a very under-researched area both in regard to why it is done and how different drugs may interact, as well as in regard to the potential harm that may arise from such practices (e.g. ethanol alters cocaine metabolism resulting in the production of *cocaethylene* which is more potent than cocaine and has greater cardiovascular toxicity). Sequential use is often intended to reduce adverse effects when coming down off the first drug (e.g. use of benzodiazepines when coming down from stimulants).

At first sight, the drugs listed in Table 48.1 form an extremely heterogeneous pharmacological group; we can find little in common at the molecular and cellular level between say, morphine, cocaine and LSD. What links them is that people find their effects pleasurable (hedonic) and tend to want to repeat the experience. The drug experience may take the form of intense euphoria, mood elevation, hallucinations, stimulation, sedation or calming depending upon the specific drug taken.

Drug use involves effects on the brain that can be both acute and chronic (Fig. 48.1). The immediate, acute effect on mood is the reason the drug is taken. For some drugs (e.g. amphetamines, Ch. 47), this may be followed by a rebound negative or depressed phase. Persistent use of a drug may lead to compulsive drug use (addiction/dependence—a complex state that involves both psychological and physiological dependence) and to the development of tolerance. Psychological dependence can give rise to intense craving even when the user has been drug-free for months or years.

DRUG ADMINISTRATION

For drugs that induce strong feelings of euphoria, there are two components to the experience: an initial rapid effect (the *rush* or *buzz*) and a more prolonged pleasurable effect (the *high*). The intensity of the initial effect is determined by how fast the drug enters the brain and activates its effector mechanism. For many casual drug users, ease of administration defines how the drug is taken (e.g. smoking, swallowing or snorting a drug is relatively easy). However, for other drug users chasing a more intense experience, the route of administration and the choice of individual drug become important. Intravenous injection or smoking results in faster absorption of a drug than when it is taken orally. Heroin (official name diamorphine), cocaine, amphetamines, tobacco and cannabis are all taken by one or other of these routes. Heroin is more popular as a drug of abuse than morphine. This is because it enters the brain more rapidly than morphine. However, heroin itself does not interact with opioid receptors but is rapidly deacetylated to 6-acetylmorphine and morphine, μ-receptor agonists (see Ch. 41).

DRUG HARM

All drugs of abuse are harmful to a varying extent. Adverse effects can be the result of drug overdose (e.g. respiratory depression produced by opioids), of effects on tissues other than the brain (e.g. necrosis of the nasal septum resulting

[1]A recent survey in one UK city showed that among Friday-night clubbers the choice of drug was associated with the type of music the clubs played (Measham & Moore, 2009).

Table 48.1 The main drugs of abuse

Type	Examples	Dependence liability	See Chapter
Opioids	Morphine	Very strong	41
	Diamorphine (heroin)	Very strong	41
	Methadone	Very strong	41
	Oxycodone	Very strong	41
General central nervous system depressants	Ethanol	Strong	This chapter
	Barbiturates	Strong	43
	General anaesthetics (e.g. N_2O, propofol)	Moderate	40
	Ketamine	Moderate	40
	Solvents	Strong	—
Anxiolytic and hypnotic drugs	Benzodiazepines	Moderate	43
	GHB	Probably moderate	37
Psychomotor stimulants	Amphetamines	Strong	47
	Cocaine	Very strong	47
	MDMA (ecstasy)	Weak or absent	47
	Nicotine	Very strong	This chapter
Psychotomimetic agents	Lysergic acid diethylamide	Weak or absent	47
	Mescaline	Weak or absent	47
	Cannabis	Weak	18

Table 48.2 Glossary of frequently used and 'abused' terms

Addict	Person for whom the desire to experience a drug's effects overrides any consideration for the serious physical, social or psychological problems that the drug may cause to the individual or others. Often used in non-scientific circles to convey criminal intent and so has fallen out of favour with those involved in treating people with drug problems
Drug misuse	Non-medicinal drug use (although some would not consider taking drugs to alter mood/induce hallucinations as 'misuse' or 'abuse')
Junkie	Pejorative term for someone who is dependent upon a drug
Narcotics	Originally used as a term to describe opioids as they induce sleep (narcosis). Subsequently this term has been used by non-scientists to describe a wide range of drugs of abuse (including cocaine which is a stimulant!)
Recreational drug use	Originally used to describe all drug abuse, it is now sometimes used to describe drug use in the bar/club/dance scene
Self-medication	Taking a drug of abuse to offset some underlying medical condition, e.g. pain, depression
Substance use	Some governments do not consider ethanol to be a drug, hence 'substance use' (or 'substance abuse') is used to include ethanol

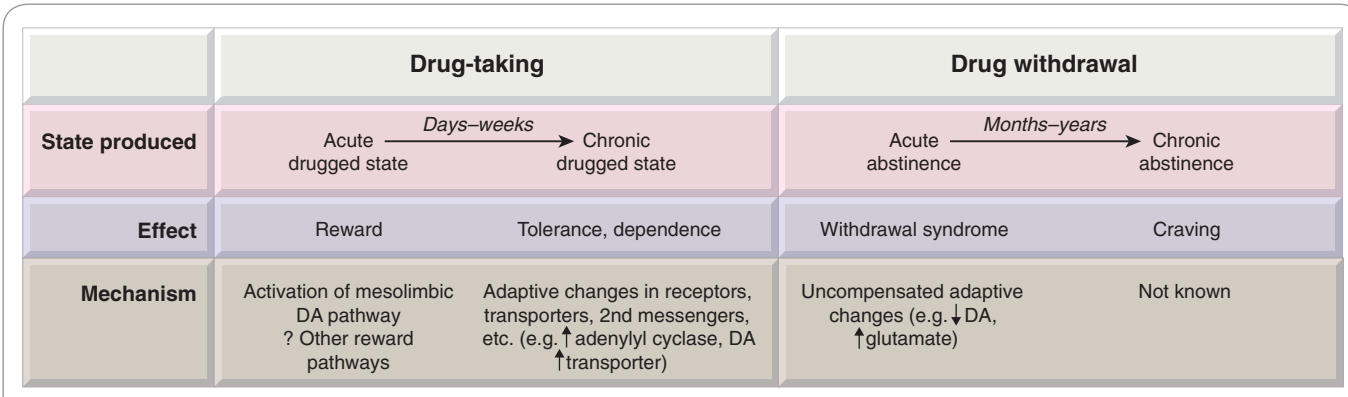

Fig. 48.1 Cellular and physiological mechanisms involved in drug dependence showing the relationship between the immediate and delayed effects of drug taking and drug withdrawal. DA, dopamine.

from chronic cocaine use), of the route of administration (e.g. HIV and other infections in drug users who share needles), of effects unrelated to the specific actions of the drug (e.g. carcinogenicity of tobacco smoke, severe bladder pain in regular ketamine users) or of use for illegal purposes (e.g. flunitrazepam or γ-hydroxybutyrate as date-rape drugs). Many major harms relate to the ability of some drugs to induce dependence (e.g. psychostimulants, opioids, ethanol and tobacco) or to reveal a susceptibility to psychotic illness in some individuals (e.g. amphetamines and cannabis).

An attempt to produce a rational scale of harm, based on assessment by an expert panel of physical risk, dependence liability and social cost was reported by Nutt et al. (2010), who have argued that such ratings should influence how governments police and punish people for supplying and using particular drugs. As expected, ethanol, heroin and cocaine were judged to be the most harmful, with cannabis, LSD and ecstasy (MDMA, see Ch. 47) much less so—an order that is not reflected in the classification of these drugs under UK law.[2]

DRUG DEPENDENCE

Drug dependence describes the human condition in which drug taking becomes compulsive, taking precedence over other needs, often with serious adverse consequences. Dependence becomes a problem when:

- the want becomes so insistent that it dominates the lifestyle of the individual and damages his or her quality of life
- the habit itself causes actual harm to the individual or the community.

Examples of the latter are the mental incapacity and liver damage caused by ethanol, the many diseases associated with smoking tobacco, the high risk of infection when injecting intravenously (especially HIV), the serious risk of overdosage with most opioids and the criminal behaviour resorted to when drug users need to finance their habit.

Dependence may involve a state of psychological as well as physical dependence. Family studies show clearly that susceptibility to dependence is an inherited characteristic, and many candidate genes have been reported, with a particular focus on genes involved in transmitter metabolism, receptors, etc. (see Mayer & Höllt, 2005). The general conclusion is that variants of many different genes each make a small contribution to the overall susceptibility of an individual to addiction—a familiar scenario that provides few pointers for therapeutic intervention. Polymorphisms in ethanol-metabolising genes (see later section on ethanol) are the best example of genes that directly affect the tendency to abuse a drug.

DRUG-INDUCED REWARD

The common feature of the various types of psychoactive drugs that are addictive is that all produce a *rewarding* experience (e.g. an elevation of mood or a feeling of euphoria or calmness).

In animal studies, where the state of mood cannot be inferred directly, reward is manifest as *positive reinforcement*, i.e. an increase in the probability of occurrence of any behaviour that is associated with the drug experience. In *conditioned place preference* studies, animals receive a drug or placebo and are then placed in different environments. Subsequently, when tested in a drug-free state, they will spend more time in the environment associated with a previous rewarding drug experience. Another way of determining if a drug is rewarding is to test whether or not animals will self-administer the drug by pressing a lever to obtain it. All dependence-producing drugs are self-administered by experimental animals. Hallucinogenic drugs are not, however, normally self-administered by animals, which may indicate that, unlike humans, they find the experience non-rewarding.

Humans, of course, self-administer drugs without necessarily becoming addicted. To model the compulsive nature of addiction more accurately, extensions to the self-administration paradigm may be employed (see Deroche-Gamonet et al., 2004). Rats treated for a short time with 'non-addictive' doses of cocaine will self-administer the drug by bar pressing, but stop bar pressing when a signal is shown to indicate that the drug injector is disconnected, or if the drug injection is accompanied by punishment in the form of a foot shock. With more intense 'addictive' pretreatment, bar pressing persists at a high rate under these conditions. Models of this sort are considered more likely to replicate the situation of addiction in humans as a basis for testing therapeutic approaches, but in humans, drug dependence represents a stable change in brain function sustained by processes that are more complex and long lasting than the neurobiological changes so far studied in experimental animals.

Humans also have a choice as to whether or not they wish to experiment with and continue taking drugs—there may therefore be an element of risk taking when experimenting with drugs. In behavioural tests, some rats are observed to be much more impulsive than others (Dalley et al., 2007). These impulsive rats also show a higher rate of cocaine self-administration. Interestingly, the impulsive rats were also observed to have a lower level of expression of D_2 and D_3 dopamine receptors in the nucleus accumbens (see below for the importance of this brain region in drug use).

Reward pathways

▼ Virtually all dependence-producing drugs so far tested, including opioids, nicotine, amphetamines, ethanol and cocaine, activate the *reward pathway*—the mesolimbic dopaminergic pathway (see Ch. 38), that runs, via the medial forebrain bundle, from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens and limbic region (see Nestler, 2001). Even though for some of these drugs their primary sites of action may be elsewhere in the brain, they all increase the extracellular level of dopamine in the nucleus accumbens, as shown by microdialysis and other techniques (see Spanagel & Weiss, 1999). Opioids enhance the firing of VTA dopaminergic neurons by reducing the level of GABAergic inhibition (disinhibition) within the VTA, whereas amphetamine and cocaine act on dopaminergic nerve terminals in the nucleus accumbens to release dopamine or prevent its reuptake (see Ch. 14). Given that dopamine release in the nucleus accumbens is also enhanced by naturally rewarding stimuli, such as food, water, sex and nurturing, it would appear that drugs are simply activating, or overactivating, the body's own pleasure system.

Chemical or surgical interruption of the VTA–accumbens dopaminergic pathway impairs drug-seeking behaviours in many experimental situations. Deletion of D_2 receptors in a transgenic mouse strain

[2]In determining society's attitude towards drugs, the media play an influential role. In the UK, deaths following consumption of ecstasy (around 60 per year) are often widely reported in the press and on television but deaths due to heroin overdose (much more prevalent at around 700 per year) are largely ignored unless the victim is famous.

eliminated the rewarding properties of morphine administration without eliminating other opioid effects, and it did not prevent the occurrence of physical withdrawal symptoms in morphine-dependent animals (Maldonado et al., 1997), suggesting that the dopaminergic pathway is responsible for the positive reward but not for the negative withdrawal effects. However, D_2-receptor antagonists (antipsychotic drugs; see Ch. 45) have not been successful in treating addiction, and more recent evidence (see Heidbreder & Hagan, 2005) suggests that D_3 receptors play an important role. The development of D_3-receptor antagonists or partial agonists as treatments for drug abuse is awaited. Other mediators, particularly 5-hydroxytryptamine, glutamate and GABA, have also been implicated in the conditioning mechanisms that reinforce drug-seeking behaviour, and a variety of pharmacological strategies based on blocking these pathways are being explored (see Heidbreder & Hagan, 2005).

PSYCHOLOGICAL DEPENDENCE

Having experienced the rewarding effects of a drug, an individual may desire to repeat the experience. The memory of previous drug-induced experiences can be very intense and long lasting, giving rise to *craving*; it may drive an individual to take the drug again—referred to as *relapse* when someone is trying to come off a drug (see Weiss, 2005). Craving may be triggered by cues such as experiencing the environment that a person associates with previously taking the drug or the sight of drug administration paraphernalia (e.g. a crack pipe or syringe). Coupled with the direct rewarding effect of the drug, cessation of drug use may be associated with an aversive psychological effect from which the subject will attempt to escape by self-administering the drug.

The psychological factors in drug dependence are discussed in detail by Koob & Le Moal (2006) and summarised in Figure 48.2.

PHYSICAL DEPENDENCE

This condition is characterised by a *withdrawal* or *abstinence syndrome* whereby on cessation of drug administration or on administering an antagonist, adverse physiological effects are experienced over a period of days or weeks, the precise withdrawal responses being characteristic of the type of drug taken. Withdrawal responses can be observed in animals after chronic drug administration. The intensity of the withdrawal syndrome also varies between drugs of the same type (e.g. withdrawal from methadone is less intense but more prolonged than that from heroin, one of the reasons behind methadone maintenance treatment of heroin users). Pharmacological intervention can be used to reduce the intensity of the withdrawal (see Table 48.3). Several types of therapeutic drug, including antidepressant and antipsychotic agents, also produce withdrawal symptoms on cessation of administration but it is important to distinguish this type of commonly observed 'rebound' phenomenon from the physical dependence associated with drugs of abuse.

Physical dependence is less important in sustaining drug-seeking behaviour than psychological dependence. A degree of physical dependence is common when patients receive opioid analgesics in hospital for several days, but this rarely leads to addiction. On the other hand, heroin users who are nursed through and recover fully from the physical abstinence syndrome are still extremely likely to revert to drug taking later. Therefore although physical dependence may influence the drive to retake a drug, it is not the major factor in long-term drug dependence.

TOLERANCE

Tolerance (see Ch. 2) describes the decrease in pharmacological effect on repeated administration of a drug—it develops over time as does the state of dependence. It does not occur with all drugs of abuse.

MECHANISMS OF DEPENDENCE AND TOLERANCE

▼ Drug users report that visual cues—such as the sight of a crack pipe or of a syringe—can evoke intense memories of the drug

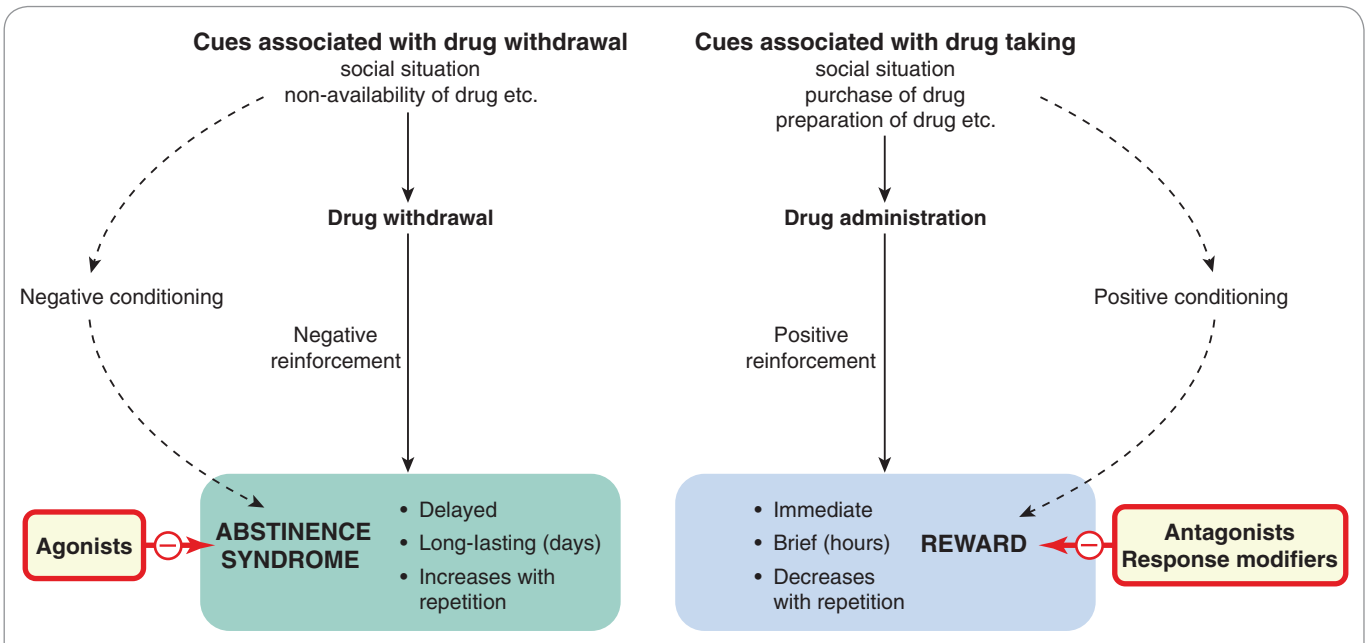

Fig. 48.2 Psychological factors involved in drug dependence.

Drug dependence

- Dependence occurs when, as a result of repeated administration of the drug, the desire to experience the effects of a drug again becomes compulsive.
- Dependence occurs with a wide range of psychotropic drugs, acting by many different mechanisms.
- Dependence can be subdivided into psychological dependence and physical dependence
- Psychological dependence (craving) is the major factor leading to relapse among treated addicts.
- The common feature of psychological dependence-inducing drugs is that they have a positive reinforcing action ('reward') associated with activation of the mesolimbic dopaminergic pathway.
- Physical dependence is characterised by an abstinence syndrome, which varies in type and intensity for different classes of drug.
- On repeated administration, tolerance may occur to the effects of the drug.
- Although genetic factors contribute to drug-seeking behaviour, no specific genes have yet been identified.

experience and induce strong craving for the drug which may precipitate relapse. This suggests that associative learning may be a major factor in psychological dependence (Robbins et al., 2008). It has been suggested that drugs alter memory formation to enhance the recollection of previous drug experience. In this regard, it is of interest that several drugs produce changes in synaptic plasticity, a cellular correlate of memory formation (see Ch. 37). While cocaine, morphine, nicotine and ethanol enhance long-term potentiation (LTP) in the VTA by increasing the expression of AMPA receptors on the plasma membrane, cocaine also increases long-term depression (LTD) in the nucleus accumbens (Hyman et al., 2006).

It was for many years assumed that physical dependence and tolerance were produced by the same underlying adaptive mechanisms. This is now generally accepted to not be the case (see Bailey & Connor, 2005).

The mechanisms responsible for the withdrawal syndrome have been most fully characterised for opioid dependence but similar mechanisms may apply to cocaine and ethanol withdrawal. At the cellular level, withdrawal of opioids results in a rebound increase in cAMP production as a result of 'superactivation' of adenylyl cyclase as well as upregulation of the amount of this enzyme. This results in activation of protein kinase A (PKA), in an increase in adenosine as a consequence of the conversion of cAMP to adenosine and in activation of a transcription factor—cAMP response element binding protein (CREB). The rise in PKA activity increases the excitability of nerve terminals by phosphorylating neurotransmitter transporters thus increasing their ionic conductance (see Bagley et al., 2005) and in an increase in neurotransmitter release by a direct action on the secretory process (Williams et al., 2001). Withdrawal results in

Table 48.3 Pharmacological approaches to treating drug dependence

Mechanism	Example(s)
To alleviate withdrawal symptoms	Methadone (orally active) used short term to blunt opioid withdrawal Ibogaine (a naturally occurring psychoactive agent) used by some to reduce opioid withdrawal α_2 Adrenoceptor agonists (e.g. clonidine, lofexidine) to diminish opioid, alcohol and nicotine withdrawal symptoms β Adrenoceptor antagonists (e.g. propranolol) to diminish excessive peripheral sympathetic activity Benzodiazepines, clomethiazole, topiramate and γ-hydroxybutyric acid (GHB) to blunt alcohol withdrawal
Long-term substitution	Methadone, buprenorphine or legal heroin to maintain opioid-dependent patients Nicotine patches or chewing gum Varenicline (α4β2 nicotinic receptor partial agonist)
Blocking response	Naltrexone to block opioid effects in drug-withdrawn patients Mecamylamine to block nicotine effects Immunisation against cocaine and nicotine to produce circulating antibody (still being developed)
Aversive therapies	Disulfiram to induce unpleasant response to ethanol
Reducing continued drug use (may act by reducing craving)	Bupropion (antidepressant with some nicotinic receptor antagonist activity) to reduce tobacco use Naltrexone to reduce ethanol use Clonidine (α_2 adrenoceptor agonist) to reduce craving for nicotine[a] Acamprosate (NMDA receptor antagonist) to treat alcoholism[a] Topiramate and lamotrogine (antiepileptic agents) to treat alcoholism and cocaine use[a] γ-Hydroxybutyric acid (GHB) reported to reduce craving for alcohol and cocaine[a] Baclofen reported to reduce opioid, alcohol and stimulant use[a] Ibogaine reported to reduce craving for stimulants and opioids[a]

[a]How effective these agents are at reducing the continued use of other drugs of abuse over and above the ones listed remains to be determined.

Notes: Antidepressant, mood stabilising, anxiolytic and antipsychotic medications are useful when treating patients who, in addition to their drug use, also suffer from other mental disorders. The cannabinoid CB_1-receptor antagonist rimonabant, in addition to its antiobesity effects, also reduces nicotine, ethanol, stimulant and opioid consumption. However, it also induces depression and its use has been discontinued. See Web links in the reference list for further information on treatments of drug dependence and Myrick & Anton (1998) for treatment of alcohol withdrawal.

enhanced GABA release in various parts of the brain, probably through the mechanisms described above. The release of other neurotransmitters is also likely to be enhanced. On the other hand, the enhanced extracellular levels of adenosine, acting on presynaptic A_1 receptors (see Ch. 16), acts to inhibit glutamate release at excitatory synapses, and thus counteracts the neuronal hyperexcitability that occurs during drug withdrawal, suggesting the possibility—not yet clinically proven—that adenosine agonists might prove useful in treating drug dependence. CREB, which is upregulated in the nucleus accumbens by prolonged administration of opioids or cocaine, plays a key role in regulating various components of cAMP signalling pathways, and transgenic animals lacking CREB show reduced withdrawal symptoms (see Chao & Nestler, 2004).

For drugs such as opioids that are agonists at specific receptors (see Ch. 41), cellular tolerance results from desensitisation of the receptor. On prolonged activation by an agonist, the μ opioid receptor (MOPr) is phosphorylated by various intracellular kinases—including G-protein-coupled receptor kinases (GRKs), protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and Ca^{2+}/calmodulin-dependent protein kinase II (CamKII)—which either directly desensitises the receptor or causes the binding to the receptor of other proteins, such as arrestins, that uncouple the receptor from its G-protein (see Bailey & Connor, 2005). In the intact animal, inhibition or knockout of these kinases reduces the level of tolerance. It has also been reported that blockade of neurokinin, calcitonin gene-related peptide (CGRP) and NMDA receptors reduces opioid tolerance in vivo. This may be because the activity of some of the kinases involved in MOPr desensitisation (e.g. PKC and CamKII) in neurons is enhanced when these other receptors are activated.

PHARMACOLOGICAL APPROACHES TO TREATING DRUG ADDICTION

From the discussion above, it will be clear that drug abuse involves many psychosocial and some genetic factors, as well as neuropharmacological mechanisms, so drug treatment is only one component of the therapeutic approaches that are used. The main pharmacological approaches (see O'Brien, 1997; Heidbreder & Hagan, 2005) are summarised in Table 48.3. For information on other approaches to the treatment of drug addiction, readers are advised to follow the Web link given at the end of this chapter to the National Institute on Drug Abuse (NIDA).

A new approach to the treatment of drug dependence, so far applied mainly to nicotine and cocaine, is the development of vaccines (see Bunce et al., 2003) consisting of the drug molecule complexed to a protein. Antibodies produced in response to injection of the complex also bind the free drug, thereby preventing it from reaching the brain. This strategy is effective in animal models involving self-administration, and clinical trials in humans are in progress.

Clinical use of drugs in substance dependence

Tobacco dependence

- Short-term **nicotine** is an adjunct to behavioural therapy in smokers committed to giving up; **varenicline** is also used as an adjunct but has been linked to suicidal ideation.
- **Bupropion** is also effective but lowers seizure threshold, so is contraindicated in people with risk factors for seizures (and also if there is a history of eating disorder).

Alcohol dependence

- Long-acting benzodiazepines (e.g. **chlordiazepoxide**) can be used to reduce withdrawal symptoms and the risk of seizures; they should be tapered over 1–2 weeks and then discontinued because of their abuse potential.
- **Disulfiram** is used as an adjunct to behavioural therapy in suitably motivated alcoholics after detoxification; it is contraindicated for patients in whom hypotension would be dangerous (e.g. those with coronary or cerebral vascular disease).
- **Acamprosate** can help to maintain abstinence; it is started as soon as abstinence has been achieved and maintained if relapse occurs, and it is continued for 1 year.

Opioid dependence

- Opioid agonists or partial agonists (e.g., respectively, **methadone** or **buprenorphine**) administered orally or sublingually may be substituted for injectable narcotics, many of whose harmful effects are attributable to the route of administration.
- **Naltrexone**, a long-acting opioid antagonist, is used as an adjunct to help prevent relapse in detoxified addicts (opioid free for at least 1 week).
- **Lofexidine**, an α_2 agonist (cf. **clonidine**; Ch. 14), is used short term (usually up to 10 days) to ameliorate symptoms of opioid withdrawal, and is then tapered over a further 2–4 days.

NICOTINE AND TOBACCO

Tobacco growing, chewing and smoking was indigenous throughout the American subcontinent and Australia at the time that European explorers first visited these places. Smoking spread through Europe during the 16th century, coming to England mainly as a result of its enthusiastic espousal by Raleigh at the court of Elizabeth I. James I strongly disapproved of both Raleigh and tobacco, and initiated the first antismoking campaign in the early 17th century with the support of the Royal College of Physicians. Parliament responded by imposing a substantial duty on tobacco, thereby setting up the dilemma (from which we show no sign of being able to escape) of giving the State an economic interest in the continuation of smoking at the same time that its official expert advisers were issuing emphatic warnings about its dangers.

Until the latter half of the 19th century, tobacco was smoked in pipes, and primarily by men. Cigarette manufacture began at the end of the 19th century, and now cigarettes account for 98% of tobacco consumption. Filter cigarettes (which give a somewhat lower delivery of tar and nicotine than standard cigarettes) and 'low-tar' cigarettes (which are also low in nicotine) constitute an increasing proportion of the total.[3] Cigarette consumption across the globe continues to rise (Fig. 48.3).[4] There are

[3]Smokers, however, adapt by smoking more low-tar cigarettes and inhaling more deeply so as to maintain their nicotine consumption.

[4]In contrast to the global picture, in the UK consumption has dropped by over 50% from its peak in the 1970s, the main factors being increased price, adverse publicity, restrictions on advertising, the compulsory publication of health warnings and, most recently, a ban on smoking in public places. Still, however, around 9.4 million adults (just over 20% of the adult population) in the UK smoke, with little difference between men and women. About 10% of children aged 10–15 are regular smokers.

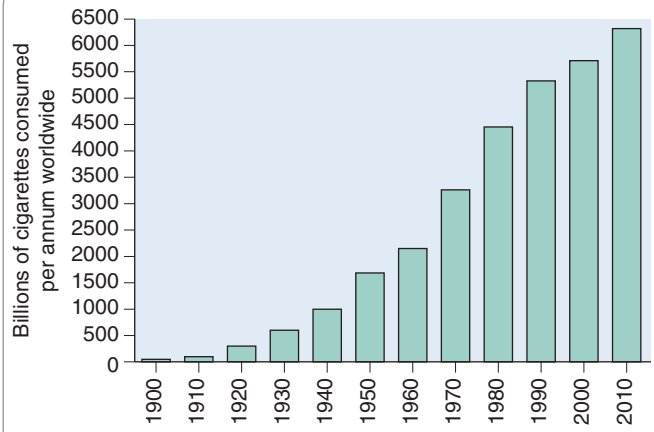

Fig. 48.3 Global cigarette consumption per annum 1900–2010. (Data from http://www.tobaccoatlas.org/consumption.html.)

Tobacco smoking

- Cigarette consumption across the world continues to rise, although in the UK it is now declining after reaching a peak in the mid-1970s.
- The worldwide prevalence of smoking is now about 18% of the adult population, each smoker using on average 5000 cigarettes per year.
- Nicotine is the main pharmacologically active agent in tobacco, apart from carcinogenic tars and carbon monoxide.
- The amount of nicotine absorbed from an average cigarette is about 1–1.5 mg, which causes the plasma nicotine concentration to reach 130–200 nmol/l. These values depend greatly on the type of cigarette and on the extent of inhalation of the smoke.

about 1.1 billion smokers in the world (18% of the population), and the number in developing countries is increasing rapidly. Six trillion (6×10^{12}) cigarettes are sold each year, more than 900 cigarettes for every man, woman and child on the planet. In 2010, 12 million cigarettes per minute will be smoked around the world.

For reviews on nicotine and smoking, see Balfour & Fagerstrom (1996) and Benowitz (1996).

PHARMACOLOGICAL EFFECTS OF SMOKING

Nicotine[5] is the main pharmacologically active substance in tobacco smoke. The acute effects of smoking can be mimicked by injection of nicotine and are blocked by **mecamylamine**, an antagonist at neuronal nicotinic acetylcholine receptors (nAChRs; see Ch. 13).

[5]From the plant *Nicotiana*, named after Jean Nicot, French ambassador to Portugal, who presented seeds to the French king in 1560, having been persuaded by natives of South America of the medical value of smoking tobacco leaves. Smoking was believed to protect against illness, particularly the plague.

Effects on the central nervous system

The central effects of nicotine are complex and cannot be summed up overall simply in terms of stimulation or inhibition. At the cellular level, nicotine acts on nAChRs (see Ch. 38), which are widely expressed in the brain, particularly in the cortex and hippocampus, and are believed to play a role in cognitive function, as well as in the VTA, from which dopaminergic neurons project to the nucleus accumbens (the reward pathway, see above). nAChRs are ligand-gated cation channels located both pre- and postsynaptically, causing, respectively, enhanced transmitter release and neuronal excitation (see Wonnacott et al., 2005). Of the various subtypes of nAChR, the $\alpha4\beta2$ and $\alpha7$ subtypes (see Ch. 13) have received most attention, but other subtypes may also be involved in the rewarding effects of nicotine. As well as activating the receptors, nicotine also causes desensitisation, which may be an important component of its effects, because the effects of a dose of nicotine are diminished in animals after sustained exposure to the drug. Chronic nicotine administration leads to a substantial increase in the number of nAChRs (an effect opposite to that produced by sustained administration of most receptor agonists), which may represent an adaptive response to prolonged receptor desensitisation. It is likely that the overall effect of nicotine reflects a balance between activation of nAChRs, causing neuronal excitation, and desensitisation, causing synaptic block.

At the spinal level, nicotine inhibits spinal reflexes, causing skeletal muscle relaxation that can be measured by electromyography. This may be due to stimulation of the inhibitory Renshaw cells in the ventral horn of the spinal cord. The higher level functioning of the brain, as reflected in the subjective sense of alertness or by the electroencephalography (EEG) pattern, can be affected in either direction by nicotine, according to dose and circumstances. Smokers report that smoking wakes them up when they are drowsy and calms them down when they are tense, and EEG recordings broadly bear this out. It also seems that small doses of nicotine tend to cause arousal, whereas large doses do the reverse. Tests of motor and sensory performance (e.g. reaction time measurements or vigilance tests) in humans generally show improvement after smoking, and nicotine enhances learning in rats.

Some elaborate tests have been conducted to see, for example, whether the effect of nicotine on performance and aggression varies according to the amount of stress. Some tests border on nasty-mindedness, such as one in which subjects played a complicated logical game with a computer that initially played fair and then began to cheat randomly, causing stress and aggression in the subjects and a decline in their performance. Smoking, it was reported, did not reduce the anger but did reduce the decline in performance.

Nicotine and other nicotinic agonists such as **epibatidine** (Ch. 41) have significant analgesic activity.

Peripheral effects

The peripheral effects of small doses of nicotine result from stimulation of autonomic ganglia (see Ch. 13) and of peripheral sensory receptors, mainly in the heart and lungs. Stimulation of these receptors elicits various autonomic reflex responses, causing tachycardia, increased cardiac output and increased arterial pressure, reduction of gastrointestinal motility and sweating. When people smoke for the first time, they usually experience nausea

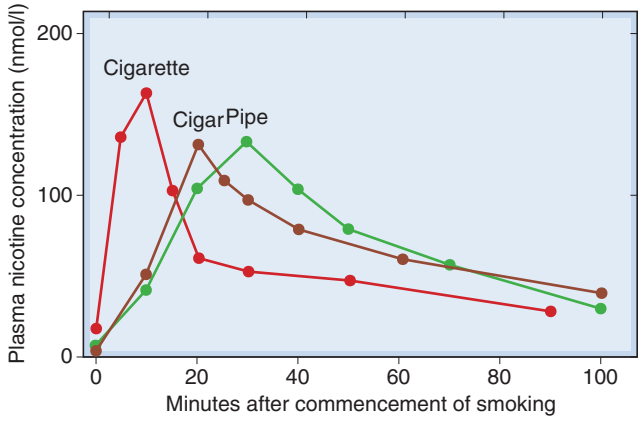

Fig. 48.4 Nicotine concentration in plasma during smoking. The subjects were habitual smokers who smoked a cigarette, cigar or pipe according to their usual habit. (From Bowman W C, Rand M 1980 Chapter 4. In: Textbook of pharmacology. Blackwell, Oxford.)

and sometimes vomit, probably because of stimulation of sensory receptors in the stomach. All these effects decline with repeated dosage, although the central effects remain. Secretion of adrenaline and noradrenaline from the adrenal medulla contributes to the cardiovascular effects, and release of antidiuretic hormone from the posterior pituitary causes a decrease in urine flow.[6] The plasma concentration of free fatty acids is increased, probably owing to sympathetic stimulation and adrenaline secretion.

Smokers weigh, on average, about 4 kg less than non-smokers, mainly because of reduced food intake; giving up smoking usually causes weight gain associated with increased food intake.

PHARMACOKINETIC ASPECTS

An average cigarette contains about 0.8 g of tobacco and 9–17 mg of nicotine, of which about 10% is normally absorbed by the smoker. This fraction varies greatly with the habits of the smoker and the type of cigarette.

Nicotine in cigarette smoke is rapidly absorbed from the lungs but poorly from the mouth and nasopharynx. Therefore, inhalation is required to give appreciable absorption of nicotine, each puff delivering a distinct bolus of drug to the CNS. Pipe or cigar smoke is less acidic than cigarette smoke, and the nicotine tends to be absorbed from the mouth and nasopharynx rather than the lungs. Absorption is considerably slower than from inhaled cigarette smoke, resulting in a later and longer-lasting peak in the plasma nicotine concentration (Fig. 48.4). An average cigarette, smoked over 10 min, causes the plasma nicotine concentration to rise to 15–30 ng/ml (100–200 nmol/l), falling to about half within 10 min and then more slowly over the next 1–2 h. The rapid decline results mainly from redistribution between the blood and other tissues; the slower decline is due to hepatic metabolism, mainly by oxidation to an inactive ketone metabolite, *cotinine*. This has a long plasma half-life, and measurement of plasma nicotine concentration provides a useful measure of smoking behaviour. A nicotine patch applied for 24 h causes the plasma concentration to rise to 75–150 nmol/l over 6 h and to remain fairly constant for about 20 h. Administration by nasal spray or chewing gum results in a time course intermediate between that of smoking and the nicotine patch.

TOLERANCE AND DEPENDENCE

As with other dependence-producing drugs, three separate processes—psychological dependence, physical dependence and tolerance—contribute to the overall state of dependence, in which taking the drug becomes compulsive.

The effects of nicotine associated with peripheral ganglionic stimulation show rapid tolerance, perhaps as a result of desensitisation of nAChRs by nicotine. With large doses of nicotine, this desensitisation produces a block of ganglionic transmission rather than stimulation (see Ch. 13). Tolerance to the central effects of nicotine (e.g. in the arousal response) is much less than in the periphery. The increase in the number of nAChRs in the brain produced by chronic nicotine administration in animals (see above) also occurs in heavy smokers. Because the cellular effects of nicotine are diminished, it is possible that the additional binding sites represent desensitised rather than functional receptors.

The addictiveness of smoking is due to the effects of nicotine combined with the ritual of smoking (see Le Foll & Goldberg, 2005). Rats choose to drink dilute nicotine solution in preference to water if given a choice, and in a situation in which lever pressing causes an injection of nicotine to be delivered—admittedly at high doses—they quickly learn to self-administer it. Similarly, monkeys who have been trained to smoke, by providing a reward in response to smoking behaviour, will continue to do so spontaneously (i.e. unrewarded) if the smoking medium contains nicotine, but not if nicotine-free tobacco is offered instead. Humans, however, are unlikely to become addicted to nicotine delivered from patches suggesting that other factors are also involved, such as the controlled pulsatile delivery associated with smoking.

Like other addictive drugs (see above), nicotine causes excitation of the mesolimbic reward pathway and increased dopamine release in the nucleus accumbens. Transgenic mice lacking the β2 subunit of the acetylcholine receptor lose the rewarding effect of nicotine and its dopamine-releasing effect, confirming the importance of the α4β2 nAChR subtype and mesolimbic dopamine release in the response to nicotine. In contrast to normal mice, the mutant mice could not be induced to self-administer nicotine, even though they did so with cocaine.

A physical withdrawal syndrome occurs in humans on cessation of smoking. Its main features are increased irritability, impaired performance of psychomotor tasks, aggressiveness and sleep disturbance. The withdrawal syndrome is much less severe than that produced by opioids, and it can be alleviated not only by nicotine but also by amphetamine, a finding consistent with the postulated role of dopamine in the reward pathway. The withdrawal syndrome lasts for 2–3 weeks, although the craving for cigarettes persists for much longer than this; relapses during attempts to give up cigarette smoking occur most commonly at a time when the physical withdrawal syndrome has long since subsided.

[6]This may explain why, in years gone by, men smoked cigars while chatting over drinks after dinner.

Pharmacology of nicotine

- At the cellular level, nicotine acts on nicotinic acetylcholine receptors (nAChRs), mainly of the $\alpha 4\beta 2$ subtype, to enhance neurotransmitter release and increase neuronal excitation. Its central effects are blocked by receptor antagonists such as **mecamylamine**.
- At the behavioural level, nicotine produces a mixture of inhibitory and excitatory effects.
- Nicotine shows reinforcing properties, associated with increased activity in the mesolimbic dopaminergic pathway, and self-administration can be elicited in animal studies.
- Electroencephalography changes show an arousal response, and subjects report increased alertness accompanied by a reduction of anxiety and tension.
- Learning, particularly under stress, is facilitated by nicotine.
- Peripheral effects of nicotine are due mainly to ganglionic stimulation: tachycardia, increased blood pressure and reduced gastrointestinal motility. Tolerance develops rapidly to these effects.
- Nicotine is metabolised, mainly in the liver, within 1–2 h.
- The inactive metabolite, cotinine, has a long plasma half-life and can be used as a measure of smoking habits.
- Nicotine gives rise to tolerance, physical dependence and psychological dependence (craving). Attempts at long-term cessation succeed in only about 20% of cases.
- Nicotine replacement therapy (chewing gum or skin patch preparations) improves the chances of giving up smoking when combined with active counselling.

HARMFUL EFFECTS OF SMOKING

The life expectancy of smokers is shorter than that of non-smokers. Smoking causes almost 90% of deaths from lung cancer, around 80% of deaths from bronchitis and emphysema, and around 17% of deaths from heart disease. About one-third of all cancer deaths can be attributed to smoking. Smoking is, by a large margin, the biggest preventable cause of death, responsible for about 1 in 10 adult deaths worldwide. Deaths from smoking are continuing to rise. In 1990, smoking was responsible for 10% (3 million out of 30 million) of deaths worldwide; by 2030, this is expected to increase to 17% (10 million out of 60 million), mainly due to the growth of smoking in Asia, Africa and Latin America (Peto et al., 1996).

The main health risks are as follows:

- *Cancer, particularly of the lung and upper respiratory tract but also of the oesophagus, pancreas and bladder.* Smoking 20 cigarettes per day is estimated to increase the risk of lung cancer about 10-fold. Pipe and cigar smoking carry much less risk than cigarette smoking, although the risk is still appreciable. Tar, rather than nicotine, is responsible for the cancer risk. Genetic variants of nicotinic receptor subunits have been associated with lung cancer although the mechanisms behind this association are unclear (see Hung et al., 2008).
- *Coronary heart disease and other forms of peripheral vascular disease*. The mortality among men aged 55–64 from coronary thrombosis is about 60% greater in men who smoke 20 cigarettes per day than in non-smokers. Although the increase in risk is less than it is for lung cancer, the actual number of excess deaths associated with smoking is larger, because coronary heart disease is so common. Other kinds of vascular disease (e.g. stroke, intermittent claudication and diabetic gangrene) are also strongly smoking related. Many studies have suggested that nicotine is mainly responsible for the adverse effect of smoking on the incidence of cardiovascular disease. Another factor may be carbon monoxide (see below). Surprisingly, there is no clear increase in ischaemic heart disease in pipe and cigar smokers, even though similar blood nicotine and carboxyhaemoglobin concentrations are reached, suggesting that nicotine and carbon monoxide may not be the only causative factors.
- *Chronic obstructive pulmonary disease* (COPD; see Ch. 27) is a major global health problem. Cigarette smoking is the main cause. Stopping smoking slows the progression of the disease. Bronchitis, inflammation of the mucous membranes of the bronchi, is much more common in smokers than in non-smokers. These effects are probably due to tar and other irritants rather than nicotine.
- *Harmful effects in pregnancy*. Smoking, particularly during the latter half of pregnancy, significantly reduces birth weight (by about 8% in women who smoke 25 or more cigarettes per day during pregnancy) and increases perinatal mortality (by an estimated 28% in babies born to mothers who smoke in the last half of pregnancy). There is evidence that children born to smoking mothers remain behind, in both physical and mental development, for at least 7 years. By 11 years of age, the difference is no longer significant. These effects of smoking, although measurable, are much smaller than the effects of other factors, such as social class and birth order. Various other complications of pregnancy are also more common in women who smoke, including spontaneous abortion (increased 30–70% by smoking), premature delivery (increased about 40%) and placenta praevia (increased 25–90%). Nicotine is excreted in breast milk in sufficient amounts to cause tachycardia in the infant.

The agents probably responsible for the harmful effects are as follows:

- Tar and irritants, such as nitrogen dioxide and formaldehyde. Cigarette smoke tar contains many known carcinogenic hydrocarbons, as well as tumour promoters, which account for the high cancer risk. It is likely that the various irritant substances are also responsible for the increase in bronchitis and emphysema.

- Nicotine probably accounts for retarded fetal development because of its vasoconstrictor properties.
- Carbon monoxide. Cigarette smoke contains about 3% carbon monoxide. Carbon monoxide has a high affinity for haemoglobin, and the average carboxyhaemoglobin content in the blood of cigarette smokers is about 2.5% (compared with 0.4% for non-smoking urban dwellers). In very heavy smokers, up to 15% of haemoglobin may be carboxylated, a level that affects fetal development in rats. This factor may also contribute to the increased incidence of heart and vascular disease. Fetal haemoglobin has a higher affinity for carbon monoxide than adult haemoglobin, and the proportion of carboxyhaemoglobin is higher in fetal than in maternal blood.
- Increased oxidative stress may be responsible for atherogenesis (Ch. 23) and chronic obstructive pulmonary disease (Ch. 27).

Low-tar cigarettes give a lower yield of both tar and nicotine than standard cigarettes. However, it has been shown that smokers puff harder, inhale more and smoke more cigarettes when low-tar brands are substituted for standard brands. The end result may be a slightly reduced intake of tar and nicotine but an increase in carbon monoxide intake, with no net gain in terms of safety.

OTHER EFFECTS OF SMOKING

Parkinson's disease is approximately twice as common in non-smokers as in smokers. It is possible that this reflects a protective effect of nicotine, but it could be that common genetic or environmental factors underlie smoking behaviour and susceptibility to Parkinson's disease. Ulcerative colitis appears to be a disease of non-smokers. Former smokers are at high risk for developing ulcerative colitis, while current smokers have the least risk. This tendency indicates that smoking cigarettes may prevent the onset of ulcerative colitis. In contrast, smoking tends to worsen the effects of Crohn's disease. Earlier reports that Alzheimer's disease is less common in smokers have not been confirmed; indeed there is evidence that smoking may increase the occurrence of Alzheimer's disease in some genetic groups.

Effects of smoking

- Smoking accounts for about 10% of deaths worldwide, mainly due to:
  - cancer, especially lung cancer, of which about 90% of cases are smoking related; carcinogenic tars are responsible
  - ischaemic heart disease; both nicotine and carbon monoxide may be responsible
  - chronic bronchitis; tars are mainly responsible.
- Smoking in pregnancy reduces birth weight and retards childhood development. It also increases abortion rate and perinatal mortality. Nicotine and possibly carbon monoxide are responsible.
- The incidence of Parkinson's disease is lower in smokers than in non-smokers.

PHARMACOLOGICAL APPROACHES TO TREATING NICOTINE DEPENDENCE

Most smokers would like to quit, but few succeed.[7] The most successful smoking cure clinics, using a combination of psychological and pharmacological treatments, achieve a success rate of about 25%, measured as the percentage of patients still abstinent after 1 year. The two main pharmacological treatments (see George & O'Malley, 2004) are **nicotine replacement therapy** and **bupropion** (also used to treat depression; see Table 46.2). A nAChR partial agonist, **varenicline** has recently been introduced.

Nicotine replacement therapy is used mainly to assist smokers to quit by reducing craving and physical withdrawal symptoms. Because nicotine is relatively short acting and not well absorbed from the gastrointestinal tract, it is given either in the form of chewing gum, used several times daily, or as a transdermal patch that is replaced daily. These preparations cause various side effects, particularly nausea and gastrointestinal cramps, cough, insomnia and muscle pains. There is a risk that nicotine may cause coronary spasm in patients with heart disease. Transdermal patches may cause local irritation and itching. The conclusion of many double-blind trials of nicotine against placebo is that these preparations, combined with professional counselling and supportive therapy, roughly double the chances of successfully breaking the smoking habit, but the success rate measured as abstinence 1 year after ceasing treatment is still only about 25%. Nicotine on its own, without counselling and support, is no more effective than placebo, so its use as an over-the-counter smoking remedy has little justification. Although of limited value as an aid to abstinence, the long-term use of nicotine can significantly reduce cigarette consumption by smokers. In Sweden, the use of 'smokeless tobacco' is encouraged and the smoking-related death rate is much lower than elsewhere in Europe or North America.

The identification of the α4β2 nAChR subtype as the main nAChR subtype in the brain involved in the rewarding properties of tobacco smoking may allow selective agonists to be developed as nicotine substitutes with fewer side effects. Varenicline is a partial agonist at the α4β2 nicotinic receptor subtype and has differing levels of efficacy at other subtypes. Being a partial agonist it may provide a level of substitution while at the same time blocking the rewarding effect of smoking. It is effective in preventing relapse but there has been some concern that it may induce suicidal thoughts, suicide attempts, aggression and homicide. However, a recent large retrospective study (Gunnell et al., 2009) found no evidence of increased suicide or suicidal thoughts with varenicline, compared with other antismoking treatments.

Bupropion (Ch. 46) appears to be as effective as nicotine replacement therapy, even in non-depressed patients, and has fewer side effects. However, bupropion lowers the seizure threshold so should not be prescribed if there are other risk factors for seizures (including other drugs that lower seizure threshold). It is also contraindicated if there is a history of eating disorders or of bipolar mood disorder, and is used only with caution in patients with liver or renal

[7]Freud tried unsuccessfully to give up cigars for 45 years before dying of cancer of the mouth at the age of 83.

disease. Because of these problems, nicotine remains the pharmacological treatment of choice in most cases.

Bupropion may act by increasing dopamine activity in the nucleus accumbens. It is a weak blocker of dopamine and noradrenaline uptake, but it is not clear that this accounts for its efficacy in treating nicotine dependence. It is usually given as a slow-release formulation.

Many other drugs have been tested clinically and shown to be useful in some cases. They include the following:

- **Clonidine**, an α_2 adrenoceptor agonist (see Ch. 14), which reduces the withdrawal effects of several dependence-producing drugs, including opioids and cocaine, as well as nicotine.[8] Clonidine may be given orally or as a transdermal patch, and is about as effective as nicotine substitution in assisting abstinence. The side effects of clonidine (hypotension, dry mouth, drowsiness) are troublesome, however, and it is not widely used.
- **Tricyclic antidepressants**, **selective serotonin reuptake inhibitors** and **monoamine oxidase inhibitors**, used mainly as antidepressants (Ch. 46). The rationale may be that depressive episodes, which often lead to resumption of smoking, are prevented.
- **Mecamylamine**, which antagonises the effects of nicotine, is not promising. Small doses actually increase smoking, presumably because its action can be overcome by increasing the amount of nicotine. Larger doses of mecamylamine, which abolish the effects of nicotine more effectively, have many autonomic side effects (see Ch. 13), and compliance is poor. The rationale is questionable because, although mecamylamine reduces the reward effect of nicotine, it does not affect the craving associated with abstinence.

ETHANOL

Judged on a molar basis, the consumption of ethanol far exceeds that of any other drug. The ethanol content of various drinks ranges from about 2.5% (weak beer) to about 55% (strong spirits), and the size of the normal measure is such that a single drink usually contains about 8–12 g (0.17–0.26 mol) of ethanol. Its low pharmacological potency is reflected in the range of plasma concentrations needed to produce pharmacological effects: minimal effects occur at about 10 mmol/l (46 mg/100 ml), and 10 times this concentration may be lethal. The average per capita ethanol consumption in the UK was 11.7 l/year (expressed as pure ethanol) in 2007, a figure that has doubled since 1970, the main changes having been a growing consumption of wine in preference to beer among adults and an increasing tendency for binge drinking, especially among young people.

For practical purposes, ethanol intake is often expressed in terms of units. One unit is equal to 8 g (10 ml) of ethanol, and is the amount contained in half a pint of normal strength beer, one measure of spirits or one small glass of wine. Based on the health risks described below, the current official recommendation is a maximum of 21 units/week for men and 14 units/week for women. It is estimated that in the UK, about 33% of men and 13% of women exceed these levels. The annual tax revenue from drink amounts to about £7 billion, whereas the health cost is estimated at £3 billion, and the social cost undoubtedly greater. Governments in most developed countries are attempting to curb alcohol consumption.

An excellent detailed review of all aspects of alcohol and alcoholism is provided by Spanagel (2009).

PHARMACOLOGICAL EFFECTS OF ETHANOL

Effects on central nervous system neurons

The main effects of ethanol are on the central nervous system (CNS; see reviews by Charness et al., 1989; Spanagel, 2009), where its depressant actions resemble those of volatile anaesthetics (Ch. 40). At a cellular level, the effect of ethanol is depressant, although it increases neuronal activity—presumably by disinhibition—in some parts of the CNS, notably in the mesolimbic dopaminergic pathway that is involved in reward. The main acute cellular effects of ethanol that occur at concentrations (5–100 mM) relevant to alcohol consumption by humans are:

- enhancement of both GABA- and glycine-mediated inhibition
- inhibition of Ca^{2+} entry through voltage-gated calcium channels
- activation of certain types of K^+ channel
- inhibition of ionotropic glutamate receptor function
- inhibition of adenosine transport.

For reviews see Tabakoff & Hoffman (1996), Lovinger (1997) and Harris et al. (2008).

Ethanol enhances the action of GABA on $GABA_A$ receptors in a similar way to benzodiazepines (see Ch. 43). Its effect is, however, smaller and less consistent than that of benzodiazepines, and no clear effect on inhibitory synaptic transmission in the CNS has been demonstrated for ethanol. This may be because the effect of ethanol is seen only on some subtypes of $GABA_A$ receptor (see Ch. 37). Exactly which $GABA_A$ receptor subtypes are sensitive to ethanol is still unclear but those containing $\alpha 6$ and δ subunits appear to be important. Ethanol may also act presynaptically to enhance GABA release. The benzodiazepine inverse agonist **flumazenil** (see Ch. 43) reverses the central depressant actions of ethanol by a non-competitive interaction on the $GABA_A$ receptor. The use of flumazenil to reverse ethanol intoxication and treat dependence has not found favour for several reasons. Because flumazenil is an inverse agonist (see Ch. 2) at benzodiazepine receptors, it carries a risk of causing seizures, and it could cause an increase in ethanol consumption and thus increase long-term toxic manifestations.

Ethanol produces a consistent enhancement of glycine receptor function. This effect is likely to be due both to a direct interaction of ethanol with the $\alpha 1$ subunit of the glycine receptor and to indirect effects of ethanol mediated through PKC activation. Ethanol can also enhance glycine release from nerve terminals.

Ethanol reduces transmitter release in response to nerve terminal depolarisation by inhibiting the opening of voltage-sensitive calcium channels in neurons. It also reduces neuronal excitability by activating G-protein-activated inwardly rectifying K^+ (GIRK) channels as well as potentiating calcium-activated potassium (BK) channel activity.

[8] It also reduces postmenopausal flushing, which may represent a physiological oestrogen withdrawal response.

The excitatory effects of glutamate are inhibited by ethanol at concentrations that produce CNS depressant effects in vivo. NMDA receptor activation is inhibited at lower ethanol concentrations than are required to affect AMPA receptors (see Ch. 37). Other effects produced by ethanol include an enhancement of the excitatory effects produced by activation of nAChRs and 5-HT_3 receptors. The relative importance of these various effects in the overall effects of ethanol on CNS function is not clear at present.

The depressant effects of ethanol on neuronal function resemble those of adenosine acting on A_1 receptors (see Ch. 16). Ethanol in cell culture systems increases extracellular adenosine by inhibiting adenosine uptake, and there is some evidence that inhibition of the adenosine transporter may account for some of its CNS effects (Melendez & Kalivas, 2004).

Endogenous opioids also play a role in the CNS effects of ethanol, because both human and animal studies show that the opioid receptor antagonist **naltrexone** reduces the reward associated with ethanol.

Behavioural effects

The effects of acute ethanol intoxication in humans are well known and include slurred speech, motor incoordination, increased self-confidence and euphoria. The effect on mood varies among individuals, most becoming louder and more outgoing, but some becoming morose and withdrawn. At higher levels of intoxication, the mood tends to become highly labile, with euphoria and melancholy, aggression and submission, often occurring successively. The association between alcohol and violence is well documented.

Intellectual and motor performance and sensory discrimination show uniform impairment by ethanol, but subjects are generally unable to judge this for themselves. For example, bus drivers were asked to drive through a gap that they selected as the minimum for their bus to pass through; ethanol caused them not only to hit the barriers more often at any given gap setting, but also to set the gap to a narrower dimension, often narrower than the bus.

Much effort has gone into measuring the effect of ethanol on driving performance in real life, as opposed to artificial tests under experimental conditions. In an American study of city drivers, it was found that the probability of being involved in an accident was unaffected at blood ethanol concentrations up to 50 mg/100 ml (10.9 mmol/l); by 80 mg/100 ml (17.4 mmol/l), the probability was increased about four-fold, and by 150 mg/100 ml (32.6 mmol/l) about 25-fold. In the UK, driving with a blood ethanol concentration greater than 80 mg/100 ml is illegal.

The relationship between plasma ethanol concentration and effect is highly variable. A given concentration produces a larger effect when the concentration is rising than when it is steady or falling. A substantial degree of cellular tolerance develops in habitual drinkers, with the result that a higher plasma ethanol concentration is needed to produce a given effect. In one study, 'gross intoxication' (assessed by a battery of tests that measured speech, gait and so on) occurred in 30% of subjects between 50 and 100 mg/100 ml and in 90% of subjects with more than 150 mg/100 ml. Coma generally occurs at about 400 mg/100 ml, and death from respiratory failure is likely at levels exceeding 500 mg/100 ml.

Ethanol significantly enhances—sometimes to a dangerous extent—the CNS depressant effects of many other drugs, including benzodiazepines, antidepressants, antipsychotic drugs and opioids. Combined use of ethanol and cocaine leads to the formation of cocaethylene, a toxic metabolite of cocaine.

Neurotoxicity

In addition to the acute effects of ethanol on the nervous system, chronic administration also causes irreversible neurological damage (see Harper & Matsumoto, 2005). This may be due to ethanol itself, or to metabolites such as acetaldehyde or fatty acid esters. Binge drinking is thought to produce greater damage; probably due to the high brain concentrations of ethanol achieved and to repeated phases of withdrawal between binges. Heavy drinkers often exhibit convulsions and may develop irreversible dementia and motor impairment associated with thinning of the cerebral cortex (apparent as ventricular enlargement) detectable by brain-imaging techniques. Degeneration in the cerebellum and other specific brain regions can also occur, as well as peripheral neuropathy. Some of these changes are not due to ethanol itself but to accompanying thiamine deficiency, which is common in alcoholics.

Effects on other systems

The main acute cardiovascular effect of ethanol is to produce cutaneous vasodilatation, central in origin, which causes a warm feeling but actually increases heat loss.[9] Paradoxically, there is a positive correlation between ethanol consumption and hypertension, possibly because ethanol withdrawal causes increased sympathetic activity. The beneficial effect of moderate drinking on cardiovascular function is discussed below.

Ethanol increases salivary and gastric secretion, perhaps a reason in some cultures for the popularity of a glass of sherry before dinner. This is partly a reflex effect produced by the taste and irritant action of ethanol. However, heavy consumption of spirits causes damage directly to the gastric mucosa, causing chronic gastritis. Both this and the increased acid secretion are factors in the high incidence of gastric bleeding in alcoholics. CNS depression predisposes to aspiration pneumonia and lung abscess formation. Acute pancreatitis may become chronic with pseudocyst formation (collections of fluid in the peritoneal sac), fat malabsorption and ultimately loss of B-cell function and insulin-dependent diabetes mellitus.

Ethanol produces a variety of endocrine effects. In particular, it increases the output of adrenal steroid hormones by stimulating the anterior pituitary gland to secrete adrenocorticotrophic hormone. However, the increase in plasma hydrocortisone usually seen in alcoholics (producing a 'pseudo-Cushing's syndrome'; Ch. 32) is due partly to inhibition by ethanol of hydrocortisone metabolism in the liver.

Diuresis is a familiar effect of ethanol. It is caused by inhibition of antidiuretic hormone secretion, and tolerance develops rapidly, so that the diuresis is not sustained. There is a similar inhibition of oxytocin secretion, which can delay parturition. Attempts have been made to use this effect in

[9]The image of a large St Bernard dog carrying a small keg of brandy around its neck to revive avalanche victims is an apocryphal one created by the English painter, Edwin Landseer, who in 1820 produced a painting called 'Alpine Mastiffs Reanimating a Distressed Traveller'. With their keen sense of smell, such dogs were useful in searching for people buried in the snow, but taking a tot of brandy would only have enhanced the victim's heat loss.

premature labour, but the dose needed is large enough to cause obvious drunkenness in the mother. If the baby is born prematurely despite the ethanol, it too may be intoxicated at birth, sufficiently for respiration to be depressed. The procedure evidently has serious disadvantages.

Acute toxic effects on muscle are exacerbated by seizures and prolonged immobility; severe myositis ('rhabdomyolysis') with myoglobinuria can cause acute renal failure. Chronic toxicity affects particularly cardiac striated muscle giving rise to alcoholic cardiomyopathy and chronic heart failure.

Chronic ethanol consumption may also result in immunosuppression, leading to increased incidence of infections such as pneumonia (immunisation with pneumococcal vaccine is important in chronic alcoholics); and increased cancer risk, particularly of the mouth, larynx and oesophagus.

Male alcoholics are often impotent and show signs of feminisation. This is associated with impaired testicular steroid synthesis, but induction of hepatic microsomal enzymes by ethanol, and hence an increased rate of testosterone inactivation, also contributes.

Effects of ethanol on the liver

Together with brain damage, liver damage is the most common serious long-term consequence of excessive ethanol consumption (see Lieber, 1995). Increased fat accumulation (fatty liver) progresses to hepatitis (i.e. inflammation of the liver) and eventually to irreversible hepatic necrosis and fibrosis. Cirrhosis is an end stage with extensive fibrosis and foci of regenerating hepatocytes that are not correctly 'plumbed in' to the blood and biliary systems. Diversion of portal blood flow around the cirrhotic liver often causes oesophageal varices to develop, which can bleed suddenly and catastrophically. Increased fat accumulation in the liver occurs, in rats or in humans, after a single large dose of ethanol. The mechanism is complex, the main factors being:

- increased release of fatty acids from adipose tissue, which is the result of increased stress, causing sympathetic discharge
- impaired fatty acid oxidation, because of the metabolic load imposed by the ethanol itself.

With chronic ethanol consumption, many other factors contribute to the liver damage. One is malnutrition, for alcoholic individuals may satisfy much of their calorie requirement from ethanol itself. Three hundred grams of ethanol (equivalent to one bottle of whisky) provides about 2000 kcal but, unlike a normal diet, it provides no vitamins, amino acids or fatty acids. Thiamine deficiency is an important factor in causing chronic neurological damage (see above). The hepatic changes occurring in alcoholics are partly due to chronic malnutrition but mainly to the cellular toxicity of ethanol, which promotes inflammatory changes in the liver.

The overall incidence of chronic liver disease is a function of cumulative ethanol consumption over many years. Therefore, overall consumption, expressed as g/kg of body weight per day multiplied by years of drinking, provides an accurate predictor of the incidence of cirrhosis. An increase in the plasma concentration of the liver enzyme γ-glutamyl transpeptidase (a marker of CYP induction) often raises the suspicion of alcohol-related liver damage, although not specific to ethanol.

Effects on lipid metabolism, platelet function and atherosclerosis

Moderate drinking reduces mortality associated with coronary heart disease, the maximum effect—about 30% reduction of mortality overall—being achieved at a level of 2–3 units/day (see Groenbaek et al., 1994). The effect is much more pronounced (> 50% reduction) in men with high plasma concentrations of low-density-lipoprotein cholesterol (see Ch. 23).[10] Most evidence suggests that ethanol, rather than any specific beverage, such as red wine, is the essential factor.

Two mechanisms have been proposed. The first involves the effect of ethanol on the plasma lipoproteins that are the carrier molecules for cholesterol and other lipids in the bloodstream (see Ch. 23). Epidemiological studies, as well as studies on volunteers, have shown that ethanol, in daily doses too small to produce obvious CNS effects, can over the course of a few weeks increase plasma high-density-lipoprotein concentration, thus exerting a protective effect against atheroma formation.

Ethanol may also protect against ischaemic heart disease by inhibiting platelet aggregation. This effect occurs at ethanol concentrations in the range achieved by normal drinking in humans (10–20 mmol/l) and probably results from inhibition of arachidonic acid formation from phospholipid. In humans, the magnitude of the effect depends critically on dietary fat intake, and it is not yet clear how important it is clinically.

The effect of ethanol on fetal development

The adverse effect of ethanol consumption during pregnancy on fetal development was demonstrated in the early 1970s, when the term *fetal alcohol syndrome (FAS)* was coined.

The features of full FAS include:

- abnormal facial development, with wide-set eyes, short palpebral fissures and small cheekbones
- reduced cranial circumference
- retarded growth
- mental retardation and behavioural abnormalities, often taking the form of hyperactivity and difficulty with social integration
- other anatomical abnormalities, which may be major or minor (e.g. congenital cardiac abnormalities, malformation of the eyes and ears).

A lesser degree of impairment, termed *alcohol-related neurodevelopmental disorder* (ARND), results in behavioural problems, and cognitive and motor deficits, often associated with reduced brain size. Full FAS occurs in about 3 per 1000 live births and affects about 30% of children born to alcoholic mothers. It is rare with mothers who drink less than about 5 units/day, and most common in binge drinkers who sporadically consume much larger amounts, resulting in high peak levels of ethanol. ARND is about three times as common. Although there is no clearly defined safe threshold, there is no evidence that amounts less than about 2 units/day are harmful. There is no critical period during pregnancy when ethanol consumption is likely to lead to FAS, although one study suggests that FAS incidence correlates most strongly with ethanol consumption

[10]This beneficial effect of moderate drinking outweighs the risk of adverse effects (e.g. accidents, cancers, liver damage) only in men over 45 and women over 55.

Effects of ethanol

- Ethanol consumption is generally expressed in units of 10 ml (8 g) of pure ethanol. Per capita consumption in the UK is more than 10 l/year.
- Ethanol acts as a general central nervous system depressant, similar to volatile anaesthetic agents, producing the familiar effects of acute intoxication.
- Several cellular mechanisms are postulated: enhancement of GABA and glycine action, inhibition of calcium channel opening, activation of potassium channels and inhibition at NMDA-type glutamate receptors.
- Effective plasma concentrations:
  - threshold effects: about 40 mg/100 ml (5 mmol/l)
  - severe intoxication: about 150 mg/100 ml
  - death from respiratory failure: about 500 mg/100 ml.
- Main peripheral effects are self-limiting diuresis (reduced antidiuretic hormone secretion), cutaneous vasodilatation and delayed labour (reduced oxytocin secretion).
- Neurological degeneration occurs with heavy and binge drinking, causing dementia and peripheral neuropathies.
- Long-term ethanol consumption causes liver disease, progressing to cirrhosis and liver failure.
- Moderate ethanol consumption has a protective effect against ischaemic heart disease.
- Excessive consumption in pregnancy causes impaired fetal development, associated with small size, abnormal facial development and other physical abnormalities, and mental retardation.
- Psychological dependence, physical dependence and tolerance all occur with ethanol.
- Drugs used to treat alcohol dependence include **disulfiram** (aldehyde dehydrogenase inhibitor), **naltrexone** (opiate antagonist) and **acamprosate** (NMDA receptor antagonist). **Topiramate** and **bupropion** are also used.

very early in pregnancy, even before pregnancy is recognised, implying that not only pregnant women, but also women who are likely to become pregnant, must be advised not to drink heavily. Experiments on rats and mice suggest that the effect on facial development may be produced very early in pregnancy (up to 4 weeks in humans), while the effect on brain development is produced rather later (up to 10 weeks).

PHARMACOKINETIC ASPECTS

Metabolism of ethanol

Ethanol is rapidly absorbed, an appreciable amount being absorbed from the stomach. A substantial fraction is cleared by first-pass hepatic metabolism. Hepatic metabolism of ethanol shows saturation kinetics (see Chs 9 and 10) at quite low ethanol concentrations, so the fraction of ethanol removed decreases as the concentration reaching the liver increases. Thus, if ethanol absorption is rapid and portal vein concentration is high, most of the ethanol escapes into the systemic circulation, whereas with slow absorption more is removed by first-pass metabolism. This is one reason why drinking ethanol on an empty stomach produces a much greater pharmacological effect. Ethanol is quickly distributed throughout the body water, the rate of its redistribution depending mainly on the blood flow to individual tissues, as with volatile anaesthetics (see Ch. 40).

Ethanol is about 90% metabolised, 5–10% being excreted unchanged in expired air and in urine. This fraction is not pharmacokinetically significant but provides the basis for estimating blood ethanol concentration from measurements on breath or urine. The ratio of ethanol concentrations in blood and alveolar air, measured at the end of deep expiration, is relatively constant, 80 mg/100 ml of ethanol in blood producing 35 µg/100 ml in expired air, this being the basis of the breathalyser test. The concentration in urine is more variable and provides a less accurate measure of blood concentration.

Ethanol metabolism occurs almost entirely in the liver, and mainly by a pathway involving successive oxidations, first to acetaldehyde and then to acetic acid (Fig. 48.5). Since ethanol is often consumed in large quantities (compared with most drugs), 1–2 mol daily being by no means unusual, it constitutes a substantial load on the hepatic oxidative systems. The oxidation of 2 mol of ethanol consumes about 1.5 kg of the co-factor nicotinamide adenine dinucleotide (NAD^+). Availability of NAD^+ limits the rate of ethanol oxidation to about 8 g/h in a normal adult, independently of ethanol concentration (Fig. 48.6), causing the process to show saturating kinetics (Ch. 10). It also leads to competition between the ethanol and other metabolic substrates for the available NAD^+ supplies, which may be a factor in ethanol-induced liver damage (see Ch. 57). The intermediate metabolite, acetaldehyde, is a reactive and toxic compound, and this may also contribute to the hepatotoxicity. A small degree of esterification of ethanol with various fatty acids also occurs in the tissues, and these esters may also contribute to long-term toxicity.

Alcohol dehydrogenase is a soluble cytoplasmic enzyme, confined mainly to liver cells, which oxidises ethanol at the same time as reducing NAD^+ to NADH (Fig. 48.5). Ethanol metabolism causes the ratio of NAD^+ to NADH to fall, and this has other metabolic consequences (e.g. increased lactate and slowing down of the Krebs cycle). The limitation on ethanol metabolism imposed by the limited rate of NAD^+ regeneration has led to attempts to find a 'sobering up' agent that works by regenerating NAD^+ from NADH. One such agent is fructose, which is reduced by an NADH-requiring enzyme. In large doses, it causes a measurable increase in the rate of ethanol metabolism, but not enough to have a useful effect on the rate of return to sobriety.

Normally, only a small amount of ethanol is metabolised by the microsomal mixed function oxidase system (see Ch. 9), but induction of this system occurs in alcoholics. Ethanol can affect the metabolism of other drugs that are metabolised by the mixed function oxidase system (e.g. **phenobarbitone**, **warfarin** and **steroids**), with an initial inhibitory effect produced by competition, followed by enhancement due to enzyme induction.

Nearly all the acetaldehyde produced is converted to acetate in the liver by *aldehyde dehydrogenase* (Fig. 48.5). Normally, only a little acetaldehyde escapes from the liver, giving a blood acetaldehyde concentration of 20–50 µmol/l after an intoxicating dose of ethanol in humans. The circulating acetaldehyde usually has little or no effect, but the concentration may become much larger under certain

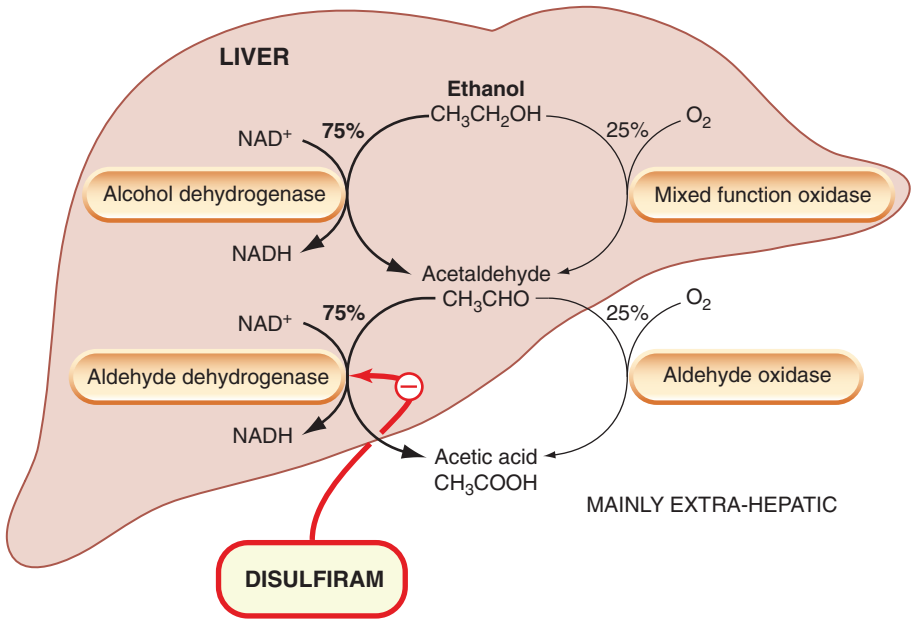

Fig. 48.5 Metabolism of ethanol. NAD, nicotinamide adenine dinucleotide.

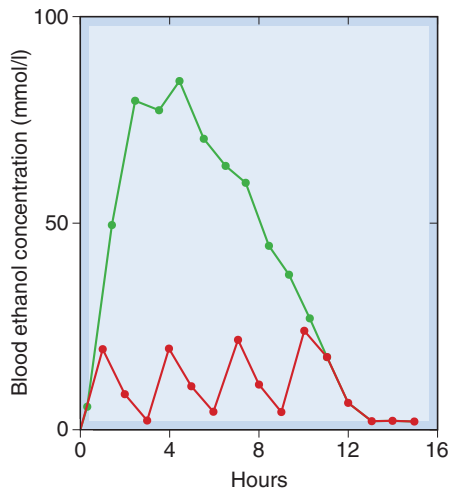

Fig. 48.6 Zero-order kinetics of ethanol elimination in rats. Rats were given ethanol orally (104 mmol/kg) either as a single dose or as four divided doses. The single dose results in a much higher and more sustained blood ethanol concentration than the same quantity given as divided doses. Note that, after the single dose, ethanol concentration declines linearly, the rate of decline being similar after a small or large dose, because of the saturation phenomenon. (From Kalant H et al. 1975 Biochem Pharmacol 24: 431.)

circumstances and produce toxic effects. This occurs if aldehyde dehydrogenase is inhibited by drugs such as **disulfiram**. In the presence of disulfiram, which produces no marked effect when given alone, ethanol consumption is followed by a severe reaction comprising flushing, tachycardia, hyperventilation, and considerable panic and distress, which is due to excessive acetaldehyde accumulation in the bloodstream. This reaction is extremely unpleasant but not harmful, and disulfiram can be used as aversion therapy to discourage people from taking ethanol. Some other drugs (e.g. **metronidazole**; see Ch. 50) produce similar reactions to ethanol. Interestingly, a Chinese herbal medicine, used traditionally to cure alcoholics, contains **daidzin**, a specific inhibitor of aldehyde dehydrogenase. In hamsters (which spontaneously consume alcohol in amounts that would defeat even the hardest two-legged drinker, while remaining, as far as one can tell in a hamster, completely sober), daidzin markedly inhibits alcohol consumption.

Genetic factors

In 50% of Asian people, an inactive genetic variant of one of the aldehyde dehydrogenase isoforms (ALDH-2) is expressed; these individuals experience a disulfiram-like reaction after alcohol, and the incidence of alcoholism in this group is extremely low (see Tanaka et al., 1997; Tyndale, 2003).

Metabolism and toxicity of methanol and ethylene glycol

▼ Methanol is metabolised in the same way as ethanol but produces formaldehyde instead of acetaldehyde from the first oxidation step. Formaldehyde is more reactive than acetaldehyde and reacts rapidly with proteins, causing the inactivation of enzymes involved in the tricarboxylic acid cycle. It is converted to another toxic metabolite, formic acid. This, unlike acetic acid, cannot be utilised in the tricarboxylic acid cycle and is liable to cause tissue damage. Conversion of alcohols to aldehydes occurs not only in the liver but also in the retina, catalysed by the dehydrogenase responsible for retinol–retinal conversion. Formation of formaldehyde in the retina accounts for one of the main toxic effects of methanol, namely blindness, which can occur after ingestion of as little as 10 g. Formic acid production and derangement of the tricarboxylic acid cycle also produce severe acidosis.

Methanol is used as an industrial solvent and also to adulterate industrial ethanol in order to make it unfit to drink. Methanol poisoning is quite common, and used to be treated by administration of large doses of ethanol, which acts to retard methanol metabolism by

Metabolism of ethanol

- Ethanol is metabolised mainly by the liver, first by alcohol dehydrogenase to acetaldehyde, then by aldehyde dehydrogenase to acetate. About 25% of the acetaldehyde is metabolised extrahepatically.
- Small amounts of ethanol are excreted in urine and expired air.
- Hepatic metabolism shows saturation kinetics, mainly because of limited availability of nicotinamide adenine dinucleotide (NAD^+). Maximal rate of ethanol metabolism is about 10 ml/h. Thus plasma concentration falls linearly rather than exponentially.
- Acetaldehyde may produce toxic effects. Inhibition of aldehyde dehydrogenase by disulfiram accentuates nausea, etc., caused by acetaldehyde, and can be used in aversion therapy.
- Methanol is similarly metabolised to formic acid, which is toxic, especially to the retina.
- Asian people show a high rate of genetic polymorphism of alcohol and aldehyde dehydrogenase, associated with alcoholism and alcohol intolerance, respectively.

competition for alcohol dehydrogenase. **Fomepizole** inhibits alcohol dehydrogenase and is now preferred if available. Such treatment may be in conjunction with haemodialysis to remove unchanged methanol, which has a small volume of distribution.

Poisoning with ethylene glycol, used in automobile antifreeze and brake fluid, is a medical emergency. It is rapidly absorbed from the gut and metabolised to glycolate and then more slowly to oxalate. Glycolate interferes with metabolic processes and produces metabolic acidosis. It affects the brain, heart and kidneys. Treatment is with alkali such as sodium bicarbonate to reverse the acidosis, pyridoxine and thiamine to promote conversion to non-toxic metabolites and haemodialysis.

TOLERANCE AND DEPENDENCE

Tolerance to the effects of ethanol can be demonstrated in both humans and experimental animals, to the extent of a two- to three-fold reduction in potency occurring over 1–3 weeks of continuing ethanol administration. A small component of this is due to the more rapid elimination of ethanol. The major component is cellular tolerance, which accounts for a roughly two-fold decrease in potency and which can be observed in vitro (e.g. by measuring the inhibitory effect of ethanol on transmitter release from synaptosomes) as well as in vivo. The mechanism of this tolerance is not known for certain (see Little, 1991). Ethanol tolerance is associated with tolerance to many anaesthetic agents, and alcoholics are often difficult to anaesthetise.

Chronic ethanol administration produces various changes in CNS neurons, which tend to oppose the acute cellular effects that it produces (see above). There is a small reduction in the density of $GABA_A$ receptors, and a proliferation of voltage-gated calcium channels and NMDA receptors.

A well-defined physical abstinence syndrome develops in response to ethanol withdrawal. As with most other dependence-producing drugs, this is probably important as a short-term factor in sustaining the drug habit, but other (mainly psychological) factors are more important in the longer term (see above). The physical abstinence syndrome usually subsides in a few days, but the craving for ethanol and the tendency to relapse last for very much longer.

The physical abstinence syndrome in humans, in severe form, develops after about 8 h. In the first stage, the main symptoms are tremor, nausea, sweating, fever and sometimes hallucinations. These last for about 24 h. This phase may be followed by seizures ('rum fits'). Over the next few days, the condition of 'delirium tremens' develops, in which the patient becomes confused, agitated and often aggressive, and may suffer much more severe hallucinations. A similar syndrome of central and autonomic hyperactivity can be produced in experimental animals by ethanol withdrawal. Treatment of this medical emergency is by sedation with large doses of a benzodiazepine such as **chlordiazepoxide** (Ch. 43) together with large doses of thiamine.

PHARMACOLOGICAL APPROACHES TO TREATING ALCOHOL DEPENDENCE

Alcohol dependence ('alcoholism') is common (4–5% of the population) and, as with smoking, difficult to treat effectively. The main pharmacological approaches (see Garbutt, 2009; Table 48.3) are the following:

- To alleviate the acute abstinence syndrome during 'drying out', **benzodiazepines** (see Ch. 43) and **clomethiazole** are effective; **clonidine** and **propranolol** are also useful. Clonidine (α_2 adrenoceptor agonist) is believed to act by inhibiting the exaggerated transmitter release that occurs during withdrawal, while propranolol (β-adrenoceptor antagonist) blocks some of the effects of excessive sympathetic activity.
- To render alcohol consumption unpleasant, **disulfiram** (see above).
- To reduce alcohol-induced reward, **naltrexone** (see above) is effective.
- To reduce craving, **acamprosate** is used. This taurine analogue is a weak antagonist at NMDA receptors, and may work by interfering in some way with synaptic plasticity. Several clinical trials have shown it to improve the success rate in achieving alcohol abstinence, with few unwanted effects.
- To alleviate both withdrawal and craving, the antiepileptic agent, **topiramate**, which has multiple effects on the brain (see Ch. 44) shows promise as does **γ-hydroxybutyric acid** (GHB), a short-chain fatty acid structurally similar to the inhibitory neurotransmitter γ-aminobutyric acid (see Ch. 37).

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http://www.ash.org.uk/. (*ASH, an antismoking organisation*)

http://www.drugscope.org.uk/. (*DrugScope, an independent organisation providing advice on various aspects of drug abuse*)

http://www.nida.nih.gov/. (*National Institute on Drug Abuse [NIDA], US government organisation providing information to scientists and the general public on various aspects of drug abuse*)

http://www.drugabuse.gov/PODAT/PODATIndex.html. (*Provides access to the NIDA publication Principles of Drug Addiction Treatment: A Research Based Guide, second ed.*)

http://www.ias.org.uk/resources/factsheets/factsheets.html. (*An excellent range of factsheets relating to all aspects of alcohol consumption and its consequences from the Institute of Alcohol Studies [UK]*)

Basic principles of antimicrobial chemotherapy

49

OVERVIEW

Chemotherapy is the term originally used to describe the use of drugs that are 'selectively toxic' to invading microorganisms while having minimal effects on the host. The term also embraces the use of drugs that target tumours and, in fact, has now come to be associated specifically with that branch of pharmacology. In this chapter, however, we intend the term to cover both usages, although, in the public mind at least, chemotherapy is usually associated with cytotoxic anticancer drugs that cause unwanted effects such as loss of hair, nausea and vomiting.

All living organisms are prey to infection. Humans, being no exception to this rule, are susceptible to diseases caused by viruses, bacteria, protozoa, fungi and helminths (collectively referred to as *pathogens*). The use of chemotherapeutic agents dates back to the work of Ehrlich and others and to the development of arsenical drugs such as salvarsan for the treatment of syphilis.[1] The successful development of such agents during the past 80 years, particularly the 'antibiotic revolution', which began in the 1940s with the advent of penicillin, constitutes one of the most important therapeutic advances in the entire history of medicine.

Clearly, the feasibility of selective toxicity depends on the ability to exploit such biochemical differences as may exist between the infecting organism (or indeed cancer cells, our internal 'invaders') and the host. Chapter 6 outlined our own 'host' defences against infection, while the bulk of the chapters in this section of the book describe the drugs used to combat such infections. In this introductory chapter we consider, very broadly, the nature of these biochemical differences and outline the molecular targets of drug action.

BACKGROUND

The term *chemotherapy* was coined by Ehrlich himself at the beginning of the 20th century to describe the use of synthetic chemicals to destroy infective agents. In recent years, the definition of the term has been broadened to include *antibiotics*—substances produced by some microorganisms (or by pharmaceutical chemists) that kill or inhibit the growth of other microorganisms. Here, we broaden it still further to include agents that kill or inhibit the growth of cancer cells.

Unhappily, our success in developing drugs to attack these invaders has been paralleled by their own success in counteracting the effects of the drugs, resulting in the emergence of drug resistance. And at present, the invaders—particularly some bacteria—seem close to getting the upper hand. This is a very important problem, and we will devote some space to the mechanisms of resistance and the means by which it is spread.

THE MOLECULAR BASIS OF CHEMOTHERAPY

Chemotherapeutic agents, then, are chemicals that are intended to be toxic to the pathogenic organism (or cancer cells) but innocuous to the host. It is important to remember that many microorganisms share our body spaces (e.g. the gut[2]) without causing disease (these are called *commensals*), although they may become pathogenic under adverse circumstances (i.e. if the host is immunocompromised).

Living organisms are classified as either *prokaryotes*, cells without nuclei (e.g. bacteria), or *eukaryotes*, cells with nuclei (e.g. protozoa, fungi, helminths). In a separate category are the viruses, which need to utilise the metabolic machinery of the host cell, and they thus present a particular kind of problem for chemotherapeutic attack. There remain those mysterious proteinaceous agents, the *prions* (see Ch. 39), which cause disease but resist all attempts at classification, and for which there is no known antidote at present.

In another category are cancer cells, which are clearly more similar to normal host cells than are any pathogenic invaders, and this makes the problem of implementing selective toxicity especially difficult. The principles of cancer chemotherapy are discussed in Chapter 55. Virtually all creatures, host and parasite alike, have the same basic DNA blueprint (an exception being the RNA viruses), so some biochemical processes are common to most, if not all, organisms. Finding agents that affect pathogens or cancers but not other human cells necessitates finding either qualitative or quantitative biochemical differences between them.

Bacteria cause most infectious diseases, and Figure 49.1 shows in simplified diagrammatic form the main structures and functions of a 'generalised' bacterial cell. Surrounding the cell is the *cell wall*, which characteristically contains *peptidoglycan* in all forms of bacteria except *Mycoplasma*. Peptidoglycan is unique to prokaryotic cells and has no counterpart in eukaryotes. Within the cell wall is the *plasma membrane*, which, like that of eukaryotic cells, consists of a phospholipid bilayer and proteins. It functions as a selectively permeable membrane with specific transport mechanisms for various nutrients. However, in bacteria the plasma membrane does not contain any *sterols*, and this may alter the penetration of some chemicals.

[1]Mercury-containing compounds were also once used for treating syphilis. 'One night with Venus, a lifetime with Mercury' was a saying of that time.

[2]Humans harbour about 2 kg of bacteria in the gut, comprising a large 'forgotten organ' in the body with important metabolic functions.

The function of the cell wall is to support the underlying plasma membrane, which is subject to an internal osmotic pressure of about 5 atmospheres in *Gram-negative* organisms, and about 20 atmospheres in *Gram-positive* organisms (see below). The plasma membrane and cell wall together comprise the *bacterial envelope*.

Bounded by the plasma membrane is the *cytoplasm*. As in eukaryotic cells, this contains soluble enzymes and other proteins, the *ribosomes* involved in protein synthesis, the small-molecule intermediates involved in metabolism as well as inorganic ions. The bacterial cell has no nucleus; instead, the genetic material, in the form of a single *chromosome* containing all the genetic information, lies in the cytoplasm with no surrounding nuclear membrane. In further contrast to eukaryotic cells, there are no *mitochondria* – cellular energy is generated by enzyme systems located in the plasma membrane.

Some bacteria have additional components such as a *capsule* and/or *flagella*, but the only additional structure with relevance for chemotherapy is the *outer membrane* outside the cell wall. The nature of this membrane enables bacteria to be classified according to whether they take up *Gram's stain* ('Gram-positive') or not ('Gram-negative'; for more details, see Ch. 50). In Gram-negative bacteria, this membrane may prevent penetration of antibacterial agents, and it also prevents easy access of *lysozyme* (a microbiocidal enzyme found in white blood cells, tears and other tissue fluids that breaks down peptidoglycan).

The biochemical reactions that are potential targets for antibacterial drugs are shown in Figure 49.1. There are three groups:

- *Class I*: the utilisation of glucose or some alternative carbon source for the generation of energy (ATP) and synthesis of simple carbon compounds used as precursors in the next class of reactions.
- *Class II*: the utilisation of these precursors in an energy-dependent synthesis of all the amino acids, nucleotides, phospholipids, amino sugars, carbohydrates and growth factors required by the cell for survival and growth.
- *Class III*: assembly of small molecules into macromolecules—proteins, RNA, DNA, polysaccharides and peptidoglycan.

Other potential targets are the *formed structures*, for example the cell membrane, or in higher organisms (e.g. fungi and cancer cells) the *microtubules* or other specific tissues (e.g. muscle tissue in helminths). In considering these targets, emphasis will be placed on bacteria, but reference will also be made to protozoa, helminths, fungi, cancer cells and viruses. The classification that follows is clearly not rigid; a drug may affect more than one class of reactions or more than one subgroup of reactions within a class.

BIOCHEMICAL REACTIONS AS POTENTIAL TARGETS

CLASS I REACTIONS

Class I reactions are not promising targets for two reasons. First, bacterial and human cells use similar mechanisms to obtain energy from glucose (the *Embden–Meyerhof pathway* and the *tricarboxylic acid cycle*). Second, even if glucose oxidation is blocked, many other compounds (amino acids, lactate, etc.) can be utilised by bacteria as an alternative energy source.

The molecular basis of antibacterial chemotherapy

- Chemotherapeutic drugs should be toxic to invading organisms and innocuous to the host. Such selective toxicity depends on the discovery of biochemical differences between the pathogen and the host that can be appropriately exploited.
- Three general classes of biochemical reaction are potential targets for chemotherapy of bacteria:
  - *class I*: reactions that utilise glucose and other carbon sources are used to produce ATP and simple carbon compounds
  - *class II*: pathways utilising energy and class I compounds to make small molecules (e.g. amino acids and nucleotides)
  - *class III*: pathways that convert small molecules into macromolecules such as proteins, nucleic acids and peptidoglycan.

CLASS II REACTIONS

Class II reactions are better targets because some pathways exist in pathogen, but not human, cells. For instance, human cells lack the ability, possessed by bacteria, to synthesise the so-called 'essential' amino acids as well as certain growth factors (termed *vitamins* in human physiology). Differences such as these represent potential targets. Another opportunity occurs when a pathway is identical in both bacteria and humans but exhibits a differential sensitivity to drugs. A prominent example is the *folic acid pathway*.

Folate

Folate biosynthesis is an example of a metabolic pathway found in bacteria but not in humans. Folate is required for DNA synthesis in both bacteria and in humans (see Chs 25 and 50). Humans cannot synthesise folate and must obtain it from the diet, and specific uptake mechanisms transport it into cells. By contrast, most species of bacteria, as well as the asexual forms of malarial protozoa, lack the necessary transport mechanisms and cannot make use of preformed folate but must synthesise their own de novo. This is a prime example of a difference that has proved to be extremely useful for chemotherapy. **Sulfonamides** contain the sulfanilamide moiety—a structural analogue of *p*-aminobenzoic acid (PABA), which is essential in the synthesis of folate (see Fig. 50.1). Sulfonamides compete with PABA for the enzyme involved in folate synthesis, and thus inhibit the metabolism of the bacteria. They are consequently *bacteriostatic*, not *bactericidal*[3] (i.e. they suppress division of the cells but do not kill them), and are therefore only really effective in the presence of adequate host defences (see Chs 6 and 17).

The utilisation of folate, in the form of *tetrahydrofolate*, as a co-factor in thymidylate synthesis is a good example of a pathway where human and bacterial enzymes exhibit a

[3]Whether a drug is bactericidal rather than bacteriostatic is determined according to a strict technical criterion, but in practice it can be difficult to differentiate the two actions during therapy.

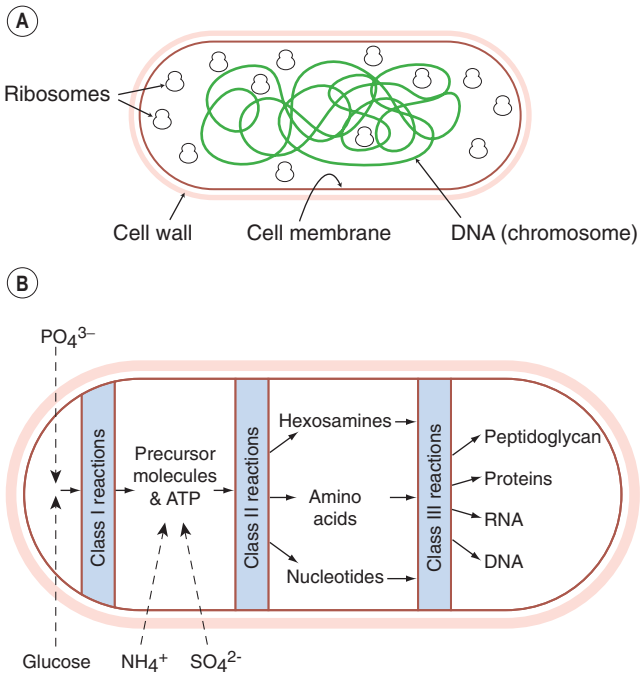


Fig. 49.1 Diagram of the structure and metabolism of a 'typical' bacterial cell. [**A**] Schematic representation of a bacterial cell. [**B**] Flow diagram showing the synthesis of the main types of macromolecule of a bacterial cell. Class I reactions result in the synthesis of the precursor molecules necessary for class II reactions, which result in the synthesis of the constituent molecules; these are then assembled into macromolecules by class III reactions. (Modified from Mandelstam J, McQuillen K, Dawes I (eds) 1982 Biochemistry of bacterial growth. Blackwell Scientific, Oxford.)

differential sensitivity to chemicals (Table 49.1; see Volpato & Pelletier, 2009). Although the pathway is virtually identical in microorganisms and humans, one of the key enzymes, *dihydrofolate reductase*, which reduces dihydrofolate to tetrahydrofolate (Fig. 50.2), is many times more sensitive to the folate antagonist **trimethoprim** in bacteria than in humans. In some malarial protozoa, this enzyme is somewhat less sensitive than the bacterial enzyme to trimethoprim but more sensitive to **pyrimethamine** and **proguanil**, which are used as antimalarial agents (Ch. 53). The relative IC_{50} values (the concentration causing 50% inhibition) for bacterial, malarial, protozoal and mammalian enzymes are given in Table 49.1. The human enzyme, by comparison, is very sensitive to the effect of the folate analogue **methotrexate** (Table 49.1) which is used to treat rheumatoid arthritis (Ch. 26) and cancer (Ch. 55). Methotrexate is inactive in bacteria because, being very similar in structure to folate, it requires active uptake by cells. Trimethoprim and pyrimethamine enter the cells by diffusion.

▼ The use of sequential blockade with a combination of two drugs that affect the same pathway at different points, for example sulfonamides and the folate antagonists, may be more successful than the use of either alone (e.g. in the treatment of *Pneumocystis jirovecii* pneumonia), and lower concentrations are effective when the two are used together. Thus, pyrimethamine and a sulfonamide (**sulfadoxine**) are used to treat *falciparum* malaria. An antibacterial formulation that contains both a sulfonamide and trimethoprim is **co-trimoxazole**; once widely used, this combination has become progressively less effective because of the development of sulfonamide resistance.

Table 49.1 Specificity of inhibitors of dihydrofolate reductase

Inhibitor	IC_{50} (μmol/l) for dihydrofolate reductase		
	Human	Protozoal	Bacterial
Trimethoprim	260	0.07	0.005
Pyrimethamine	0.7	0.0005	2.5
Methotrexate	0.001	~0.1[a]	Inactive

[a]Tested on *Plasmodium berghei*, a rodent malaria.

Pyrimidine and purine analogues

Another example of a drug that interferes with a class II reaction is the pyrimidine analogue **fluorouracil**, which is used in cancer chemotherapy (Ch. 55). Fluorouracil is converted to a fraudulent nucleotide that interferes with thymidylate synthesis. Other cancer chemotherapy agents that give rise to fraudulent nucleotides are the purine analogues **mercaptopurine** and **thioguanine**. **Flucytosine**, an antifungal drug (Ch. 52), is deaminated to fluorouracil within fungal cells but to a much lesser extent in human cells, conferring a degree of selectivity.

CLASS III REACTIONS

As pathogen cells cannot take up their own unique macromolecules from the environment, class III reactions are particularly good targets for selective toxicity, and there are distinct differences between mammalian cells and parasitic cells in this respect.

The synthesis of peptidoglycan

The cell wall of bacteria contains peptidoglycan, a substance that does not occur in eukaryotes. It is the equivalent of a non-stretchable string bag enclosing the whole bacterium. In Gram-negative bacteria, this bag consists of a single thickness, but in Gram-positive bacteria there may be as many as 40 layers of peptidoglycan. Each layer consists of multiple backbones of amino sugars—alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues (Fig. 49.2)—the latter having short peptide side-chains that are crosslinked to form a polymeric lattice, which is strong enough to resist the high internal osmotic pressure and may constitute up to 10–15% of the dry weight of the cell. The cross-links differ in different species. In staphylococci, they consist of five glycine residues.

▼ To build up this very large insoluble peptidoglycan layer on the outside of the cell membrane, the bacterial cell has the problem of how to transport the hydrophilic cytoplasmic 'building blocks' through the hydrophobic cell membrane structure. This is accomplished by linking them to a very large lipid carrier, containing 55 carbon atoms, which 'tows' them across the membrane. The process of peptidoglycan synthesis is outlined in Figure 49.3. First, *N*-acetylmuramic acid, attached to uridine diphosphate (UDP) and a pentapeptide, is transferred to the C_{55} lipid carrier in the membrane, with the release of uridine monophosphate. This is followed by a reaction with UDP–*N*-acetylglucosamine, resulting in the formation of a disaccharide pentapeptide complex attached to the carrier. This complex is the basic building block of the peptidoglycan. In *Staphylococcus aureus*, the five glycine residues are attached to the peptide

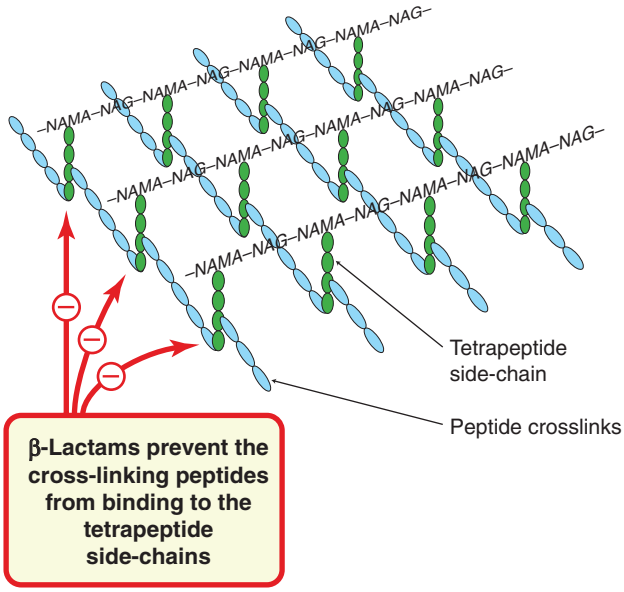


Fig. 49.2 Schematic diagram of a single layer of peptidoglycan from a bacterial cell (e.g. *Staphylococcus aureus*), showing the site of action of the β-lactam antibiotics. In *S. aureus*, the peptide crosslinks consist of five glycine residues. Gram-positive bacteria have several layers of peptidoglycan. (NAG, *N*-acetylglucosamine; NAMA, *N*-acetylmuramic acid; more detail in Fig. 49.3.)

chain at this stage. The building block is now transported out of the cell and added to the growing end of the peptidoglycan, the 'acceptor', with the release of the C_{55} lipid, which still has two phosphates attached. The lipid carrier then loses one phosphate group and thus becomes available for another cycle. Crosslinking between the peptide side-chains of the sugar residues in the peptidoglycan layer then occurs, the hydrolytic removal of the terminal alanine supplying the requisite energy.

This synthesis of peptidoglycan is a vulnerable step and can be blocked at several points by antibiotics (Fig. 49.3; Ch. 50). **Cycloserine**, which is a structural analogue of D-alanine, prevents the addition of the two terminal alanine residues to the initial tripeptide side-chain on *N*-acetylmuramic acid by competitive inhibition. **Vancomycin** inhibits the release of the building block unit from the carrier, thus preventing its addition to the growing end of the peptidoglycan. **Bacitracin** interferes with the regeneration of the lipid carrier by blocking its dephosphorylation. **Penicillins**, **cephalosporins** and other **β-lactams** inhibit the final transpeptidation by forming covalent bonds with *penicillin-binding proteins* that have transpeptidase and carboxypeptidase activities, thus preventing formation of the crosslinks.

Protein synthesis

Protein synthesis takes place in the ribosomes. Eukaryotic and prokaryotic ribosomes are different, and this provides the basis for the selective antimicrobial action of some antibiotics. The bacterial ribosome consists of a 50S subunit and a 30S subunit (Fig. 49.4), whereas in the mammalian ribosome the subunits are 60S and 40S. The other elements involved in peptide synthesis are messenger RNA (mRNA), which forms the template for protein synthesis, and transfer RNA (tRNA), which specifically transfers the individual amino acids to the ribosome. The ribosome has three binding sites for tRNA, termed the A, P and E sites.

A simplified version of protein synthesis in bacteria is shown in Figure 49.4. To initiate translation, mRNA, transcribed from the DNA template (see below), is attached to the 30S subunit of the ribosome. The 50S subunit then binds to the 30S subunit to form a 70S subunit,[4] which moves along the mRNA such that successive codons of the messenger pass along the ribosome from the A position to the P position. Antibiotics may affect protein synthesis at any one of these stages (Fig. 49.4; Ch. 50).

Nucleic acid synthesis

The nucleic acids of the cell are DNA and RNA. There are three types of RNA: mRNA, tRNA and ribosomal RNA (rRNA). The last of these is an integral part of the ribosome and is necessary for its assembly as well as for facilitating mRNA binding. The assembled ribosome also exhibits peptidyl transferase activity.

DNA is the template for the synthesis of both DNA and RNA. It exists in the cell as a double helix, each strand of which is a linear polymer of nucleotides. Each nucleotide consists of a base linked to a sugar (deoxyribose) and a phosphate. There are two purine bases, adenine (A) and guanine (G), and two pyrimidine bases, cytosine (C) and thymine (T). Single-strand DNA comprises alternating sugar and phosphate groups with the bases attached (Fig. 49.5). Specific hydrogen bonding between G and C and between A and T on each strand (i.e. complementary base pairing) is the basis of the double-stranded helical structure of DNA. The DNA helix is itself further coiled. In the test tube, the coil has 10 base pairs per turn. In vivo, the coil is unwound by about 1 turn in 20, forming a *negative supercoil*.

Initiation of DNA synthesis requires first the activity of a protein that causes separation of the strands. The replication process inserts a positive supercoil, which is relaxed by *DNA gyrase* (also called *topoisomerase II*; Fig. 49.6). During the synthesis of DNA, nucleotide units—each consisting of a base linked to a sugar and three phosphate groups—are added by base pairing with the complementary residues on the template. Condensation occurs by elimination of two phosphate groups, catalysed by *DNA polymerase*.

RNA exists only in single-stranded form. The sugar moiety here is ribose, and the ribonucleotides contain the bases adenine, guanine, cytosine and uracil (U).

It is possible to interfere with nucleic acid synthesis in five different ways:

- by inhibiting the synthesis of the nucleotides
- by altering the base-pairing properties of the template
- by inhibiting either DNA or RNA polymerase
- by inhibiting DNA gyrase
- by a direct effect on DNA itself.

Inhibition of the synthesis of the nucleotides

This can be accomplished by an effect on the metabolic pathways that generate nucleotide precursors. Examples of agents that have such an effect have been described under class II reactions.

[4]You query whether 30S + 50S = 70S? Yes it does, because we are talking about *Svedberg units*, which measure sedimentation *rate*, not *mass*.

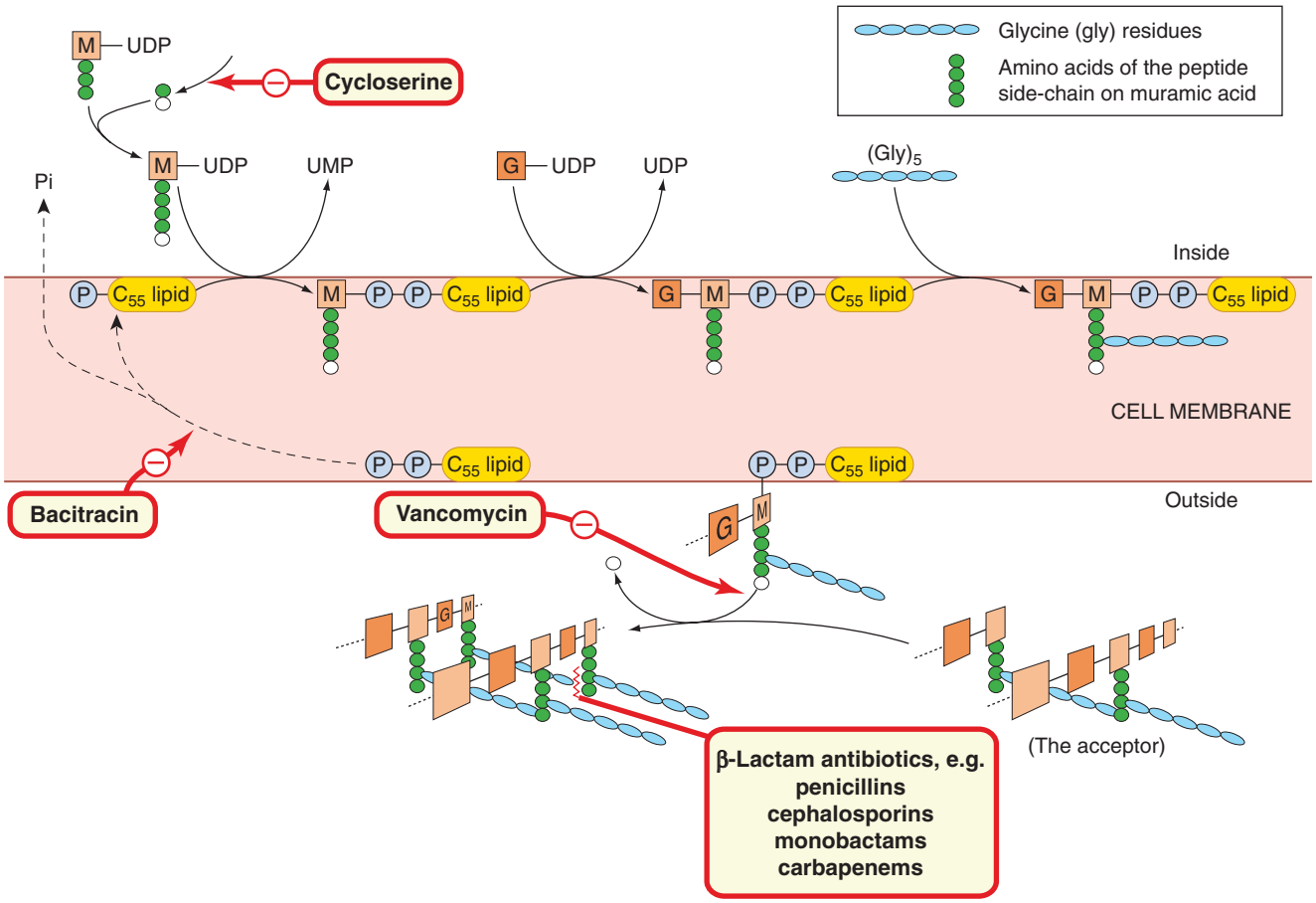


Fig. 49.3 Schematic diagram of the biosynthesis of peptidoglycan in a bacterial cell (e.g. *Staphylococcus aureus*), with the sites of action of various antibiotics. The hydrophilic disaccharide–pentapeptide is transferred across the lipid cell membrane attached to a large lipid (C_{55} lipid) by a pyrophosphate bridge (–P–P–). On the outside, it is enzymically attached to the 'acceptor' (the growing peptidoglycan layer). The final reaction is a transpeptidation, in which the loose end of the (Gly) 5 chain is attached to a peptide side-chain of an M in the acceptor and during which the terminal amino acid (alanine) is lost. The lipid is regenerated by loss of a phosphate group (Pi) before functioning again as a carrier. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid; UDP, uridine diphosphate; UMP, uridine monophosphate.

Alteration of the base-pairing properties of the template

Agents that intercalate in the DNA have this effect. Examples include acridines (**proflavine** and **acriflavine**), which are used topically as antiseptics. The acridines double the distance between adjacent base pairs and cause a *frameshift mutation* (Fig. 49.7), whereas some purine and pyrimidine analogues cause base *mispairing*.

Inhibition of either DNA or RNA polymerase

Dactinomycin (actinomycin D) binds to the guanine residues in DNA and blocks the movement of RNA polymerase, thus preventing transcription and inhibiting protein synthesis. The drug is used in cancer chemotherapy in humans (Ch. 55) and also as an experimental tool, but it is not useful as an antibacterial agent. Specific inhibitors of bacterial RNA polymerase that act by binding to this enzyme in prokaryotic but not in eukaryotic cells include **rifamycin** and **rifampicin**, which are particularly useful for treating tuberculosis (see Ch. 50). **Aciclovir** (an analogue of guanine) is phosphorylated in cells infected with herpes virus, the initial phosphorylation being by a virus-specific kinase to give the aciclovir trisphosphate, which has an inhibitory action on the DNA polymerase of the herpes virus (Ch. 51; Fig. 49.8).

RNA retroviruses have a *reverse transcriptase* (*viral RNA-dependent DNA polymerase*) that copies the viral RNA into DNA that integrates into the host cell genome as a provirus. Various agents (**zidovudine**, **didanosine**) are phosphorylated by cellular enzymes to the trisphosphate forms, which compete with the host cell precursors essential for the formation by the viral reverse transcriptase of proviral DNA.

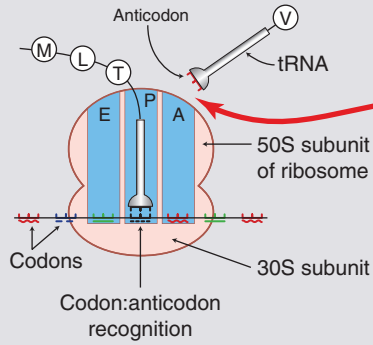
Cytarabine (cytosine arabinoside) is used in cancer chemotherapy (Ch. 55). Its trisphosphate derivative is a potent inhibitor of DNA polymerase in mammalian cells. **Foscarnet** inhibits viral RNA polymerase by attaching to the pyrophosphate-binding site.

Inhibition of DNA gyrase

Figure 49.6 is a simplified scheme showing the action of DNA gyrase. The *fluoroquinolones* (**cinoxacin**, **ciprofloxacin**, **nalidixic acid** and **norfloxacin**) act by inhibiting DNA gyrase, and these chemotherapeutic agents are used particularly in infections with Gram-negative organisms

A

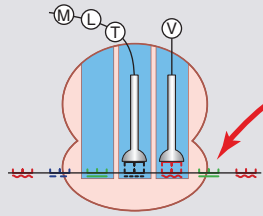
The elements involved in protein synthesis are shown: a ribosome (with 3 binding sites for transfer RNA (tRNA): the P, A and E sites), messenger RNA (mRNA) and tRNA. The different mRNA codons (triplets of 3 nucleotides which code for specific amino acids) are represented by dots, dashes and straight or wavy lines and are shown in different colours. A tRNA with the growing peptide chain (consisting so far of Met–Leu–Trp: MLT) is in the P site, bound by codon:anticodon recognition (i.e. by complementary base-pairing). The incoming tRNA carries valine (V), covalently linked.



Competition with tRNA for the A site, e.g. tetracyclines; selectivity largely through selective uptake by active transport into prokaryotic cells

B

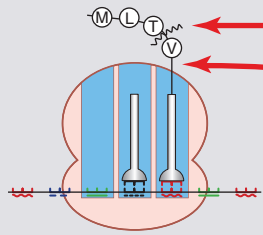
The incoming tRNA binds to the A site by complementary base-pairing.



Abnormal codon:anticodon leads to misreading of the message, e.g. aminoglycosides, gentamicin, amikacin, etc.

C

Transpeptidation occurs, i.e. the peptide chain on the tRNA in the P site is transferred to the tRNA on the A site. The peptide chain attached to the tRNA in the A site now consists of Met–Leu–Trp–Val (MLTV). The tRNA in the P site has been 'discharged', i.e. has lost its peptide.

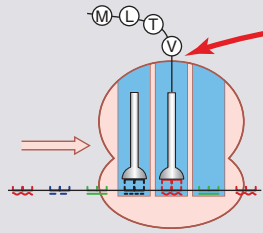


Inhibition of transpeptidation, e.g. chloramphenicol

Premature termination of peptide chain, e.g. puromycin, which resembles the amino acid end of tRNA (it also affects mammalian cells; used as an experimental tool)

D

The discharged tRNA is now transferred from the P site to the E site; the tRNA with the growing peptide chain is translocated from the A site to the P site and the ribosome moves on one codon, relative to the messenger.



Inhibition of translocation, e.g. erythromycin (also spectinomycin, fusidic acid)

E

The tRNA from which the peptide chain has been removed is ejected. A new tRNA, with amino acid (M) attached and with the relevant anticodon, now moves into the A site, and the whole process is repeated.

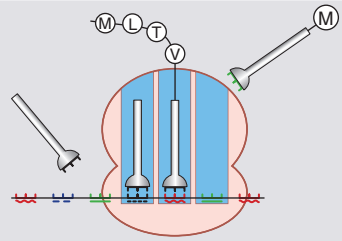


Fig. 49.4 Schematic diagram of bacterial protein synthesis, indicating the points at which antibiotics inhibit the process.

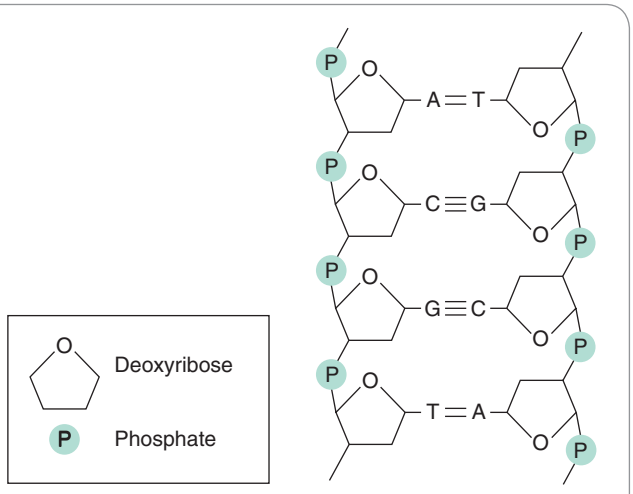


Fig. 49.5 Structure of DNA. Each strand of DNA consists of a sugar–phosphate backbone with purine or pyrimidine bases attached. The purines are adenine (A) or guanine (G) and the pyrimidines are cytosine (C) or thymine (T). The sugar is deoxyribose. Complementarity between the two strands of DNA is maintained by hydrogen bonds (either two or three) between bases.

(Ch. 50). These drugs are selective for the bacterial enzyme because it is structurally different from the mammalian enzyme. Some anticancer agents, for example **doxorubicin**, act on the mammalian topoisomerase II.

Direct effects on DNA itself

Alkylating agents form covalent bonds with bases in DNA and prevent replication. Compounds with this action are used only in cancer chemotherapy and include *nitrogen mustard* derivatives and *nitrosoureas* (Ch. 55). **Mitomycin** also binds covalently to DNA. No antibacterial agents work by these mechanisms. **Bleomycin**, an anticancer drug, causes fragmentation of the DNA strands following free radical formation (Ch. 55).

THE FORMED STRUCTURES OF THE CELL AS POTENTIAL TARGETS

THE MEMBRANE

The plasma membrane of bacterial cells is similar to that in mammalian cells in that it consists of a phospholipid bilayer in which proteins are embedded, but it can be more easily disrupted in certain bacteria and fungi.

Polymixins are cationic peptide antibiotics, containing both hydrophilic and lipophilic groups, which have a selective effect on bacterial cell membranes. They act as detergents, disrupting the phospholipid components of the membrane structure, thus killing the cell.

Unlike mammalian and bacterial cells, fungal cell membranes comprise large amounts of *ergosterol*. This facilitates the attachment of *polyene antibiotics* (e.g. **nystatin** and **amphotericin**; Ch. 52), which act as ionophores and cause leakage of cations.

Azoles such as **itraconazole** kill fungal cells by inhibiting ergosterol synthesis, thereby disrupting the function of membrane-associated enzymes. The azoles also affect Gram-positive bacteria, their selectivity being associated

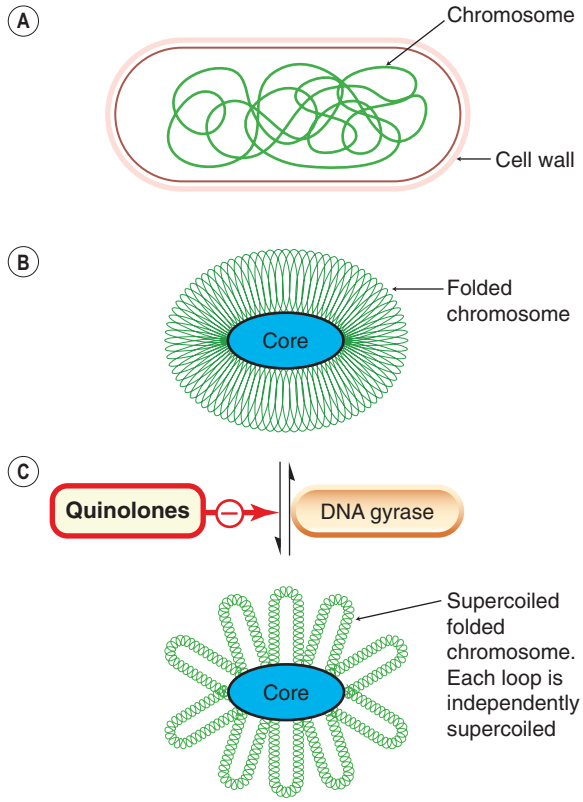


Fig. 49.6 Schematic diagram of the action of DNA gyrase: the site of action for quinolone antibacterials. [**A**] Conventional diagram used to depict a bacterial cell and chromosome (e.g. *Escherichia coli*). Note that the *E. coli* chromosome is 1300 mm long and is contained in a cell envelope of 2 μm × 1 μm; this is approximately equivalent to a 50 m length of cotton folded into a matchbox. [**B**] Chromosome folded around RNA core, and then [**C**], supercoiled by DNA gyrase (topoisomerase II). Quinolone antibacterials interfere with the action of this enzyme. (Modified from Smith J T 1985 In: Greenwood D, O'Grady F (eds) Scientific basis of antimicrobial therapy. Cambridge University Press, Cambridge, p. 69.)

mRNA (normal)	UCU	UUU	CUU	AUU	GUU	UCU...
	Ser	Phe	Leu	Ile	Val	Ser
mRNA (mutant)	UCU	UUG	UCU	UAU	UGU	UUC...
	Ser	Leu	Ser	Tyr	Cys	Phe

Fig. 49.7 An example of the effect on RNA and protein synthesis of a frameshift mutation in DNA. A frameshift mutation is one that involves a deletion of a base or an insertion of an extra base. In the above example, an extra cytosine has been inserted in the DNA template, with the result that when mRNA is formed it has an additional guanine (G), as indicated in orange. The effect is to alter that codon and all the succeeding ones (shown in blue), so that a completely different protein is synthesised, as indicated by the different amino acids (Leu instead of Phe, Ser instead of Leu, etc.). A, adenine; C, cytosine; U, uracil.

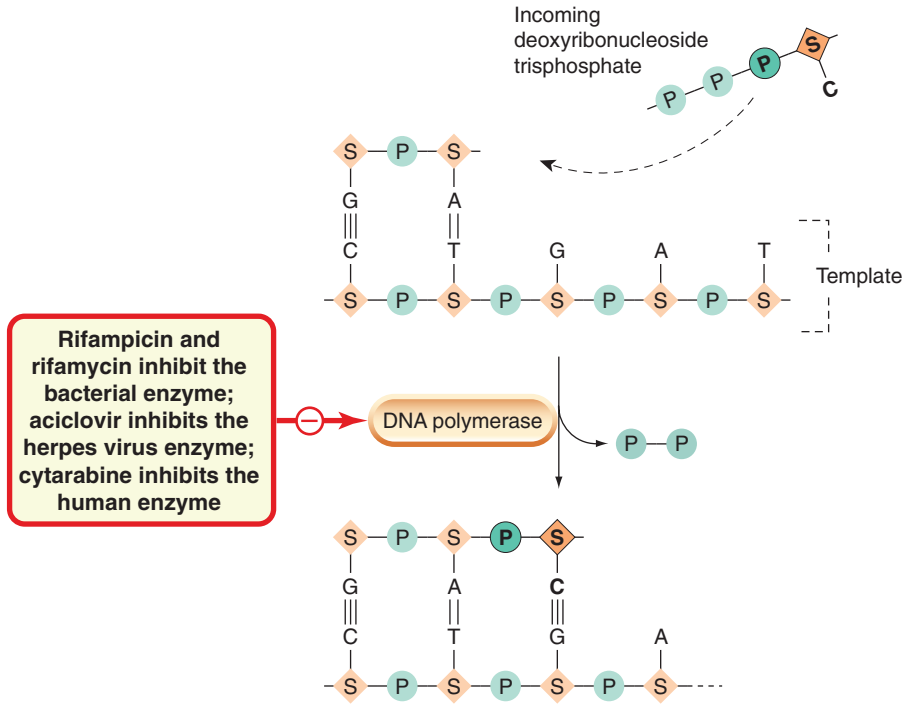


Fig. 49.8 Schematic diagram of DNA replication, showing some antibiotics that inhibit it by acting on DNA polymerase. Nucleotides are added, one at a time, by base pairing to an exposed template strand, and are then covalently joined together in a reaction catalysed by DNA polymerase. The units that pair with the complementary residues in the template consist of a base linked to a sugar and three phosphate groups. Condensation occurs with the elimination of two phosphates. The elements added to the template are shown in darker colours and bold type. A, adenine; C, cytosine; G, guanine; P, phosphate; S, sugar; T, thymine.

Biochemical reactions as potential targets for chemotherapy

- Class I reactions are poor targets.
- Class II reactions are better targets:
  - *folate synthesis* in bacteria is inhibited by sulfonamides
  - *folate utilisation* is inhibited by folate antagonists, for example **trimethoprim** (bacteria), **pyrimethamine** (malarial parasite), **methotrexate** (cancer cells)
  - pyrimidine analogues (e.g. **fluorouracil**) and purine analogues (e.g. **mercaptopurine**) give rise to *fraudulent nucleotides* and are used to treat cancer.
- Class III reactions are important targets:
  - *peptidoglycan synthesis* in bacteria can be selectively inhibited by β-lactam antibiotics (e.g. **penicillin**)
  - *bacterial protein synthesis* can be selectively inhibited by antibiotics that prevent binding of tRNA (e.g. tetracyclines), promote misreading of mRNA (e.g. aminoglycosides), inhibit transpeptidation (e.g. **chloramphenicol**) or inhibit translocation of tRNA from A site to P site (e.g. **erythromycin**)
  - *nucleic acid synthesis* can be inhibited by altering base pairing of DNA template (e.g. the antiviral **vidarabine**), by inhibiting DNA polymerase (e.g. the antivirals **aciclovir** and **foscarnet**) or by inhibiting DNA gyrase (e.g. the antibacterial **ciprofloxacin**).

with the presence of high levels of free fatty acids in the membrane of susceptible organisms (Ch. 52).

INTRACELLULAR ORGANELLES

Microtubules and/or microfilaments

The benzimidazoles (e.g. **albendazole**) exert their anthelminthic action by binding selectively to parasite tubulin and preventing microtubule formation (Ch. 54). The vinca alkaloids **vinblastine** and **vincristine** are anticancer agents that disrupt the functioning of microtubules during cell division (Ch. 55).

Food vacuoles

The erythrocytic form of the malaria plasmodium feeds on host haemoglobin, which is digested by proteases in the parasite food vacuole, the final product, haem, being detoxified by polymerisation. **Chloroquine** exerts its antimalarial action by inhibiting plasmodial haem polymerase (Ch. 53).

MUSCLE FIBRES

Some anthelminthic drugs have a selective action on helminth muscle cells (Ch. 54). **Piperazine** acts as an agonist on parasite-specific chloride channels gated by GABA in nematode muscle, hyperpolarising the muscle fibre membrane and paralysing the worm; **avermectins** increase Cl^- permeability in helminth muscle—possibly by a similar mechanism. **Pyrantel** (now seldom used) and **levamisole** are agonists at nematode acetylcholine nicotinic receptors on muscle, causing contraction followed by paralysis (Ch. 54).

Formed structures of the cell that are targets for chemotherapy

- The plasma membrane is affected by:
  - **amphotericin**, which acts as an ionophore in fungal cells
  - azoles, which inhibit fungal membrane ergosterol synthesis.
- Microtubule function is disrupted by:
  - vinca alkaloids (anticancer drugs)
  - benzimidazoles (anthelminthics).
- Muscle fibres are affected by:
  - avermectins (anthelminthics), which increase Cl^- permeability
  - **pyrantel** (anthelminthic), which stimulates nematode nicotinic receptors, eventually causing muscle paralysis.

RESISTANCE TO ANTIBACTERIAL DRUGS

Since the 1940s, the development of effective and safe drugs to deal with bacterial and other infections has revolutionised medical treatment, and the morbidity and mortality associated with these diseases has been dramatically reduced. Unfortunately, the development of effective antibacterial drugs has been accompanied by the emergence of drug-resistant organisms. This is not unexpected, because the short generation time of many bacterial species affords ample opportunity for evolutionary adaptation. The phenomenon of resistance imposes serious constraints on the options available for the medical treatment of many bacterial infections. Resistance to chemotherapeutic agents can also develop in protozoa, in multicellular parasites (see, for example, Martin & Robertson, 2000; St Georgiev, 2000) and in populations of malignant cells (discussed in Ch. 55). Here, however, we will confine our discussion mainly to the mechanisms of resistance in bacteria.

Antibiotic resistance in bacteria spreads in three ways:

1. by transfer of bacteria between people
2. by transfer of resistance genes between bacteria (usually on plasmids)
3. by transfer of resistance genes between genetic elements within bacteria, on transposons.

Understanding the mechanisms involved in antibiotic resistance is crucial for the sensible clinical use of existing medicines and in the design of new antibacterial drugs. One byproduct of the studies of resistance in bacteria was the development of plasmid-based techniques for DNA cloning, leading to the use of bacteria to produce recombinant proteins for therapeutic use (see Ch. 59).

GENETIC DETERMINANTS OF ANTIBIOTIC RESISTANCE

CHROMOSOMAL DETERMINANTS: MUTATIONS

▼ The spontaneous mutation rate in bacterial populations for any particular gene is very low, and the probability is that approximately only 1 cell in 10 million will, on division, give rise to a daughter cell containing a mutation in that gene. However, as there are likely to be very many more cells than this over the course of an infection, the probability of a mutation causing a change from drug sensitivity to drug resistance can be quite high with some species of bacteria and with some drugs. Fortunately, the presence of a few mutants is not generally sufficient to produce resistance: despite the selective advantage that the resistant mutants possess, the drastic reduction of the population by the antibiotic usually enables the host's natural defences (see Ch. 6) to prevail. However, the outcome may not be quite so happy if the primary infection is caused by a drug-resistant strain.

Resistance resulting from chromosomal mutation is important in some instances, notably infections with **meticillin**-resistant *S. aureus* (MRSA; see below) and in tuberculosis, but apart from these examples this type of resistance is of limited clinical relevance, possibly because the mutants often have reduced pathogenicity.

GENE AMPLIFICATION

▼ Recently it has been discovered that *gene duplication* and *amplification* are important mechanisms for resistance in some organisms (Sandegren & Andersson, 2009). According to this idea, treatment with antibiotics can induce an increased number of copies for pre-existing resistance genes such as antibiotic-destroying enzymes and efflux pumps.

EXTRACHROMOSOMAL DETERMINANTS: PLASMIDS

▼ In addition to the chromosome itself, many species of bacteria contain extrachromosomal genetic elements called *plasmids* that exist free in the cytoplasm. These are also genetic elements that can replicate independently. Structurally, they are closed loops of DNA that may comprise a single gene or as many as 500 or even more. Only a few plasmid copies may exist in the cell but often multiple copies are present, and there may also be more than one type of plasmid in each bacterial cell. Plasmids that carry genes for resistance to antibiotics (*r genes*) are referred to as *R plasmids*. Much of the drug resistance encountered in clinical medicine is plasmid determined. It is not known how these genes arose.

The whole process can occur with frightening speed. *S. aureus*, for example, is a past master of the art of antibiotic resistance. Having become completely resistant to penicillin through plasmid-mediated mechanisms, this organism, within only 1–2 years, was able to adapt to its replacement, meticillin (de Lancastre et al., 2007).

THE TRANSFER OF RESISTANCE GENES BETWEEN GENETIC ELEMENTS WITHIN THE BACTERIUM

Transposons

▼ Some stretches of DNA are readily transferred (transposed) from one plasmid to another and also from plasmid to chromosome or vice versa. This is because integration of these segments of DNA, which are called *transposons*, into the acceptor DNA can occur independently of the normal mechanism of homologous genetic recombination. Unlike plasmids, transposons are not able to replicate independently, although some may replicate during the process of integration (Fig. 49.9), resulting in a copy in both the donor and the acceptor DNA molecules. Transposons may carry one or more resistance genes (see below) and can 'hitch-hike' on a plasmid to a new species of bacterium. Even if the plasmid is unable to replicate in the new host, the transposon may integrate into the new host's chromosome or into its indigenous plasmids. This probably accounts for the widespread distribution of certain of the resistance genes on different R plasmids and among unrelated bacteria.

Gene cassettes and integrons

▼ Plasmids and transposons do not complete the tally of mechanisms that natural selection has provided to confound the hopes of the microbiologist/chemotherapist. Resistance—in fact, *multidrug resistance*—can also be spread by another mobile element, the *gene cassette*, which consists of a resistance gene attached to a small recognition site. Several cassettes may be packaged together in a *multicassette array*, which can, in turn, be integrated into a larger mobile DNA unit termed an *integron*. The integron (which may be located on a

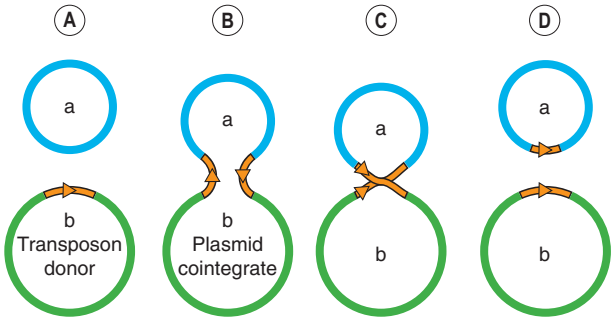


Fig. 49.9 An example of the transfer and replication of a transposon (which may carry genes coding for resistance to antibiotics). [**A**] Two plasmids, a and b, with plasmid b containing a transposon (shown in brown). [**B**] An enzyme encoded by the transposon cuts DNA of both donor plasmid and target plasmid a to form a *cointegrate*. During this process, the transposon replicates. [**C**] An enzyme encoded by the transposon resolves the cointegrate. [**D**] Both plasmids now contain the transposon DNA.

transposon) contains a gene for an enzyme, *integrase* (*recombinase*), which inserts the cassette(s) at unique sites on the integron. This system—transposon/integron/multiresistance cassette array—allows particularly rapid and efficient transfer of multidrug resistance between genetic elements both within and between bacteria.

THE TRANSFER OF RESISTANCE GENES BETWEEN BACTERIA

▼ The transfer of resistance genes between bacteria of the same and indeed of different species is of fundamental importance in the spread of antibiotic resistance. The most important mechanism in this context is *conjugation*. Other gene transfer mechanisms, *transduction* and *transformation*, are of little importance in spreading resistance genes.

Conjugation

▼ Conjugation involves cell-to-cell contact during which chromosomal or extrachromosomal DNA is transferred from one bacterium to another, and is the main mechanism for the spread of resistance. The ability to conjugate is encoded in *conjugative plasmids*; these are plasmids that contain transfer genes that, in coliform bacteria, code for the production by the host bacterium of proteinaceous surface tubules, termed *sex pili*, which connect the two cells. The conjugative plasmid then passes across from one bacterial cell to another (generally of the same species). Many Gram-negative and some Gram-positive bacteria can conjugate. Some *promiscuous plasmids* can cross the species barrier, accepting one host as readily as another. Many R plasmids are conjugative. Non-conjugative plasmids, if they co-exist in a 'donor' cell with conjugative plasmids, can hitch-hike from one bacterium to the other with the conjugative plasmids. The transfer of resistance by conjugation is significant in populations of bacteria that are normally found at high densities, as in the gut.

Transduction

▼ *Transduction* is a process by which plasmid DNA is enclosed in a bacterial virus (or *phage*) and transferred to another bacterium of the same species. It is a relatively ineffective means of transfer of genetic material but is clinically important in the transmission of resistance genes between strains of staphylococci and of streptococci.

Transformation

▼ A few species of bacteria can, under natural conditions, undergo *transformation* by taking up DNA from the environment and incorporating it into the genome by normal homologous recombination. Transformation is probably not of importance clinically.

Resistance to antibiotics

- Drug resistance in bacterial populations can be spread from person to person by bacteria, from bacterium to bacterium by plasmids and from plasmid to plasmid (or chromosome) by transposons.
- Plasmids are extrachromosomal genetic elements that can replicate independently and can carry genes coding for resistance to antibiotics (r genes).
- The main method of transfer of r genes from one bacterium to another is by conjugative plasmids. The bacterium forms a connecting tube with other bacteria through which the plasmids pass.
- A less common method of transfer is by transduction, i.e. the transmission by a bacterial virus (phage) of a plasmid bearing an r gene into another bacterium.
- Transposons are stretches of DNA that can be transposed from one plasmid to another, from a plasmid to a chromosome or vice versa. A plasmid containing an r gene-bearing transposon may code for enzymes that cause the plasmid to be integrated with another. Following their separation, this transposon replicates so that both plasmids then contain the r gene.

BIOCHEMICAL MECHANISMS OF RESISTANCE TO ANTIBIOTICS

THE PRODUCTION OF AN ENZYME THAT INACTIVATES THE DRUG

Inactivation of β-lactam antibiotics

The most important example of resistance caused by inactivation is that of the *β-lactam antibiotics*. The enzymes concerned are *β-lactamases*, which cleave the β-lactam ring of penicillins and **cephalosporins** (see Ch. 50). Cross-resistance between the two classes of antibiotic is not complete, because some β-lactamases have a preference for penicillins and some for cephalosporins.

▼ *Staphylococci* are the principal bacterial species producing β-lactamase, and the genes coding for the enzymes are on plasmids that can be transferred by transduction. In staphylococci, the enzyme is inducible (i.e. its synthesis is not expressed in the absence of the drug) and minute, subinhibitory, concentrations of antibiotics de-repress the gene and result in a 50- to 80-fold increase in expression. The enzyme passes through the bacterial envelope and inactivates antibiotic molecules in the surrounding medium. The grave clinical problem posed by resistant staphylococci secreting β-lactamase was tackled by developing semisynthetic penicillins (such as meticillin) and new β-lactam antibiotics (the **monobactams** and **carbapenems**), and cephalosporins (such as **cephamandole**), that are less susceptible to inactivation. The growing problem of MRSA is discussed below.

Gram-negative organisms can also produce β-lactamases, and this is a significant factor in their resistance to the semisynthetic broad-spectrum β-lactam antibiotics. In these organisms, the enzymes may be coded by either chromosomal or plasmid genes. In the former case, the enzymes may be inducible, but in the latter they are produced constitutively. When this occurs, the enzyme does not inactivate the drug in the surrounding medium but instead remains attached to the cell wall, preventing access of the

drug to membrane-associated target sites. Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics.

Inactivation of chloramphenicol

Chloramphenicol is inactivated by *chloramphenicol acetyltransferase*, an enzyme produced by resistant strains of both Gram-positive and Gram-negative organisms, the resistance gene being plasmid borne. In Gram-negative bacteria, the enzyme is produced constitutively, resulting in levels of resistance five-fold higher than in Gram-positive bacteria, in which the enzyme is inducible.

Inactivation of aminoglycosides

Aminoglycosides are inactivated by phosphorylation, adenylation or acetylation, and the requisite enzymes are found in both Gram-negative and Gram-positive organisms. The resistance genes are carried on plasmids, and several are found on transposons.

Many other examples of this kind are given by Wright (2005).

ALTERATION OF DRUG-SENSITIVE OR DRUG-BINDING SITE

The aminoglycoside-binding site on the 30S subunit of the ribosome may be altered by chromosomal mutation. A plasmid-mediated alteration of the binding site protein on the 50S subunit also underlies resistance to **erythromycin**, and decreased binding of fluoroquinolones because of a point mutation in DNA gyrase A has recently been described. An altered DNA-dependent RNA polymerase determined by a chromosomal mutation is reported to be the basis for **rifampicin** resistance.

In addition to acquiring resistance to β-lactams susceptible to β-lactamase, some strains of *S. aureus* have even become resistant to some antibiotics that are not significantly inactivated by β-lactamase (e.g. meticillin), because they express an additional β-lactam-binding protein coded for by a mutated chromosomal gene.

See Lambert (2005) for other examples of this type of action.

DECREASED DRUG ACCUMULATION IN THE BACTERIUM

An important example of decreased drug accumulation is the plasmid-mediated resistance to **tetracyclines** encountered in both Gram-positive and Gram-negative bacteria. In this case, resistance genes in the plasmid code for inducible proteins in the bacterial membrane, which promote energy-dependent efflux of the tetracyclines, and hence resistance. This type of resistance is common and has greatly reduced the therapeutic value of the tetracyclines in human and veterinary medicine. Resistance of *S. aureus* to erythromycin and the other macrolides, and to fluoroquinolones, is also brought about by energy-dependent efflux. Inhibitors of such pumps may be useful adjuncts to antibiotics (Van Bambeke et al., 2006).

There is also recent evidence of plasmid-determined inhibition of *porin* synthesis, which could affect those hydrophilic antibiotics that enter the bacterium through these water-filled channels in the outer membrane. Altered permeability as a result of chromosomal mutations involving the polysaccharide components of the outer membrane of Gram-negative organisms may confer enhanced resistance to **ampicillin**. Mutations affecting envelope components have been reported to affect the accumulation of aminoglycosides, β-lactams, chloramphenicol, peptide antibiotics and tetracycline.

Biochemical mechanisms of resistance to antibiotics

The principal mechanisms are as follow:

- *Production of enzymes that inactivate the drug*: for example, β-lactamases, which inactivate **penicillin**; acetyltransferases, which inactivate **chloramphenicol**; kinases and other enzymes, which inactivate aminoglycosides.
- *Alteration of the drug-binding sites*: this occurs with aminoglycosides, **erythromycin**, penicillin.
- *Reduction of drug uptake by the bacterium*: for example, tetracyclines.
- *Alteration of enzyme pathways*: for example, dihydrofolate reductase becomes insensitive to **trimethoprim**.

THE DEVELOPMENT OF A PATHWAY THAT BYPASSES THE REACTION INHIBITED BY THE ANTIBIOTIC

Resistance to trimethoprim is the result of plasmid-directed synthesis of a *dihydrofolate reductase* with low or zero affinity for trimethoprim. It is transferred by transduction and may be spread by transposons.

Sulfonamide resistance in many bacteria is plasmid mediated and results from the production of a form of *dihydropteroate synthetase* with a low affinity for sulfonamides but no change in affinity for PABA. Bacteria causing serious infections have been found to carry plasmids with resistance genes to both sulfonamides and trimethoprim.

CURRENT STATUS OF ANTIBIOTIC RESISTANCE IN BACTERIA

The most disturbing development of resistance has been in staphylococci, one of the commonest causes of hospital bloodstream infections, many strains of which are now resistant to almost all currently available antibiotics (de Lencastre et al., 2007). In addition to resistance to some β-lactams through production of β-lactamase and the production of an additional β-lactam-binding protein that also renders them resistant to meticillin, *S. aureus* may also manifest resistance to other antibiotics as follows:

- to **streptomycin** (because of chromosomally determined alterations of target site)
- to aminoglycosides in general (because of altered target site and plasmid-determined inactivating enzymes)
- to **chloramphenicol** and the macrolides (because of plasmid-determined enzymes)
- to **trimethoprim** (because of transposon-encoded drug-resistant dihydrofolate reductase)
- to sulfonamides (because of chromosomally determined increased production of PABA)
- to **rifampicin** (because of chromosomally and plasmid determined increases in efflux of the drug)

- to **fusidic acid** (because of chromosomally determined decreased affinity of the target site or a plasmid-encoded decreased permeability to the drug)
- to quinolones, for example **ciprofloxacin** and **norfloxacin** (because of chromosomally determined reduced uptake).

Infections with MRSA have become a major problem, particularly in hospitals, where they can spread rapidly among elderly and/or seriously ill patients, and patients with burns or wounds. Until recently, the glycopeptide **vancomycin** was the antibiotic of last resort against MRSA but, ominously, strains of MRSA showing decreased susceptibility to this drug were isolated from hospitalised patients in the USA and Japan in 1997[5] and, more recently, in the community. MRSA infections are rising; Bax et al. (2000) report prevalence in US hospitals as rising from 11–13% in 1985/6 to 26% in 1998.

The fact that vancomycin resistance seems to have developed spontaneously could have major clinical implications — and not only for *nosocomial* (those contracted in hospital) MRSA infections. It had been thought that antibiotic-resistant bacteria were dangerous only to seriously ill, hospitalised patients, in that the genetic burden of multiple resistance genes would lead to reduced virulence. Distressingly, however, there is now evidence that the spectrum and frequency of disease produced by meticillin-susceptible and meticillin-resistant staphylococci are similar.

▼ In the past few years, *enterococci* have been rapidly developing resistance to many chemotherapeutic agents and have emerged as the second most common nosocomial pathogen. Non-pathogenic enterococci are ubiquitous in the intestine, have intrinsic resistance to many antibacterial drugs, and can readily become resistant to other agents by taking up plasmids and transposons carrying the relevant resistance genes. Such resistance is easily transferred to invading pathogenic enterococci.

Enterococci, already multiresistant, have recently developed resistance to vancomycin. This is apparently achieved by substitution of D-Ala-D-Ala with D-Ala-D-lactate in the peptide chain attached to *N*-acetylglucosamine-*N*-acetylmuramic acid (G-M) during the first steps of peptidoglycan synthesis (see Fig. 49.3; Ch. 50). This is becoming a major problem in hospitalised patients, and in the USA vancomycin resisitance has increased from 0.5% to 18% in less than a decade (Bax et al., 2000). A particular concern is the possibility of transfer of vancomycin resistance from enterococci to staphylococci, because they can co-exist in the same patient.

Many other pathogens are developing or have developed resistance to commonly used drugs. This list includes *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *N. gonorrhoeae*, *Haemophilius influenzae* and *H. ducreyi*, as well as *Mycobacterium*, *Campylobacter* and *Bacteroides* species. Some strains of *M. tuberculosis* are now able to evade every antibiotic in the clinician's armamentarium, and tuberculosis, once easily treatable, is now reported to be causing more deaths worldwide than malaria and AIDS together. Fortunately, some glycopeptide and other antibiotics (e.g. **teicoplanin**, **daptomycin** and **linezolide** see Ch. 50) that are used to treat resistant Gram-positive strains have largely maintained their potency. Even so, there is a danger of resistance arising if they are wrongly utilised.

[5]Noble et al. (1992) have reported transfer of vancomycin resistance from enterococci to staphylococci. If this occurred in a clinical environment, it would be disastrous. Some microbiologists have suggested that Noble and his team should be autoclaved.

Multidrug resistance

Many pathogenic bacteria have developed resistance to the commonly used antibiotics. Examples include the following:

- Some strains of staphylococci and enterococci that are resistant to virtually all current antibiotics, the resistance being transferred by transposons and/or plasmids; such organisms can cause serious and virtually untreatable nosocomial infections.
- Some strains of *Mycobacterium tuberculosis* that have become resistant to most antituberculosis agents.

Prescribers and consumers must also bear a responsibility for the burgeoning problem of resistance. Indiscriminate use of antibiotics in human and veterinary medicine, and their use in animal foodstuffs, has undoubtedly encouraged the growth of resistant strains. Some governmental and regulatory bodies (e.g. the European Union) have devised political and social measures to curb such excesses, and these have been at least partly successful.

The issue around declining antibiotic efficacy is, however, not solely to do with bacterial countermeasures. There has been a declining interest in the pharmaceutical industry in researching novel antibiotics. Historically, the area has been one of the mainstays of the industry, but most of the drugs available today are the result of incremental changes in the structures of a relatively small number of basic molecular structures, such as the β-lactam nucleus. By common consent, the days when it was possible to discover new and effective drugs in this way are long gone.

Hubris has also played a part. In 1967, the US Surgeon General effectively announced that infectious diseases had been vanquished, and that the researchers should turn their attention to chronic diseases instead. As a result, many pharmaceutical companies scaled down their efforts in the area, and only in the past few years has activity been resumed as the pressing need for novel compounds has been recognised (Barrett & Barrett, 2003).

However, nature has endowed microorganisms with fiendishly effective adaptive mechanisms for outwitting our best therapeutic strategies, and so far several have been effortlessly keeping pace with our attempts to eradicate them. This challenging situation has been reviewed in depth by Shlaes (2003) and Barrett & Barrett (2003).

REFERENCES AND FURTHER READING

General reading

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Knodler, L.A., Celli, J., Finlay, B.B., 2001. Pathogenic trickery: deception of host cell processes. Mol. Cell. Biol. 2, 578–588. (*Discusses bacterial ploys to subvert or block normal host cellular processes: mimicking the ligands for host cell receptors or signalling pathways. Useful list of examples*)

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clamp technique to study ion channels in resistant and non-resistant nematode tissue)

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Drug resistance

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Wright, G.D., 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv. Drug Deliv. Rev. 57, 1451–1470. (*This comprehensive review gives details of the many pathways that have evolved in bacteria to destroy antibiotics. A little complex but fascinating reading*)

50 Antibacterial drugs

OVERVIEW

In this chapter, we continue to develop the ideas we introduced in the previous chapter. A detailed discussion of the bacteria of medical importance is beyond the scope of this book and the reader is referred to more specialist texts. However, information about some clinically significant pathogens is provided in Table 50.1 and an overview of the principal antibiotic 'families', together with their mechanisms of action, is given in Table 50.2. The major classes of antibacterial drugs are described, along with their mechanism of action, relevant pharmacokinetic properties and side effects.

INTRODUCTION

In 1928, Alexander Fleming, working at St Mary's Hospital in London, observed that a culture plate on which staphylococci were being grown had become contaminated with a mould of the genus *Penicillium*, and that bacterial growth in the vicinity of the mould had been inhibited. He isolated the mould in pure culture and demonstrated that it produced an antibacterial substance, which he called **penicillin**. This substance was subsequently prepared in bulk, extracted and its antibacterial effects analysed by Florey, Chain and their colleagues at Oxford in 1940. They showed that it had powerful chemotherapeutic properties in infected mice, and that it was non-toxic, thus ushering in the 'antibiotic era'. Seventy years later, the number of different types of antibiotics has grown 10-fold and the practice of medicine would be unthinkable without them.

Many organisms can be classified as being either *Gram-positive* or *Gram-negative* depending on whether or not they stain with *Gram's stain*.[1] This is not merely a taxonomic device, as it reflects several fundamental differences in (for example) the structure of their cell walls, and this in turn has implications for the action of antibiotics.

The cell wall of Gram-positive organisms is a relatively simple structure, 15–50 nm thick. It comprises about 50% peptidoglycan (see Ch. 49), 40–45% acidic polymer (which results in the cell surface being highly polar and carrying a negative charge) and 5–10% proteins and polysaccharides. The strongly polar polymer layer influences the penetration of ionised molecules and favours the penetration into the cell of positively charged compounds such as **streptomycin**.

The cell wall of Gram-negative organisms is much more complex. From the plasma membrane outwards, it consists of the following:

- A *periplasmic space* containing enzymes and other components.
- A *peptidoglycan layer* 2 nm in thickness, forming 5% of the cell wall mass, that is often linked to outwardly projecting lipoprotein molecules.
- An *outer membrane* consisting of a lipid bilayer, similar in some respects to the plasma membrane, that contains protein molecules and (on its inner aspect) lipoproteins linked to the peptidoglycan. Other proteins form transmembrane water-filled channels, termed *porins*, through which hydrophilic antibiotics can move freely.
- *Complex polysaccharides* forming important components of the outer surface. These differ between strains of bacteria and are the main determinants of their antigenicity. They are the source of *endotoxin*, which, in vivo, triggers various aspects of the inflammatory reaction by activating complement, causing fever, etc. (see Ch. 17).

Difficulty in penetrating this complex outer layer is probably the reason why some antibiotics are less active against Gram-negative than Gram-positive bacteria. This is one reason for the extraordinary antibiotic resistance exhibited by *Pseudomonas aeruginosa*, a pathogen that can cause life-threatening infections in neutropenic patients and those with burns and wounds.

The cell wall lipopolysaccharide is also a major barrier to penetration. Antibiotics affected include **benzylpenicillin** (**penicillin G**), **meticillin**, the macrolides, **rifampicin** (**rifampin**), **fusidic acid**, **vancomycin**, **bacitracin** and **novobiocin**.

ANTIMICROBIAL AGENTS THAT INTERFERE WITH FOLATE SYNTHESIS OR ACTION

SULFONAMIDES

In a landmark discovery in the 1930s, Domagk demonstrated that it was possible for a drug to influence the course of a bacterial infection. The agent was **prontosil**, a dye that proved to be an inactive prodrug but which is metabolised in vivo to give the active product, **sulfanilamide** (Fig. 50.1). Many sulfonamides have been developed since, but their importance has declined in the face of increasing resistance. The only drugs still commonly used are **sulfamethoxazole** (usually in combination with **trimethoprim** as **co-trimoxazole**), **sulfasalazine** (poorly absorbed in the gastrointestinal tract, used to treat ulcerative colitis and Crohn's disease; see Chs 26 and 29) and occasionally **sufadiazine**.

Mechanism of action

Sulfanilamide is a structural analogue of *p*-aminobenzoic acid (PABA; see Fig. 50.1), which is an essential precursor in the synthesis of folic acid, required for the synthesis of DNA and RNA in bacteria (see Ch. 49). Sulfonamides compete with PABA for the enzyme *dihydropteroate synthetase*, and the effect of the sulfonamide may be overcome

[1]Named after the Danish physician who devised the technique.

Table 50.1 Some clinically significant pathogenic bacteria

Genus	Morphology	Species	Disease
Gram-negative			
Bordetella	Cocci	*B. pertussis*	Whooping cough
Brucella	Curved rods	*B. abortus*	Brucellosis (cattle and humans)
Campylobacter	Spiral rods	*C. jejuni*	Food poisoning
Escherichia	Rods	*E. coli*	Septicaemia, wound infections, UTIs
Haemophilus	Rods	*H. influenzae*	Acute respiratory tract infection, meningitis
Helicobacter	Motile rods	*H. pylori*	Peptic ulcers, gastric cancer
Klebsiella	Capsulated rods	*K. pneumoniae*	Pneumonia, septicaemia
Legionella	Flagellated rods	*L. pneumophila*	Legionnaires' disease
Neisseria	Cocci, paired	*N. gonorrhea*	Gonorrhoea
Pseudomonas	Flagellated rods	*P. aeruginosa*	Septicaemia, respiratory infections, UTIs
Rickettsiae	Cocci or threads	*Several spp.*	Tick- and insect-borne infections
Salmonella	Motile rods	*S. typhimurium*	Food poisoning
Shigella	Rods	*S. dysenteriae*	Bacillary dysentry
Yersinia	Rods	*Y. pestis*	Bubonic plague
Vibrio	Flagellated rods	*V. cholerae*	Cholera
Gram-positive			
Bacillus	Rods, chains	*B. anthrax*	Anthrax
Clostridium	Rods	*Cl. tetani*	Tetanus
Corynebacterium	Rod	*C. diphtheriae*	Diphtheria
Mycobacterium	Rods	*M. tuberculosis* *M. leprae*	Tuberculosis Leprosy
Staphylococcus	Cocci, clusters	*Staph. aureus*	Wound infections, boils, septicaemia
Streptococcus	Cocci, pairs Cocci, chains	*Strept. pneumoniae* *Strept. pyogenes*	Pneumonia, meningitis Scarlet fever, rheumatic fever, cellulitis
Other			
Chlamydia	Gram 'uncertain'	*C. trachomatis*	Eye disease, infertility
Treponema	Flagellated spiral rods	*T. pallidum*	Syphillis

UTI, urinary tract infection.

by adding excess PABA. This is why some local anaesthetics, which are PABA esters (such as **procaine**; see Ch. 42), can antagonise the antibacterial effect of these agents.

▼ While not necessarily clinically relevant, the action of a sulfonamide is to inhibit *growth* of the bacteria, not to kill them; that is to say, it is *bacteriostatic* rather than *bactericidal*. The action is vitiated in the presence of pus or products of tissue breakdown, because these contain thymidine and purines, which bacteria utilise directly, bypassing the requirement for folic acid. Resistance to the drugs, which is common, is plasmid mediated (see Ch. 49) and results from the synthesis of a bacterial enzyme insensitive to the drug.

Pharmacokinetic aspects

Most sulfonamides are given orally and, apart from sulfasalazine, are well absorbed and widely distributed in the body. There is a risk of sensitisation or allergic reactions when these drugs are given topically.

The drugs pass into inflammatory exudates and cross both placental and blood–brain barriers. They are metabolised mainly in the liver, the major product being an acetylated derivative that lacks antibacterial action.

Table 50.2 A general overview of antibacterials and their mechanism of action

Family/class	Examples	Main organisms	Major cellular target
Sulphonamides	Sulfadiazine, sulfamethoxazole, trimethoprim	*T. gondii, P. jirovecii*	Bacterial folate synthesis or action
β-Lactams			
Penicillins	Benzylpenicillin, phenoxymethylpenicillin	Overall, mainly Gram-positive spp.; some Gram-negative spp.	Bacterial cell wall peptidoglycan synthesis
Penicillinase resistant	Flucloxacillin, temocillin	Used for staphylococcal infections	
Broad-spectrum penicillins	Amoxicillin, ampicillin	A wide range of Gram-positive and Gram-negative spp.	
Antipseudomonal penicillins	Piperacillin, ticarcillin	Selected Gram-negative spp., especially *P. aeruginosa*	
Mecillinams	Pivmecillinam	Mainly Gram-negative spp.	
Cephalosporins	Cefalcor, cefadroxil, cefalexin, cefixime, cefotaxime, cefpodoxime, cefradine, ceftazidime, ceftriaxone, cefuroxime	Broad spectrum of activity against Gram-negative and positive spp.	
Carbapenems and monobactams	Ertapenem, impenem, meropenem Aztreonam	Many Gram-negative and positive spp. Gram-negative rods	
Glycopeptides	Vancomycin, teicoplanin, (daptomycin)	Gram-positive spp.	
Polymixins	Colistin, polymixin B	Gram-negative spp.	Bacterial outer cell membrane structure
Tetracyclines	Tetracycline, demeclocycline, doycycline, lymecycline, minocycline, oxytetracycline (tigecycline)	Many Gram-negative and Gram-positive spp.	Bacterial protein synthesis (multiple mechanisms inhibited including initiation, transpeptidation and translocation; see text)
Aminogycosides	Gentamicin, amikacin, neomycin, tobramycin	Many Gram-negative, some Gram-positive spp.	
Macrolides	Erythromycin, azithromycin, clarithromycin, telithromycin	Similar to penicillin	
Oxazolidinones	Linezolid	Gram-positive spp.	
Lincosamides	Clindamycin	Gram-positive spp.	
Amphenicols	Chloramphenicol	Gram-negative and Gram-positive spp.	
Streptogramins	Quinupristin, dalfopristin	Gram-positive spp.	
Antimycobacterials	Capreomycin, cycloserine, ethambutol, isoniazid, pyrazinamide, rifabutin, rifampicin, dapsone, clofazimine	Most used for mycobacterial infections only	Various unrelated mechanisms (see text)
Quinolones	Ciprofloxacin, levofloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin	Gram-negative and Gram-positive spp.	Bacterial DNA synthesis
Miscellaneous	Fusidic acid Nitrofurantoin Methenamine	Gram-positive spp. Gram-negative UTIs Gram-negative UTIs	Bacterial protein synthesis Damages bacterial DNA Formaldehyde prodrug

Unwanted effects

Serious adverse effects necessitating cessation of therapy include hepatitis, hypersensitivity reactions (rashes including Stevens–Johnson syndrome and toxic epidermal necrolysis, fever, anaphylactoid reactions—see Ch. 57), bone marrow depression and acute renal failure due to interstitial nephritis or crystalluria. This last effect results from the precipitation of acetylated metabolites in the urine (Ch. 28). Cyanosis caused by methaemoglobinaemia may occur but is a lot less alarming than it looks. Mild to moderate side effects include nausea and vomiting, headache and mental depression.

Clinical uses of sulfonamides

- Combined with **trimethoprim** (**co-trimoxazole**) for *Pneumocystis carinii* (now known as *P. jirovecii*).
- Combined with **pyrimethamine** for drug-resistant *malaria* (Table 53.1) and for *toxoplasmosis*.
- In *inflammatory bowel disease*: **sulfasalazine** (sulfapyridine–aminosalicylate combination) is used (see Ch. 26).
- For infected *burns* (**silver sulfadiazine** given topically).
- For some sexually transmitted infections (e.g. *trachoma*, *chlamydia*, *chancroid*).
- For *respiratory infections*: use now confined to a few special problems (e.g. infection with *Nocardia*).
- For acute *urinary tract infection* (now seldom used).

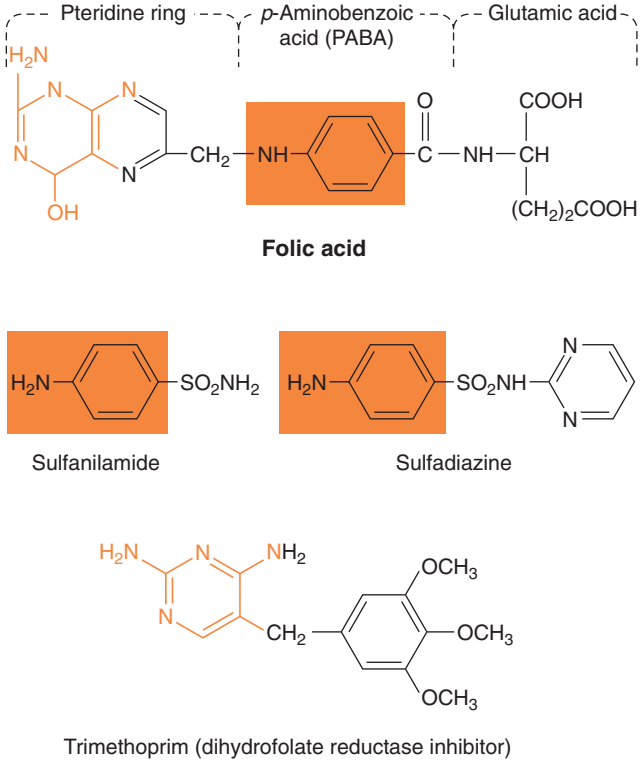

Fig. 50.1 Structures of two representative sulfonamides and trimethoprim. The structures illustrate the relationship between the sulfonamides and the *p*-aminobenzoic acid moiety in folic acid (orange box), as well as the possible relationship between the antifolate drugs and the pteridine moiety (orange). Co-trimoxazole is a mixture of sulfamethoxazole and trimethoprim.

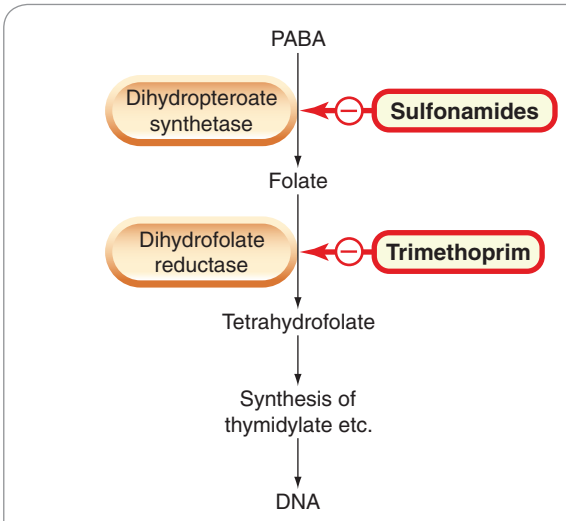

Fig. 50.2 The action of sulfonamides and trimethoprim on bacterial folate synthesis. See Figure 25.2 for more detail of tetrahydrofolate synthesis, and Table 49.1 for comparisons of antifolate drugs. PABA, *p*-aminobenzoic acid.

TRIMETHOPRIM

Mechanism of action

Trimethoprim is chemically related to the antimalarial drug **pyrimethamine** (Fig. 53.3), both being folate antagonists. Structurally (Fig. 50.1), it resembles the pteridine moiety of folate and the similarity is close enough to fool the bacterial dihydrofolate reductase, which is many times more sensitive to trimethoprim than the equivalent enzyme in humans.

Trimethoprim is active against most common bacterial pathogens as well as protozoa, and it too is bacteriostatic. It is sometimes given as a mixture with sulfamethoxazole as co-trimoxazole (Fig. 50.1). Because sulfonamides inhibit the same bacterial metabolic pathway, but upstream from dihydrofolate reductase, they can potentiate the action of trimethoprim (see Fig. 50.2). In the UK, its use is generally restricted to the treatment of *Pneumocystis carinii* (now known as *P. jirovecii*) pneumonia (a fungal infection), toxoplasmosis (a protozoan infection) as well as nocardiasis (a bacterial infection).

Pharmacokinetic aspects

Trimethoprim is well absorbed orally, and widely distributed throughout the tissues and body fluids. It reaches high concentrations in the lungs and kidneys, and fairly high concentrations in the cerebrospinal fluid (CSF). When given with sulfamethoxazole, about half the dose of each is excreted within 24 h. Because trimethoprim is a weak base, its elimination by the kidney increases with decreasing urinary pH.

Unwanted effects

Folate deficiency, with resultant *megaloblastic anaemia* (see Ch. 25)—a toxic effect related to the pharmacological action of trimethoprim, can be prevented by giving folinic acid. Other unwanted effects include nausea, vomiting, blood disorders and rashes.

β-LACTAM ANTIBIOTICS

PENICILLINS

The remarkable antibacterial effects of penicillin in humans were clearly demonstrated in 1941. A small amount of penicillin, extracted laboriously from crude cultures in the laboratories of the Dunn School of Pathology in Oxford,

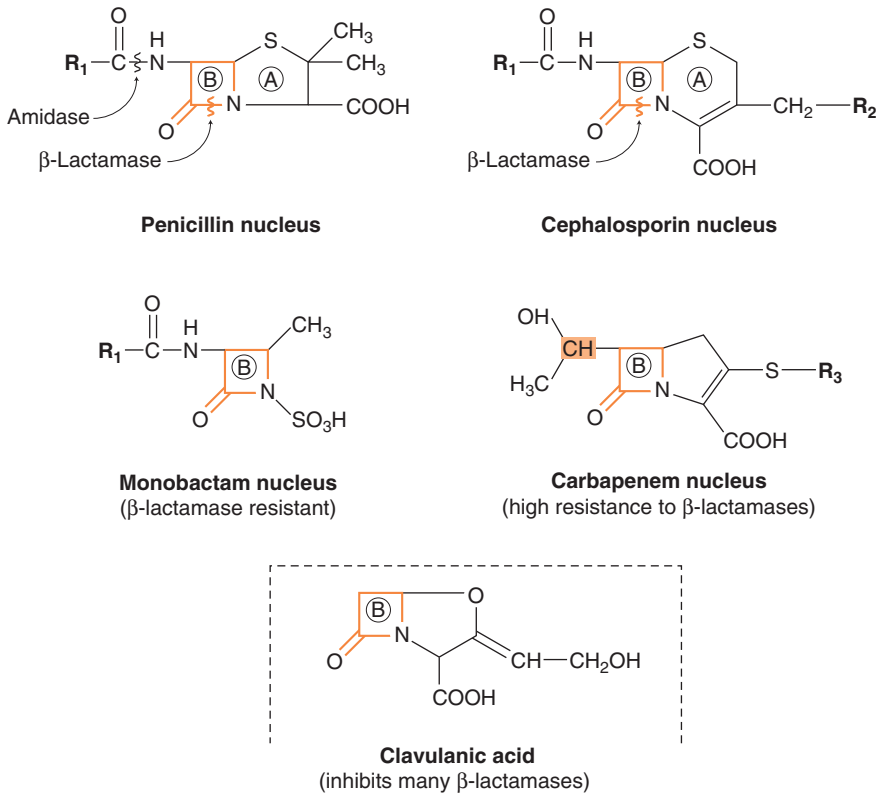

Fig. 50.3 Basic structures of four groups of β-lactam antibiotics and clavulanic acid. The structures illustrate the β-lactam ring (marked B) and the sites of action of bacterial enzymes that inactivate these antibiotics (A, thiazolidine ring). Various substituents are added at R_1, R_2 and R_3 to produce agents with different properties. In carbapenems, the stereochemical configuration of the part of the β-lactam ring shown shaded in orange here is different from the corresponding part of the penicillin and cephalosporin molecules; this is probably the basis of the β-lactamase resistance of the carbapenems. The β-lactam ring of clavulanic acid is thought to bind strongly to β-lactamase, meanwhile protecting other β-lactams from the enzyme.

Antimicrobial agents that interfere with the synthesis or action of folate

- Sulfonamides are bacteriostatic; they act by interfering with folate synthesis and thus with nucleotide synthesis. Unwanted effects include crystalluria and hypersensitivities.
- **Trimethoprim** is bacteriostatic. It acts by antagonising folate.
- **Co-trimoxazole** is a mixture of trimethoprim with **sulfamethoxazole**, which affects bacterial nucleotide synthesis at two points in the pathway.
- Pyrimethamine and proguanil are antimalarial agents (see Ch. 53).

Clinical uses of trimethoprim/co-trimoxazole

- For *urinary tract* and *respiratory infections*: **trimethoprim**, used on its own, is usually preferred.
- For infection with *Pneumocystis carinii* (now known as *P. jirovecii*), which causes *pneumonia* in patients with *AIDS*: **co-trimoxazole** is used in high dose.

was given to a desperately ill policeman who had septicaemia with multiple abscesses. Although sulfonamides were available, they would have had no effect in the presence of pus. Intravenous injections of penicillin were given every 3 h. All the patient's urine was collected, and each day the bulk of the excreted penicillin was extracted and reused. After 5 days, the patient's condition was vastly improved, and there was obvious resolution of the abscesses. Furthermore, there seemed to be no toxic effects of the drug.[2]

[2]Although this was the first evidence of the dramatic antibacterial effect of penicillin when given systemically in humans, topical penicillin had actually been used with success in five patients with eye infections 10 years previously by Paine, a graduate of St Mary's who had obtained some penicillin mould from Fleming.

Unfortunately, when the supply of penicillin was finally exhausted his condition gradually deteriorated and he died a month later.

While the penicillins are extremely effective antibiotics and are very widely used, they can be destroyed by bacterial *amidases* and *β-lactamases* (*penicillinases*; see Fig. 50.3). This forms the basis of one of the principal types of antibiotic resistance. Penicillins, often combined with other antibiotics, remain crucially important in antibacterial chemotherapy, and are the drugs of choice for many infections. A list of clinical uses is given in the clinical box.

Mechanisms of action

All β-lactam antibiotics interfere with the synthesis of the bacterial cell wall peptidoglycan (see Ch. 49, Fig. 49.3). After attachment to *penicillin-binding proteins* on bacteria (there may be seven or more types in different organisms), they inhibit the transpeptidation enzyme that crosslinks the peptide chains attached to the backbone of the peptidoglycan.

The final bactericidal event is the inactivation of an inhibitor of autolytic enzymes in the cell wall, leading to lysis of the bacterium. Some organisms, referred to as 'tolerant', have defective autolytic enzymes and are inhibited but not lysed in the presence of the drug. Resistance to penicillin may result from a number of different causes and is discussed in detail in Chapter 49.

Types of penicillin and their antimicrobial activity

The first penicillins were the naturally occurring benzylpenicillin (**penicillin G**) and its congeners, including **phenoxymethylpenicillin** (**penicillin V**). Benzylpenicillin is active against a wide range of organisms and is the drug of first choice for many infections (see clinical box). Its main drawbacks are poor absorption in the gastrointestinal tract (which means it must be given by injection) and its susceptibility to bacterial β-lactamases.

Semisynthetic penicillins, incorporating different side-chains attached to the penicillin nucleus (at R_1 in Fig. 50.3), include *β-lactamase-resistant* penicillins (e.g. **meticillin**,[3] **flucloxacillin**, **temocillin**) and *broad-spectrum* penicillins (e.g. **ampicillin**, **amoxicillin**). *Extended-spectrum* penicillins (e.g. **ticarcillin**, **piperacillin**) with antipseudomonal activity have gone some way to overcoming the problem of serious infections caused by *P. aeruginosa*. Amoxicillin and ticarcillin are sometimes given in combination with the β-lactamase inhibitor **clavulanic acid** (e.g. **co-amoxiclav**). **Pivmecillinam** is a prodrug of **mecillinam**, which also has a wide spectrum of action.

Pharmacokinetic aspects

Oral absorption of penicillins varies, depending on their stability in acid and their adsorption to foodstuffs in the gut. Penicillins can also be given by intravenous injection. Preparations for intramuscular injection are also available, including slow-release preparations such as benzathine penicillin (cf. long-lived preparations of insulin, Ch. 30). Benzathine penicillin may be useful in treating syphilis since *Treponema pallidum* is a very slowly dividing organism. Intrathecal administration (used historically to treat meningitis) is no longer used, as it can cause convulsions.[4]

The penicillins are widely distributed in body fluids, passing into joints; into pleural and pericardial cavities; into bile, saliva and milk; and across the placenta. Being lipid insoluble, they do not enter mammalian cells, and cross the blood–brain barrier only if the meninges are inflamed, when they reach therapeutically effective concentrations in the CSF.

Elimination of most penicillins occurs rapidly and is mainly renal, 90% being through tubular secretion. The relatively short plasma half-life is a potential problem in the clinical use of benzylpenicillin, although because penicillin works by preventing cell wall synthesis in dividing organisms, intermittent rather than continuous exposure to the drug can be an advantage.

[3]Meticillin (previous name: methicillin) was the first β-lactamase-resistant penicillin; it is now not used clinically because it was particularly associated with interstitial nephritis but is remembered in the acronym 'MRSA'—meticillin-resistant *Staphylococcus aureus*.

[4]Indeed, penicillins applied topically to the cortex are used to induce convulsions in an experimental setting.

Clinical uses of the penicillins

- Penicillins are given by mouth or, in more severe infections, intravenously, and often in combination with other antibiotics.
- Uses are for sensitive organisms and may (but may not: individual sensitivity testing is often appropriate depending on local conditions—see below) include:
  - *bacterial meningitis* (e.g. caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*): **benzylpenicillin**, high doses intravenously
  - *bone* and *joint infections* (e.g. with *Staphylococcus aureus*): **flucloxacillin**
  - *skin* and *soft tissue infections* (e.g. with *Strep. pyogenes* or *Staph. aureus*): **benzylpenicillin**, **flucloxacillin**; animal bites: **co-amoxiclav**
  - *pharyngitis* (from *Strep. pyogenes*): **phenoxylmethylpenicillin**
  - *otitis media* (organisms commonly include *Strep. pyogenes*, *Haemophilus influenzae*): **amoxicillin**
  - *bronchitis* (mixed infections common): **amoxicillin**
  - *pneumonia*: **amoxicillin**
  - *urinary tract infections* (e.g. with *Escherichia coli*): **amoxicillin**
  - *gonorrhea*: **amoxicillin** (plus **probenecid**)
  - *syphilis*: **procaine benzylpenicillin**
  - *endocarditis* (e.g. with *Strep. viridans* or *Enterococcus faecalis*): high-dose intravenous **benzylpenicillin** sometimes with an aminoglycoside
  - serious infections with *Pseudomonas aeruginosa*: **ticarcillin**, **piperacillin**.
- This list is not exhaustive. Treatment with penicillins is sometimes started empirically, if the likely causative organism is one thought to be susceptible to penicillin, while awaiting the results of laboratory tests to identify the organism and determine its antibiotic susceptibility.

Unwanted effects

Penicillins are relatively free from direct toxic effects (other than their proconvulsant effect when given intrathecally). The main unwanted effects are hypersensitivity reactions caused by the degradation products of penicillin, which combine with host protein and become antigenic. Skin rashes and fever are common; a delayed type of serum sickness occurs infrequently. Much more serious is acute anaphylactic shock which, although rare, may be fatal. When given orally, penicillins, particularly the broad-spectrum type, alter the bacterial flora in the gut. This can be associated with gastrointestinal disturbances and in some cases with suprainfection by other, penicillin-insensitive, microorganisms leading to problems such as pseudomembranous colitis (caused by *C. difficile*, see below).

CEPHALOSPORINS AND CEPHAMYCINS

Cephalosporins N and C, which are chemically related to penicillin, and cephalosporin P, a steroidal antibiotic that resembles **fusidic acid** (see below), were first isolated from *Cephalosporium* fungus. The cephamycins are β-lactam

antibiotics produced by *Streptomyces* organisms, and they are closely related to the cephalosporins. They have the same mechanism of action as penicillins (see above).

Semisynthetic broad-spectrum cephalosporins have been produced by addition, to the cephalosporin C nucleus, of different side-chains at R_1 and/or R_2 (see Fig. 50.3). These agents are water soluble and relatively acid stable. They vary in susceptibility to β-lactamases. There are now a very large number of cephalosporins and cephamycins available for clinical use. Original members of the group such as **cefradine**, **cefalexin** and **cefadroxil** have largely been replaced with 'second-generation' drugs such as **cefuroxime** and **cefaclor**, or 'third-generation' drugs such as **cefotaxime**, **ceftazidime**, **cefixime**, **cefpodoxime** and **ceftriaxone**.

Resistance to this group of drugs has increased because of plasmid-encoded or chromosomal β-lactamase. Nearly all Gram-negative bacteria have a chromosomal gene coding for a β-lactamase that is more active in hydrolysing cephalosporins than penicillins, and in several organisms a single mutation can result in high-level constitutive production of this enzyme. Resistance also occurs when there is decreased penetration of the drug as a result of alterations to outer membrane proteins, or mutations of the binding-site proteins.

Pharmacokinetic aspects

Some cephalosporins may be given orally, but most are given parenterally, intramuscularly (which may be painful) or intravenously. After absorption, they are widely distributed in the body and some, such as cefotaxime, cefuroxime and ceftriaxone, cross the blood–brain barrier. Excretion is mostly via the kidney, largely by tubular secretion, but 40% of ceftriaxone is eliminated in the bile.

Unwanted effects

Hypersensitivity reactions, very similar to those seen with penicillin, may occur, and there may be some cross-sensitivity; about 10% of penicillin-sensitive individuals will have allergic reactions to cephalosporins. Nephrotoxicity has been reported (especially with cefradine), as has drug-induced alcohol intolerance. Diarrhoea is common and can be due to *C. difficile*.

Clinical uses of the cephalosporins

- Cephalosporins are used to treat infections caused by sensitive organisms. As with other antibiotics, patterns of sensitivity vary geographically, and treatment is often started empirically. Many different kinds of infection may be treated, including:
  - *septicaemia* (e.g. **cefuroxime**, **cefotaxime**)
  - *pneumonia* caused by susceptible organisms
  - *meningitis* (e.g. **ceftriaxone**, **cefotaxime**)
  - *biliary tract infection*
  - *urinary tract infection* (especially in pregnancy or in patients unresponsive to other drugs)
  - *sinusitis* (e.g. **cefadroxil**).

OTHER β-LACTAM ANTIBIOTICS

Carbapenems and monobactams (see Fig. 50.3) were developed to deal with β-lactamase-producing Gram-negative organisms resistant to penicillins.

CARBAPENEMS

Imipenem, an example of a carbapenem, acts in the same way as the other β-lactams (see Fig. 50.3). It has a very broad spectrum of antimicrobial activity, being active against many aerobic and anaerobic Gram-positive and Gram-negative organisms. However, many of the 'meticillin-resistant' staphylococci are less susceptible, and resistant strains of *P. aeruginosa* have emerged during therapy. Imipenem was originally resistant to all β-lactamases, but some organisms now have chromosomal genes that code for imipenem-hydrolysing β-lactamases. It is sometimes given together with **cilastatin**, which inhibits its inactivation by renal enzymes. **Meropenem** is similar but is not metabolised by the kidney. **Ertapenem** has a broad spectrum of antibacterial actions but is licensed only for a limited range of indications. Most carbapenems are not orally active, and are used only in special situations.

Unwanted effects are generally similar to those seen with other β-lactams, nausea and vomiting being the most frequently seen. Neurotoxicity can occur with high plasma concentrations.

MONOBACTAMS

The main monobactam is **aztreonam** (see Fig. 50.3), which is resistant to most β-lactamases. It is given by injection and has a plasma half-life of 2 h. Aztreonam has an unusual spectrum of activity and is effective only against Gram-negative aerobic bacilli such as pseudomonads, *Neisseria meningitidis* and *Haemophilus influenzae*. It has no action against Gram-positive organisms or anaerobes.

Unwanted effects are, in general, similar to those of other β-lactam antibiotics, but this agent does not necessarily cross-react immunologically with penicillin and its products, and so does not usually cause allergic reactions in penicillin-sensitive individuals.

GLYCOPEPTIDES

Vancomycin is a glycopeptide antibiotic, and **teicoplanin** is similar but longer lasting. Vancomycin acts by inhibiting cell wall synthesis (see Fig. 49.3). It is effective mainly against Gram-positive bacteria and has been used against MRSA. Vancomycin is not absorbed from the gut and is only given by the oral route for treatment of gastrointestinal infection with *C. difficile*. For parenteral use, it is given intravenously and has a plasma half-life of about 8 h.

The clinical use of vancomycin is limited mainly to *pseudomembranous colitis* (a clostridial infection sometimes associated with antibiotic therapy) and the treatment of some multiresistant staphylococcal infections. It is also valuable in severe staphylococcal infections in patients allergic to both penicillins and cephalosporins, and in some forms of endocarditis.

Unwanted effects include fever, rashes and local phlebitis at the site of injection. Ototoxicity and nephrotoxicity can occur, and hypersensitivity reactions are occasionally seen.

Daptomycin is a new lipopeptide antibacterial with a similar spectrum of actions to vancomycin. It is usually used, in combination with other drugs, for the treatment of MRSA.

β-Lactam antibiotics

- Bactericidal by interference with peptidoglycan synthesis.

Penicillins

- The first choice for many infections.
- Benzylpenicillin:
  - given by injection, short half-life and is destroyed by β-lactamases
  - spectrum: Gram-positive and Gram-negative cocci and some Gram-negative bacteria
  - many staphylococci are now resistant.
- β-Lactamase-resistant penicillins (e.g. flucloxacillin):
  - given orally
  - spectrum: as for benzylpenicillin
  - many staphylococci are now resistant.
- Broad-spectrum penicillins (e.g. amoxicillin):
  - given orally; they are destroyed by β-lactamases
  - spectrum: as for benzylpenicillin (although less potent); they are also active against Gram-negative bacteria.
- Extended-spectrum penicillins (e.g. ticarcillin):
  - given orally; they are susceptible to β-lactamases
  - spectrum: as for broad-spectrum penicillins; they are also active against pseudomonads.
- Unwanted effects of penicillins: mainly hypersensitivities.
- A combination of clavulanic acid plus amoxicillin or ticarcillin is effective against many β-lactamase-producing organisms.

Cephalosporins and cephamycins

- Second choice for many infections.
- Oral drugs (e.g. cefaclor) are used in urinary infections.
- Parenteral drugs (e.g. cefuroxime, which is active against *S. aureus*, *H. influenzae*, Enterobacteriaceae).
- Unwanted effects: mainly hypersensitivities.

Carbapenems

- Imipenem is a broad-spectrum antibiotic.
- Imipenem is used with cilastin, which blocks its breakdown in the kidney.

Monobactams

- Aztreonam is active only against Gram-negative aerobic bacteria and is resistant to most β-lactamases.

Clinical uses of tetracyclines

- The use of tetracyclines declined because of widespread drug resistance, but has staged something of a comeback, e.g. for respiratory infections, as resistance has receded with reduced use. Most members of the group are microbiologically similar; **doxycycline** is given once daily and may be used in patients with renal impairment. Uses (sometimes in combination with other antibiotics) include:
  - *rickettsial* and *chlamydial infections*, *brucellosis*, *anthrax* and *Lyme disease*
  - as useful second choice, for example in patients with *allergies*, for several infections (see Table 50.1), including *mycoplasma* and *leptospira*
  - respiratory tract infections (e.g. exacerbations of *chronic bronchitis*, *community-aquired pneumonia*)
  - *acne*
  - inappropriate secretion of antidiuretic hormone (e.g. by some *malignant lung tumours*), causing hyponatraemia: **demeclocycline** inhibits the action of this hormone by an entirely distinct action from its antibacterial effect (Ch. 32).

ANTIMICROBIAL AGENTS AFFECTING BACTERIAL PROTEIN SYNTHESIS

TETRACYCLINES

Tetracyclines are broad-spectrum antibiotics. The group includes **tetracycline**, **oxytetracycline**, **demeclocycline**, **lymecycline**, **doxycycline** and **minocycline**. **Tigelcycline** is structurally related to the tetracycline family and has similar therapeutic and unwanted effects.

Mechanism of action

Following uptake into susceptible organisms by active transport, tetracyclines act by inhibiting protein synthesis (see Ch. 49, Fig. 49.4). The tetracyclines are regarded as bacteriostatic, not bactericidal.

Antibacterial spectrum

The spectrum of antimicrobial activity of the tetracyclines is very wide and includes Gram-positive and Gram-negative bacteria, *Mycoplasma*, *Rickettsia*, *Chlamydia* spp., spirochaetes and some protozoa (e.g. amoebae). Minocycline is also effective against *N. meningitidis* and has been used to eradicate this organism from the nasopharynx of carriers. However, widespread resistance to these agents has decreased their usefulness. Resistance is transmitted mainly by plasmids and, because the genes controlling resistance to tetracyclines are closely associated with genes for resistance to other antibiotics, organisms may develop resistance to many drugs simultaneously. The clinical use of the tetracyclines is given in the clinical box.

Pharmacokinetic aspects

The tetracyclines are generally given orally but can also be administered parenterally. Minocycline and doxycycline are virtually completely absorbed. The absorption of most other tetracyclines is irregular and incomplete but is improved in the absence of food. Because tetracyclines chelate metal ions (calcium, magnesium, iron, aluminium), forming non-absorbable complexes, absorption is decreased in the presence of milk, certain antacids and iron preparations.

Unwanted effects

The commonest unwanted effects are gastrointestinal disturbances caused initially by direct irritation and later by modification of the gut flora. Vitamin B complex deficiency can occur, as can suprainfection. Because they chelate Ca^{2+}, tetracyclines are deposited in growing bones and teeth, causing staining and sometimes dental hypoplasia and bone deformities. They should therefore not be given to children, pregnant women or nursing mothers. Another hazard to pregnant women is hepatotoxicity. Phototoxicity (sensitisation to sunlight) has also been seen, particularly

Clinical uses of chloramphenicol

- Chloramphenicol should be reserved for serious infections in which the benefit of the drug outweighs its uncommon but serious haematological toxicity. Such uses may include:
  - infections caused by *Haemophilus influenzae* resistant to other drugs
  - *meningitis* in patients in whom penicillin cannot be used.
- It is also safe and effective in bacterial *conjunctivitis* (given topically).
- It is effective in *typhoid fever*, but **ciprofloxacin** or **amoxicillin** and **co-trimoxazole** are similarly effective and less toxic.

with demeclocycline. Minocycline can produce vestibular disturbances (dizziness and nausea). High doses of tetracyclines can decrease protein synthesis in host cells, an antianabolic effect that may result in renal damage. Long-term therapy can cause disturbances of the bone marrow.

AMPHENICOLS

The principal agent is **chloramphenicol** which was originally isolated from cultures of *Streptomyces*. It inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit (see Ch. 49, Fig. 49.4). The clinical uses of chloramphenicol are given in the box.

Antibacterial spectrum

Chloramphenicol has a wide spectrum of antimicrobial activity, including Gram-negative and Gram-positive organisms and rickettsiae. It is bacteriostatic for most organisms but kills *H. influenzae*. Resistance, caused by the production of *chloramphenicol acetyltransferase*, is plasmid mediated.

Pharmacokinetic aspects

Given orally, chloramphenicol is rapidly and completely absorbed and reaches its maximum concentration in the plasma within 2 h; it can also be given parenterally. The drug is widely distributed throughout the tissues and body fluids including the CSF. Its half-life is approximately 2 h. About 10% is excreted unchanged in the urine, and the remainder is inactivated in the liver.

Unwanted effects

The most important unwanted effect of chloramphenicol is severe, idiosyncratic depression of the bone marrow, resulting in *pancytopenia* (a decrease in all blood cell elements)—an effect that, although rare, can occur even with low doses in some individuals. Chloramphenicol must be used with great care in newborns, with monitoring of plasma concentrations, because inadequate inactivation and excretion of the drug (see Ch. 56) can result in the 'grey baby syndrome'—vomiting, diarrhoea, flaccidity, low temperature and an ashen-grey colour—which carries 40% mortality. Hypersensitivity reactions can occur, as can gastrointestinal disturbances secondary to alteration of the intestinal microbial flora.

AMINOGLYCOSIDES

The aminoglycosides are a group of antibiotics of complex chemical structure, resembling each other in antimicrobial activity, pharmacokinetic characteristics and toxicity. The main agents are **gentamicin**, **streptomycin**, **amikacin**, **tobramycin** and **neomycin**.

Mechanism of action

Aminoglycosides inhibit bacterial protein synthesis by blocking initiation (see Ch. 49). Their penetration through the cell membrane of the bacterium depends partly on oxygen-dependent active transport by a polyamine carrier system, and they have minimal action against anaerobic organisms. Chloramphenicol blocks this transport system. The effect of the aminoglycosides is bactericidal and is enhanced by agents that interfere with cell wall synthesis.

Resistance

Resistance to aminoglycosides is becoming a problem. It occurs through several different mechanisms, the most important being inactivation by microbial enzymes, of which nine or more are known. Amikacin was purposefully designed as a poor substrate for these enzymes, but some organisms have acquired enzymes that inactivate this agent as well. Resistance as a result of failure of penetration can be largely overcome by the concomitant use of penicillin and/or vancomycin.

Antibacterial spectrum

The aminoglycosides are effective against many aerobic Gram-negative and some Gram-positive organisms. They are most widely used against Gram-negative enteric organisms and in sepsis. They may be given together with a penicillin in streptococcocal infections and those caused by *Listeria* spp. and *P. aeruginosa* (see Table 50.1). Gentamicin is the aminoglycoside most commonly used, although tobramycin is the preferred member of this group for *P. aeruginosa* infections. Amikacin has the widest antimicrobial spectrum and can be effective in infections with organisms resistant to gentamicin and tobramycin.

Pharmacokinetic aspects

The aminoglycosides are polycations and therefore highly polar. They are not absorbed from the gastrointestinal tract and are usually given intramuscularly or intravenously. They cross the placenta but do not cross the blood–brain barrier, although high concentrations can be attained in joint and pleural fluids. The plasma half-life is 2–3 h. Elimination is virtually entirely by glomerular filtration in the kidney, 50–60% of a dose being excreted unchanged within 24 h. If renal function is impaired, accumulation occurs rapidly, with a resultant increase in those toxic effects (such as ototoxicity and nephrotoxicity; see below) that are dose related.

Unwanted effects

Serious, dose-related toxic effects, which may increase as treatment proceeds, can occur with the aminoglycosides, the main hazards being ototoxicity and nephrotoxicity.

The ototoxicity involves progressive damage to, and eventually destruction of, the sensory cells in the cochlea and vestibular organ of the ear. The result, usually irreversible, may manifest as vertigo, ataxia and loss of balance in the case of vestibular damage, and auditory disturbances or deafness in the case of cochlear damage.

Any aminoglycoside may produce both types of effect, but streptomycin and gentamicin are more likely to interfere with vestibular function, whereas neomycin and amikacin mostly affect hearing. Ototoxicity is potentiated by the concomitant use of other ototoxic drugs (e.g. loop diuretics; Ch. 28) and susceptibility is genetically determined via mitochondrial DNA (see Ch. 11).

The nephrotoxicity consists of damage to the kidney tubules, and function recovers if the use of the drugs is stopped. Nephrotoxicity is more likely to occur in patients with pre-existing renal disease or in conditions in which urine volume is reduced, and concomitant use of other nephrotoxic agents (e.g. first-generation cephalosporins) increases the risk. As the elimination of these drugs is almost entirely renal, their nephrotoxic action can impair their own excretion, and a vicious cycle may develop. Plasma concentrations should be monitored regularly and the dose adjusted accordingly.

A rare but serious toxic reaction is paralysis caused by neuromuscular blockade. This is usually seen only if the agents are given concurrently with neuromuscular-blocking agents. It results from inhibition of the Ca^{2+} uptake necessary for the exocytotic release of acetylcholine (see Ch. 13).

MACROLIDES

The term *macrolide* relates to the structure—a many-membered lactone ring to which one or more deoxy sugars are attached. The main macrolide and related antibiotics are **erythromycin**, **clarithromycin** and **azithromycin**. **Spiramycin** and **telithromycin** are of minor utility.

Mechanism of action

The macrolides inhibit bacterial protein synthesis by an effect on translocation (Fig. 49.4). The drugs bind to the same 50S subunit of the bacterial ribosome as chloramphenicol and clindamycin, and any of these drugs may compete if given concurrently.

Antimicrobial spectrum

The antimicrobial spectrum of erythromycin is very similar to that of penicillin, and it is a safe and effective alternative for penicillin-sensitive patients. Erythromycin is effective against Gram-positive bacteria and spirochaetes but not against most Gram-negative organisms, exceptions being *N. gonorrhoeae* and, to a lesser extent, *H. influenzae*. *Mycoplasma pneumoniae*, *Legionella* spp. and some chlamydial organisms are also susceptible (see Table 50.1). Resistance can occur and results from a plasmid-controlled alteration of the binding site for erythromycin on the bacterial ribosome (Fig. 49.4).

Azithromycin is less active against Gram-positive bacteria than erythromycin but is considerably more effective against *H. influenzae* and may be more active against *Legionella*. It has excellent action against *Toxoplasma gondii*, killing the cysts. Clarithromycin is as active, and its metabolite is twice as active, against *H. influenzae* as erythromycin. It is also effective against *Mycobacterium avium-intracellulare* (which can infect immunologically compromised individuals and elderly patients with chronic lung disease), and it may also be useful in leprosy and against *Helicobacter pylori* (see Ch. 29). Both these macrolides are also effective in Lyme disease.

Antimicrobial agents affecting bacterial protein synthesis

- *Tetracyclines* (e.g. minocycline). These are orally active, bacteriostatic, broad-spectrum antibiotics. Resistance is increasing. Gastrointestinal disorders are common. They chelate calcium and are deposited in growing bone. They are contraindicated in children and pregnant women.
- *Chloramphenicol*. This is an orally active, bacteriostatic, broad-spectrum antibiotic. Serious toxic effects are possible, including bone marrow depression and 'grey baby syndrome'. It should be reserved for life-threatening infections.
- *Aminoglycosides* (e.g. gentamicin). These are given by injection. They are bactericidal, broad-spectrum antibiotics (but with low activity against anaerobes, streptococci and pneumococci). Resistance is increasing. The main unwanted effects are dose-related nephrotoxicity and ototoxicity. Serum levels should be monitored. (Streptomycin is an antituberculosis aminoglycoside.)
- *Macrolides* (e.g. erythromycin). Can be given orally and parenterally. They are bactericidal/bacteriostatic. The antibacterial spectrum is the same as for penicillin. Erythromycin can cause jaundice. Newer agents are clarithromycin and azithromycin.
- *Clindamycin*. Can be given orally and parenterally. It can cause pseudomembranous colitis.
- *Quinupristin/dalfopristin*. Given by intravenous infusion as a combination. Considerably less active when administered separately. Active against several strains of drug-resistant bacteria.
- *Fusidic acid*. This is a narrow-spectrum antibiotic that acts by inhibiting protein synthesis. It penetrates bone. Unwanted effects include gastrointestinal disorders.
- *Linezolid*. Given orally or by intravenous injection. Active against several strains of drug-resistant bacteria.

Pharmacokinetic aspects

The macrolides are administered orally. Erythromycin can also be given parenterally, although intravenous injections can be followed by local thrombophlebitis. All three diffuse readily into most tissues but do not cross the blood–brain barrier, and there is poor penetration into synovial fluid. The plasma half-life of erythromycin is about 90 min; that of clarithromycin is three times longer, and that of azithromycin 8–16 times longer. Macrolides enter and indeed are concentrated within phagocytes—azithromycin concentrations in phagocyte lysosomes can be 40 times higher than in the blood—and they can enhance intracellular phagocyte killing of bacteria.

Erythromycin is partly inactivated in the liver; azithromycin is more resistant to inactivation, and clarithromycin is converted to an active metabolite. Their inhibition of the P450 cytochrome system can affect the bioavailability of other drugs leading to clinically important interactions, for example with **theophylline** (see Ch. 56). The major route of elimination is in the bile.

Unwanted effects

Gastrointestinal disturbances are common and unpleasant but not serious. With erythromycin, the following have also been reported: hypersensitivity reactions such as rashes and fever, transient hearing disturbances and, rarely, following treatment for longer than 2 weeks, cholestatic jaundice. Opportunistic infections of the gastrointestinal tract or vagina can occur.

ANTIMICROBIAL AGENTS AFFECTING TOPOISOMERASE

QUINOLONES

The **quinolones** include the broad-spectrum agents **ciprofloxacin**, **levofloxacin**, **ofloxacin**, **norfloxacin** and **moxifloxacin** as well as a narrow-spectrum drug used in urinary tract infections—**nalidixic acid**. These agents inhibit topoisomerase II (a bacterial DNA gyrase), the enzyme that produces a negative supercoil in DNA and thus permits transcription or replication (see Fig. 50.4).

Antibacterial spectrum and clinical use

The fluoroquinolone ciprofloxacin is the most commonly used and typical of the group. It is a broad-spectrum antibiotic effective against both Gram-positive and Gram-negative organisms, and also against the Enterobacteriaceae (the enteric Gram-negative bacilli), including many organisms resistant to penicillins, cephalosporins and aminoglycosides, and against *H. influenzae*, penicillinase-producing *N. gonorrhoeae*, *Campylobacter* spp. and pseudomonads. Of the Gram-positive organisms, streptococci and pneumococci are only weakly inhibited, and there is a high incidence of staphylococcal resistance. Ciprofloxacin should be avoided in MRSA infections. Clinically, the fluoroquinolones are best reserved for infections with facultative and aerobic Gram-negative bacilli and cocci.[5] Resistant strains of *S. aureus* and *P. aeruginosa* have emerged. Further details of the clinical use of the fluoroquinolones are given in the box.

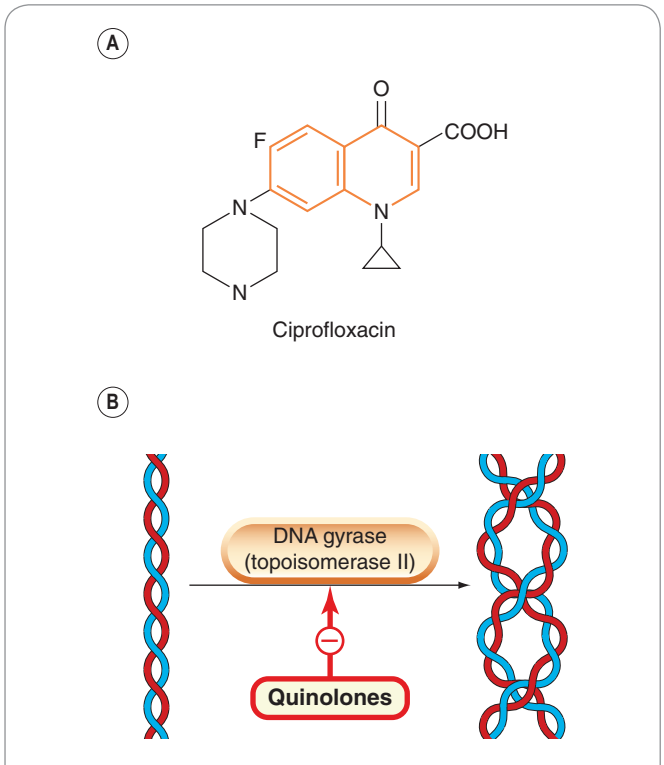

Fig. 50.4 A simplified diagram of the mechanism of action of the fluoroquinolones. [**A**] An example of a quinolone (the quinolone moiety is shown in orange). [**B**] Schematic diagram of (left) the double helix and (right) the double helix in supercoiled form (see also Fig. 49.6). In essence, the DNA gyrase unwinds the RNA-induced positive supercoil (not shown) and introduces a negative supercoil.

Pharmacokinetic aspects

Fluoroquinolones are well absorbed orally. The drugs accumulate in several tissues, particularly in the kidney, prostate and lung. All quinolones are concentrated in phagocytes. Most fail to cross the blood–brain barrier, but ofloxacin does so. Aluminium and magnesium antacids interfere with the absorption of the quinolones. Elimination of ciprofloxacin and norfloxacin is partly by hepatic

Antimicrobial agents affecting DNA topoisomerase II

- The quinolones interfere with the supercoiling of DNA.
- **Ciprofloxacin** has a wide antibacterial spectrum, being especially active against Gram-negative enteric coliform organisms, including many organisms resistant to penicillins, cephalosporins and aminoglycosides; it is also effective against *H. influenzae*, penicillinase-producing *N. gonorrhoeae*, *Campylobacter* spp. and pseudomonads. There is a high incidence of staphylococcal resistance.
- Unwanted effects include gastrointestinal tract upsets, hypersensitivity reactions and, rarely, central nervous system disturbances.

Clinical uses of the fluoroquinolones

- Complicated *urinary tract infections* (**norfloxacin**, **ofloxacin**).
- *Pseudomonas aeruginosa* respiratory infections in patients with cystic fibrosis.
- Invasive external otitis ('malignant otitis') caused by *P. aeruginosa*.
- Chronic Gram-negative bacillary osteomyelitis.
- Eradication of *Salmonella typhi* in carriers.
- *Gonorrhoea* (**norfloxacin**, **ofloxacin**).
- Bacterial *prostatitis* (**norfloxacin**).
- *Cervicitis* (**ofloxacin**).
- *Anthrax*.

[5]When ciprofloxacin was introduced, clinical pharmacologists and microbiologists sensibly suggested that it should be reserved for organisms already resistant to other drugs so as to prevent emergence of resistance. However, by 1989 it was already estimated that it was prescribed for 1 in 44 of Americans, so it would seem that the horse had not only left the stable but had bolted into the blue!

metabolism by P450 enzymes (which they can inhibit, giving rise to interactions with other drugs; see below) and partly by renal excretion. Ofloxacin is excreted in the urine.

Unwanted effects

In hospitals, infection with *C. difficile* may prove hazardous (see below) but otherwise unwanted effects are infrequent, usually mild and reversible. The most frequent manifestations are gastrointestinal disorders and skin rashes. Arthropathy has been reported in young individuals. Central nervous system symptoms—headache and dizziness—have occurred, as have, less frequently, convulsions associated with central nervous system pathology or concurrent use of **theophylline** or a non-steroidal anti-inflammatory drug.

There is a clinically important interaction between ciprofloxacin and theophylline (through inhibition of P450 enzymes), which can lead to theophylline toxicity in asthmatics treated with the fluoroquinolones. The topic is discussed further in Chapter 27.

MISCELLANEOUS AND LESS COMMON ANTIBACTERIAL AGENTS

METRONIDAZOLE

▼ **Metronidazole** was introduced as an antiprotozoal agent (see Ch. 53), but it is also active against anaerobic bacteria such as *Bacteroides*, *Clostridia* spp. and some streptococci. It is effective in the therapy of pseudomembranous colitis (see below), and is important in the treatment of serious anaerobic infections (e.g. sepsis secondary to bowel disease). It has a disulfiram-like action (see Ch. 48), so patients must avoid alcohol while taking metronidazole.

STREPTOGRAMINS

▼ **Quinupristin** and **dalfopristin** are cyclic peptides, which inhibit bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome. Dalfopristin changes the structure of the ribosome so as to promote the binding of quinupristin. Individually, they exhibit only very modest bacteriostatic activity, but combined together as an intravenous injection they are active against many Gram-positive bacteria.

The combination is used to treat serious infections, usually where no other antibacterial is suitable. For example, the combination is effective against MRSA and is also active against vancomycin-resistant *Enterococcus faecium*.

Both drugs undergo extensive first-pass hepatic metabolism and must therefore be given as an intravenous infusion. The half-life of each compound is 1–2 h.

Unwanted effects include inflammation and pain at the infusion site, arthralgia, myalgia and nausea, vomiting and diarrhoea. To date, resistance to quinupristin and dalfopristine does not seem to be a major problem.

CLINDAMYCIN

▼ The lincosamide, **clindamycin**, is active against Gram-positive cocci, including many penicillin-resistant staphylococci and many anaerobic bacteria such as *Bacteroides* spp. It acts in the same way as macrolides and chloramphenicol (Fig. 49.4). In addition to its use in infections caused by *Bacteroides* organisms, it is used to treat staphylococcal infections of bones and joints. It is also given topically, as eye drops, for staphylococcal conjunctivitis.

Unwanted effects consist mainly of gastrointestinal disturbances, and a potentially lethal condition, *pseudomembranous colitis*, may develop. This is an acute inflammation of the colon caused by a necrotising toxin produced by a clindamycin-resistant organism, *Clostridium difficile*, which may form part of the normal faecal flora.[6] Metronidazole (see above) is usually effective in the treatment of this condition; vancomycin, given orally, is an alternative.

OXAZOLIDINONES

▼ Hailed as the 'first truly new class of antibacterial agents to reach the marketplace in several decades' (Zurenko et al., 2001), the oxazolidinones inhibit bacterial protein synthesis by a novel mechanism: inhibition of *N*-formylmethionyl-tRNA binding to the 70S ribosome. **Linezolid** is the first member of this new antibiotic family to be introduced. It is active against a wide variety of Gram-positive bacteria and is particularly useful for the treatment of drug-resistant bacteria such as MRSA, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant enterococci. The drug is also effective against some anaerobes, such as *Clostridium difficile*. Most common Gram-negative organisms are not susceptible to the drug. Linezolid can be used to treat pneumonia, septicaemia, and skin and soft tissue infections. Its use is restricted to serious bacterial infections where other antibiotics have failed, and there have so far been few reports of linezolid resistance.

Unwanted effects include thrombocytopenia, diarrhoea, nausea and, rarely, rash and dizziness. Linezolid is a non-selective inhibitor of monoamine oxidase, and appropriate precautions need to be observed (see Ch. 46).

FUSIDIC ACID

▼ Fusidic acid is a narrow-spectrum steroid antibiotic active mainly against Gram-positive bacteria. It acts by inhibiting bacterial protein synthesis (Fig. 49.4). As the sodium salt, the drug is well absorbed from the gut and is distributed widely in the tissues. Some is excreted in the bile and some metabolised. It is used in combination with other antistaphylococcal agents in staphylococcal sepsis, and topically for staphylococcal infections (e.g. as eye drops).

Unwanted effects such as gastrointestinal disturbances are fairly common. Skin eruptions and jaundice can occur. Resistance occurs if it is used systemically as a single agent.

NITROFURANTOIN

▼ **Nitrofurantoin** is a synthetic compound active against a range of Gram-positive and Gram-negative organisms. The development of resistance in susceptible organisms is rare, and there is no cross-resistance. Its mechanism of action is not known. It is given orally and is rapidly and totally absorbed from the gastrointestinal tract and just as rapidly excreted by the kidney. Its use is confined to the treatment of urinary tract infections.

Unwanted effects such as gastrointestinal disturbances are relatively common, and hypersensitivity reactions involving the skin and the bone marrow (e.g. leukopenia) can occur. Hepatotoxicity and peripheral neuropathy have also been reported.

Methanamine has a similar clinical utility to nitrofurantoin and shares several of its unwanted effects. It exerts its effects by conversion (in acidic urine) to formaldehyde.

POLYMIXINS

▼ The polymixin antibiotics in use are **polymixin B** and **colistin** (polymixin E). They have cationic detergent properties and exert their antibacterial action by disrupting the outer cell membrane (Ch. 49). They have a selective, rapidly bactericidal action on Gram-negative bacilli, especially pseudomonads and coliform organisms. They are not absorbed from the gastrointestinal tract. Clinical use of these drugs is limited by their toxicity and is confined largely to gut sterilisation and topical treatment of ear, eye or skin infections caused by susceptible organisms.

Unwanted effects may be serious and include neurotoxicity and nephrotoxicity.

[6]This may also occur with some penicillins and cephalosporins.

Miscellaneous antibacterial agents

- *Glycopeptide antibiotics* (e.g. **vancomycin**). Vancomycin is bactericidal, acting by inhibiting cell wall synthesis. It is used intravenously for multiresistant staphylococcal infections and orally for pseudomembranous colitis. Unwanted effects include ototoxicity and nephrotoxicity.
- *Polymixins* (e.g. **colistin**). They are bactericidal, acting by disrupting bacterial cell membranes. They are highly neurotoxic and nephrotoxic, and are only used topically.

ANTIMYCOBACTERIAL AGENTS

The main mycobacterial infections in humans are tuberculosis and leprosy, chronic infections caused by *Mycobacterium tuberculosis* and *M. leprae*, respectively. A particular problem with both these organisms is that they can survive inside macrophages after phagocytosis, unless these cells are 'activated' by cytokines produced by T-helper (Th)1 lymphocytes (see Ch. 17).

DRUGS USED TO TREAT TUBERCULOSIS

For centuries, tuberculosis was a major killer disease, but the introduction of **streptomycin** in the late 1940s followed by **isoniazid** and, in the 1960s, of **rifampicin** and **ethambutol** revolutionised therapy, and tuberculosis came to be regarded as an easily treatable condition. Regrettably, this is so no longer; strains with increased virulence or exhibiting multidrug resistance are now common (Bloom & Small, 1998). Tuberculosis is again a major threat; the World Health Organization estimates that one-third of the world's population (2 billion people) are currently infected with the bacillus, 10% of whom will develop the disease at some point in their lifetime. Infection rates are falling very slowly, but in 2008 there were over 9 million new cases and the disease killed about 1.8 million people. Poverty-stricken countries in Africa and Asia bear the brunt of the disease, partly because of an ominous synergy between mycobacteria (e.g. *M. tuberculosis*, *M. avium-intercellulare*) and HIV. About one-third of HIV-associated deaths are caused by tuberculosis. The disease is out of control in many countries, and it is now the world's leading cause of death from a single agent.

Our counterattack is led by the first-line drugs **isoniazid**, **rifampicin**, **rifabutin**, **ethambutol** and **pyrazinamide**. Some second-line drugs available are **capreomycin**, **cycloserine**, **streptomycin** (rarely used now in the UK), **clarithromycin** and **ciprofloxacin**. These are used to treat infections likely to be resistant to first-line drugs, or when the first-line agents have to be abandoned because of unwanted reactions.

To decrease the probability of the emergence of resistant organisms, compound drug therapy is a frequent strategy. This commonly involves:

- an initial phase of treatment (about 2 months) with a combination of isoniazid, rifampicin and pyrazinamide (plus ethambutol if the organism is suspected to be resistant)
- a second, continuation phase (about 4 months) of therapy, with isoniazid and rifampicin; longer-term treatment is needed for patients with meningitis, bone/joint involvement or drug-resistant infection.

ISONIAZID

The antibacterial activity of isoniazid is limited to mycobacteria. It halts the growth of resting organisms (i.e. is bacteriostatic) but can kill dividing bacteria. It passes freely into mammalian cells and is thus effective against intracellular organisms. Isoniazid is a prodrug that must be activated by bacterial enzymes before it can exert its inhibitory activity on the synthesis of *mycolic acids*, important constituents of the cell wall peculiar to mycobacteria. Resistance to the drug, caused by reduced penetration into the bacterium, may be encountered, but cross-resistance with other tuberculostatic drugs does not occur.

Isoniazid is readily absorbed from the gastrointestinal tract and is widely distributed throughout the tissues and body fluids, including the CSF. An important point is that it penetrates well into 'caseous' tuberculous lesions (i.e. necrotic lesions with a cheese-like consistency). Metabolism, which involves acetylation, depends on genetic factors that determine whether a person is a slow or rapid acetylator of the drug (see Chs 11 and 56), with slow inactivators enjoying a better therapeutic response. The half-life in slow inactivators is 3 h and in rapid inactivators, 1 h. Isoniazid is excreted in the urine partly as unchanged drug and partly in the acetylated or otherwise inactivated form.

Unwanted effects depend on the dosage and occur in about 5% of individuals, the commonest being allergic skin eruptions. A variety of other adverse reactions have been reported, including fever, hepatotoxicity, haematological changes, arthritic symptoms and vasculitis. Adverse effects involving the central or peripheral nervous systems are largely consequences of pyridoxine deficiency and are common in malnourished patients unless prevented by administration of this substance. Isoniazid may cause haemolytic anaemia in individuals with glucose 6-phosphate dehydrogenase deficiency, and it decreases the metabolism of the antiepileptic agents **phenytoin**, **ethosuximide** and **carbamazepine**, resulting in an increase in the plasma concentration and toxicity of these drugs (Ch. 57).

RIFAMPICIN

Rifampicin acts by binding to, and inhibiting, DNA-dependent RNA polymerase in prokaryotic but not in eukaryotic cells (Ch. 49). It is one of the most active antituberculosis agents known, and is also effective against leprosy (see below) and most Gram-positive bacteria as well as many Gram-negative species. It enters phagocytic cells and can therefore kill intracellular microorganisms including the tubercle bacillus. Resistance can develop rapidly in a one-step process and is thought to be caused by chemical modification of microbial DNA-dependent RNA polymerase, resulting from a chromosomal mutation (see Ch. 49).

Rifampicin is given orally and is widely distributed in the tissues and body fluids (including CSF), giving an orange tinge to saliva, sputum, tears and sweat. It is excreted partly in the urine and partly in the bile, some of it undergoing enterohepatic cycling. The metabolite retains antibacterial activity but is less well absorbed from the gastrointestinal tract. The half-life is 1–5 h, becoming

shorter during treatment because of induction of hepatic microsomal enzymes.

Unwanted effects are relatively infrequent. The commonest are skin eruptions, fever and gastrointestinal disturbances. Liver damage with jaundice has been reported and has proved fatal in a very small proportion of patients, and liver function should be assessed before treatment is started. Rifampicin causes induction of hepatic metabolising enzymes (Ch. 10), resulting in an increase in the degradation of **warfarin**, **glucocorticoids**, narcotic analgesics, oral antidiabetic drugs, **dapsone** and **oestrogens**, the last effect leading to failure of oral contraceptives.

ETHAMBUTOL

Ethambutol has no effect on organisms other than mycobacteria. It is taken up by the bacteria and exerts a bacteriostatic effect after a period of 24 h, although the mechanism by which this occurs is unknown. Resistance emerges rapidly if the drug is used alone. Ethambutol is given orally and is well absorbed. It can reach therapeutic concentrations in the CSF in tuberculous meningitis. In the blood, it is taken up by erythrocytes and slowly released. Ethambutol is partly metabolised and is excreted in the urine.

Unwanted effects are uncommon, the most important being optic neuritis, which is dose related and is more likely to occur if renal function is decreased. It results in visual disturbances manifesting initially as red–green colour blindness progressing to a decreased visual acuity. Colour vision should be monitored during prolonged treatment.

PYRAZINAMIDE

Pyrazinamide is inactive at neutral pH but tuberculostatic at acid pH. It is effective against the intracellular organisms in macrophages because, after phagocytosis, the organisms are contained in phagolysosomes where the pH is low. Resistance develops rather readily, but cross-resistance with isoniazid does not occur. The drug is well absorbed after oral administration and is widely distributed, penetrating well into the meninges. It is excreted through the kidney, mainly by glomerular filtration.

Unwanted effects include gout, which is associated with high concentrations of plasma urates. Gastrointestinal upsets, malaise and fever have also been reported. Serious hepatic damage due to high doses was once a problem but is less likely with lower dose/shorter course regimens now used; nevertheless, liver function should be assessed before treatment.

CAPREOMYCIN

▼ Capreomycin is a peptide antibiotic given by intramuscular injection. *Unwanted effects* include kidney damage and injury to the auditory nerve, with consequent deafness and ataxia. The drug should not be given at the same time as streptomycin or other drugs that may cause deafness.

CYCLOSERINE

▼ Cycloserine is a broad-spectrum antibiotic that inhibits the growth of many bacteria, including coliforms and mycobacteria. It is water soluble and destroyed at acid pH. It acts by competitively inhibiting bacterial cell wall synthesis. It does this by preventing the formation of D-alanine and the D-Ala-D-Ala dipeptide that is added to the initial tripeptide side-chain on *N*-acetylmuramic acid, i.e. it prevents completion of the major building block of peptidoglycan (see Fig. 49.3). It is absorbed orally and distributed throughout the tissues and body fluids, including CSF. Most of the drug is eliminated in active form in the urine, but approximately 35% is metabolised.

Cycloserine has unwanted effects mainly on the central nervous system. A wide variety of disturbances may occur, ranging from headache and irritability to depression, convulsions and psychotic states. Its use is limited to tuberculosis that is resistant to other drugs.

Antituberculosis drugs

- To avoid the emergence of resistant organisms, compound therapy is used (e.g. three drugs initially, followed by a two-drug regimen later).

First-line drugs

- **Isoniazid** kills actively growing mycobacteria within host cells; mechanism of action unknown. Given orally, it penetrates necrotic lesions, also the cerebrospinal fluid (CSF). 'Slow acetylators' (genetically determined) respond well. It has low toxicity. Pyridoxine deficiency increases risk of neurotoxicity. No cross-resistance with other agents.
- **Rifampicin** (rifampin) is a potent, orally active drug that inhibits mycobacterial RNA polymerase. It penetrates CSF. Unwanted effects are infrequent (but serious liver damage has occurred). It induces hepatic drug-metabolising enzymes. Resistance can develop rapidly.
- **Ethambutol** inhibits growth of mycobacteria by an unknown mechanism. It is given orally and can penetrate CSF. Unwanted effects are uncommon, but optic neuritis can occur. Resistance can emerge rapidly.
- **Pyrazinamide** is tuberculostatic against intracellular mycobacteria by an unknown mechanism. Given orally, it penetrates CSF. Resistance can develop rapidly. Unwanted effects include increased plasma urate and liver toxicity with high doses.

Second-line drugs

- **Capreomycin** is given intramuscularly. Unwanted effects include damage to the kidney and to the eighth nerve.
- **Cycloserine** is a broad-spectrum agent. It inhibits an early stage of peptidoglycan synthesis. Given orally, it penetrates the CSF. Unwanted effects affect mostly the central nervous system.
- **Streptomycin**, an aminoglycoside antibiotic, acts by inhibiting bacterial protein synthesis. It is given intramuscularly. Unwanted effects are ototoxicity (mainly vestibular) and nephrotoxicity.

DRUGS USED TO TREAT LEPROSY

Leprosy is one of the most ancient diseases known to mankind and has been mentioned in texts dating back to 600 BC. It is a chronic disfiguring illness with a long latency, and historically sufferers have been ostracised and forced to live apart from their communities although, in fact, the disease is not particularly contagious. Once viewed as incurable, the introduction in the 1940s of **dapsone**, and subsequently **rifampicin** (see above) and **clofazimine** in the 1960s, completely changed our perspective on leprosy. It is now generally curable, and the global figures show

that the prevalence rates for the disease have dropped by 90% since 1985 and there has been a 20% annual decrease in the number of new cases detected since 2001. The disease has been eliminated from 113 out of 122 countries where it was considered to be a major health problem. In 2009, some 200 000 new cases were reported. The bulk of these (75%) are in the Indian subcontinent, Brazil or Mozambique.

Multidrug treatment regimens (which fortunately seem to defy drug resistance) initiated by the World Health Organization in 1982 are now the mainstay of treatment. *Paucibacillary leprosy*, leprosy characterised by one to five numb patches, is mainly *tuberculoid*[7] in type and is treated for 6 months with dapsone and rifampicin. *Multibacillary leprosy*, characterised by more than five numb skin patches, is mainly *lepromatous* in type and is treated for at least 2 years with rifampicin, dapsone and clofazimine. The effect of therapy with minocycline or the fluoroquinolones is being investigated.

Antileprosy drugs

- For *tuberculoid leprosy*: **dapsone** and **rifampicin** (rifampin).
- Dapsone is sulfonamide-like and may inhibit folate synthesis. It is given orally. Unwanted effects are fairly frequent; a few are serious. Resistance is increasing.
- Rifampicin (see *Antituberculosis drugs* box).
- For lepromatous leprosy: dapsone, rifampicin and **clofazimine**.
- Clofazimine is a dye that is given orally and can accumulate by sequestering in macrophages. Action is delayed for 6–7 weeks, and its half-life is 8 weeks. Unwanted effects include red skin and urine, sometimes gastrointesinal disturbances.

DAPSONE

Dapsone is chemically related to the sulfonamides and, because its action is antagonised by PABA, probably acts through inhibition of bacterial folate synthesis. Resistance to the drug has steadily increased since its introduction and treatment in combination with other drugs is now recommended.

Dapsone is given orally; it is well absorbed and widely distributed through the body water and in all tissues. The plasma half-life is 24–48 h, but some dapsone persists in certain tissues (liver, kidney and, to some extent, skin and muscle) for much longer periods. There is enterohepatic recycling of the drug, but some is acetylated and excreted in the urine. Dapsone is also used to treat *dermatitis herpetiformis*, a chronic blistering skin condition associated with coeliac disease.

Unwanted effects occur fairly frequently and include haemolysis of red cells (usually not severe enough to lead to frank anaemia), methaemoglobinaemia, anorexia, nausea and vomiting, fever, allergic dermatitis and neuropathy. *Lepra reactions* (an exacerbation of lepromatous lesions) can occur, and a potentially fatal syndrome resembling infectious mononucleosis has occasionally been seen.

CLOFAZIMINE

Clofazimine is a dye of complex structure. Its mechanism of action against leprosy bacilli may involve an action on DNA. It also has anti-inflammatory activity and is useful in patients in whom dapsone causes inflammatory side effects.

Clofazimine is given orally and accumulates in the body, being sequestered in the mononuclear phagocyte system. The plasma half-life may be as long as 8 weeks. The antileprotic effect is delayed and is usually not evident for 6–7 weeks.

Unwanted effects may be related to the fact that clofazimine is a dye. The skin and urine can develop a reddish colour and the lesions a blue–black discoloration. Dose-related nausea, giddiness, headache and gastrointestinal disturbances can also occur.

[7]The basis of the difference between *tuberculoid* and *lepromatous* disease appears to be that the T cells from patients with the former vigorously produce interferon-γ, which enables macrophages to kill intracellular microbes, whereas in the latter case the immune response is dominated by interleukin-4, which blocks the action of interferon-γ (see Ch. 17).

POSSIBLE NEW ANTIBACTERIAL DRUGS

In contrast to the rapid discoveries and developments that characterised the 'heroic' years of antibiotic research spanning approximately 1950–1980, and which produced virtually all our existing drugs, the flow has since dried up, with only two totally novel antibiotics introduced during this period (Jagusztyn-Krynicka & Wyszynska, 2008). However, the problem of resistance is now acute and a major worry. Novel antibiotic candidates continue to be discovered in plants (Limsuwan et al., 2009) and bacteria (Sit & Vederas, 2008) as well as through traditional medicinal chemistry. In addition, researchers in the front line of this important field are pressing all the latest conceptual technologies into the fray: bioinformatics, utilising information derived from pathogen genome sequencing, is one such approach (Bansal, 2008). The hunt for, and targeting of, bacterial *virulence factors* is showing some promise (Escaich, 2008). New types of screening procedures have been devised (Falconer & Brown, 2009) which would reveal novel types of targets, and sophisticated pharmacodynamic profiling brought to bear on the problem (Lister, 2006). The world awaits developments with bated breath.

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(*These papers have been provided for those who want to learn more about the work underway to develop novel antibacterials. Some are quite technical in nature*)

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Useful Web site

http://www.who.int (*Once again, the World Health Organization Web site is a mine of information about the demographics and treatment of infectious diseases. The sections on leprosy and tuberculosis are especially worthwhile studying. The site includes photographs, maps and much statistical information, as well as information on drug resistance. Highly recommended*)