36 Chemical transmission and drug action in the central nervous system

OVERVIEW

Brain function is the single most important aspect of physiology that defines the difference between humans and other species. Disorders of brain function, whether primary or secondary to malfunction of other systems, are a major concern of human society, and a field in which pharmacological intervention plays a key role. In this chapter, we introduce some basic principles of neuropharmacology that underlie much of the material in the rest of this section.

INTRODUCTION

There are two reasons why understanding the action of drugs on the central nervous system (CNS) presents a particularly challenging problem. The first is that centrally acting drugs are of special significance to humankind. Not only are they of major therapeutic importance,[1] but they are also the drugs that humans most commonly administer to themselves for non-medical reasons (e.g. alcohol, tea and coffee, cannabis, nicotine, opioids, amphetamines and so on). The second reason is that the CNS is functionally far more complex than any other system in the body, and this makes the understanding of drug effects very much more difficult. The relationship between the behaviour of individual cells and that of the organ as a whole is far less direct in the brain than in other organs. Currently, the links between a drug's action at the biochemical and cellular level and its effects on brain function remain largely mysterious. Functional brain imaging is beginning to reveal relationships between brain activity in specific regions and mental function, and this tool is being used increasingly to probe drug effects. Nevertheless, the fairly gross (millimetre scale) resolution currently achievable with imaging methods is far from being able to reveal events at the level of individual neurons and synapses. Despite sustained progress in understanding the cellular and biochemical effects produced by centrally acting drugs, and the increasing use of brain imaging to study brain function and drug effects, the gulf between our understanding of drug action at the cellular level and at the functional and behavioural level remains, for the most part, very wide.

In some instances, our understanding of brain function and how drugs alter it is more advanced. Thus, the relationship between dopaminergic pathways in the extrapyramidal system and the effects of drugs in alleviating or exacerbating the symptoms of Parkinson's disease (see Ch. 39) is clear cut. Many CNS drugs are used to treat psychiatric disorders that are defined according to their symptomatology rather than on the basis of causative factors or clinical signs and investigations. What is called 'schizophrenia' or 'depression' on the basis of particular symptoms is likely to consist of several distinct disorders caused by different mechanisms and responding to drugs in different ways. Much effort is going into pinning down the biological basis of psychiatric disorders—a necessary step to improve the design of better drugs for clinical use—but the task is daunting and progress is slow.

In this chapter, we outline the general principles governing the action of drugs on the CNS. Most neuroactive drugs work by interfering with the chemical signals that underlie brain function, and the next two chapters discuss the major CNS transmitter systems and the ways in which drugs affect them. In Chapter 39, we focus on neurodegenerative diseases, and the remaining chapters in this section deal with the main classes of neuroactive drugs that are currently in use.

Background information will be found in neurobiology textbooks such as Kandel et al. (2000) and Bear et al. (2006), and in texts on neuropharmacology such as Nestler et al. (2008) and Iversen et al. (2009).

CHEMICAL SIGNALLING IN THE NERVOUS SYSTEM

The brain (like every other organ in the body!) is basically a chemical machine; it controls the main functions of a higher animal across timescales ranging from milliseconds (e.g. returning a 100 mph tennis serve) to years (e.g. remembering how to ride a bicycle).[2] The chemical signalling mechanisms cover a correspondingly wide dynamic range, as summarised, in a very general way, in Figure 36.1. Currently, we understand much about drug effects on events at the fast end of the spectrum—synaptic transmission and neuromodulation—but much less about long-term adaptive processes, although it is quite evident that the latter are of great importance for the neurological and psychiatric disorders that are susceptible to drug treatment.

The original concept of neurotransmission envisaged a substance released by one neuron and acting rapidly, briefly and at short range on the membrane of an adjacent (postsynaptic) neuron, causing excitation or inhibition. The principles outlined in Chapter 12 apply to the central as well as the peripheral nervous system. It is now clear that chemical mediators within the brain can produce slow and

[1] In Britain in 2008/2009, 145 million prescriptions (about 20% of all prescriptions), costing £1.7 billion, were for CNS drugs as defined by the *British National Formulary*. This amounted to over two per person across the whole population.

[2] Memory of drug names and the basic facts of pharmacology seems to come somewhere in the middle of this range (skewed towards the short end).

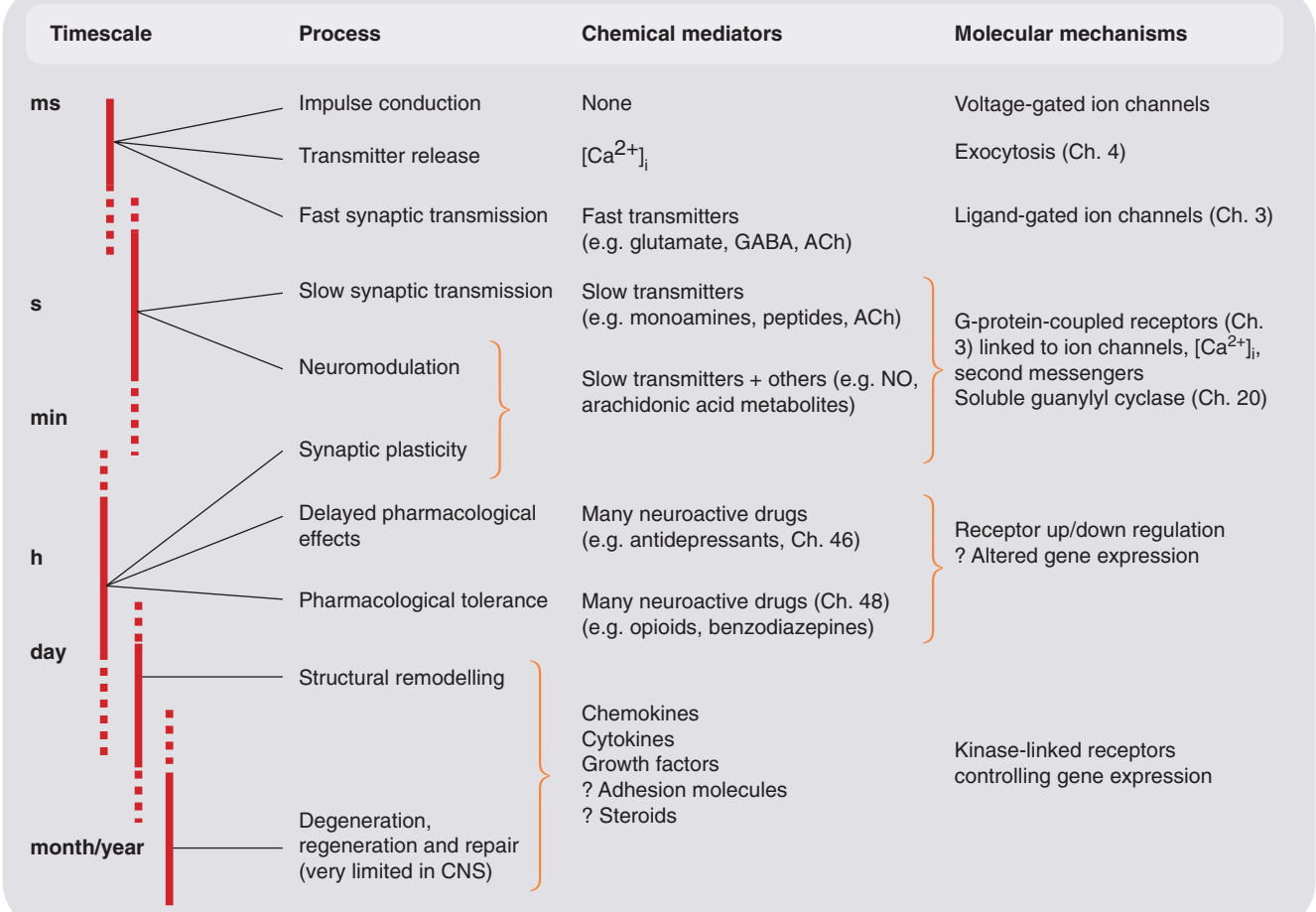

Fig. 36.1 Chemical signalling in the nervous system. Knowledge of the mediators and mechanisms becomes sparser as we move from the rapid events of synaptic transmission to the slower ones involving remodelling and alterations of gene expression. ACh, acetylcholine; CNS, central nervous system; NO, nitric oxide.

long-lasting effects; that they can act rather diffusely, at a considerable distance from their site of release; and that they can produce diverse effects, for example on transmitter synthesis, on the expression of neurotransmitter receptors and on neuronal morphology, in addition to affecting the ionic conductance of the postsynaptic cell membrane. The term *neuromodulator* is often used to denote a mediator, the actions of which do not conform to the original neurotransmitter concept. The term is not clearly defined, and it covers not only the diffusely acting neuropeptide mediators, but also mediators such as nitric oxide (NO) and arachidonic acid metabolites, which are not stored and released like conventional neurotransmitters, and may come from non-neuronal cells, particularly glia, as well as neurons. In general, *neuromodulation* relates to synaptic plasticity, including short-term physiological events such as the regulation of presynaptic transmitter release or postsynaptic excitability. Longer-term *neurotrophic* effects are involved in regulating the growth and morphology of neurons, as well as their functional properties. Table 36.1 summarises the types of chemical mediator that operate in the CNS.

Glial cells, particularly astrocytes, which are the main non-neuronal cells in the CNS and outnumber neurons by 10 to 1, also play an important signalling role. Once thought of mainly as housekeeping cells, whose function was merely to look after the fastidious neurons, they are increasingly seen as 'inexcitable neurons' with a major communications role (see Barres, 2008), albeit on a slower timescale than that of neuronal communication. These cells express a range of receptors and transporters similar to those present in neurons, and also release a wide variety of mediators, including glutamate, D-serine, lipid mediators and growth factors. They respond to chemical signals from neurons, and also from neighbouring astrocytes and microglial cells (the CNS equivalent of macrophages, which function much like inflammatory cells in peripheral tissues). Electrical coupling between astrocytes causes them often to respond in concert in a particular brain region, thus controlling the chemical environment in which the neurons operate. Although they do not conduct action potentials, and do not send signals to other parts of the body, astrocytes are otherwise very similar to neurons and play a crucial communication role within the brain. Because they are difficult to study in situ, however, our knowledge of how they function, and how they respond to drugs, is still fragmentary. It is an area to watch closely.

Table 36.1 Types of chemical mediators in the central nervous system

Mediator type[a]	Examples	Targets	Main functional role
Conventional small-molecule mediators	Glutamate, GABA, acetylcholine, dopamine, 5-hydroxytryptamine, etc.	Ligand-gated ion channels G-protein-coupled receptors	Fast and slow synaptic neurotransmission Neuromodulation
Neuropeptides	Substance P, neuropeptide Y, endorphins, corticotrophin-releasing factor, etc.	G-protein-coupled receptors	Neuromodulation
Lipid mediators	Prostaglandins, endocannabinoids	G-protein-coupled receptors	Neuromodulation
Nitric oxide	—	Guanylyl cyclase	Neuromodulation
Neurotrophins, cytokines	Nerve growth factor, brain-derived neurotrophic factor, interleukin-1	Kinase-linked receptors	Neuronal growth, survival and functional plasticity
Steroids	Androgens, oestrogens	Nuclear and membrane receptors	Functional plasticity

[a] Most central nervous system pharmacology is currently centred on small-molecule mediators and, less commonly, neuropeptides. Other mediator types have yet to be targeted successfully for therapeutic purposes.

Chemical transmission in the central nervous system

- The basic processes of synaptic transmission in the central nervous system are essentially similar to those operating in the periphery (Ch. 12).
- Glial cells, particularly astrocytes, participate actively in chemical signalling, functioning essentially as 'inexcitable neurons'.
- The terms *neurotransmitter*, *neuromodulator* and *neurotrophic factor* refer to chemical mediators that operate over different timescales. In general:
  - *neurotransmitters* are released by presynaptic terminals and produce rapid excitatory or inhibitory responses in postsynaptic neurons
  - fast neurotransmitters (e.g. glutamate, GABA) operate through ligand-gated ion channels
  - slow neurotransmitters and neuromodulators (e.g. dopamine, neuropeptides, prostanoids) operate mainly through G-protein-coupled receptors
  - *neuromodulators* are released by neurons and by astrocytes, and produce slower pre- or postsynaptic responses
  - *neurotrophic factors* are released mainly by non-neuronal cells and act on tyrosine kinase-linked receptors that regulate gene expression and control neuronal growth and phenotypic characteristics.
- The same agent (e.g. glutamate, 5-hydroxytryptamine, acetylcholine) may act through both ligand-gated channels and G-protein-coupled receptors, and function as both neurotransmitter and neuromodulator.
- Many chemical mediators, including glutamate, nitric oxide and arachidonic acid metabolites, are produced by glia as well as neurons.
- Many mediators (e.g. cytokines, chemokines, growth factors and steroids) control long-term changes in the brain (e.g. synaptic plasticity and remodelling), mainly by affecting gene transcription.

TARGETS FOR DRUG ACTION

▼ To recapitulate what was discussed in Chapters 2 and 3, neuroactive drugs act on one of four types of target proteins, namely ion channels, receptors, enzymes and transport proteins. Of the four main receptor families—ionotropic receptors, G-protein-coupled receptors, kinase-linked receptors and nuclear receptors—current drugs target mainly the first two.

In the last two or three decades, knowledge about these targets in the CNS has accumulated rapidly, particularly as follows.

- As well as 40 or more small-molecule and peptide mediators, the importance of other 'non-classical' mediators—nitric oxide, eicosanoids, growth factors, etc.—has become apparent (see Barañano et al., 2001).
- Considerable molecular diversity of known receptor molecules and ion channels (see Ch. 3) has been revealed.
- All the receptors and channels are expressed in at least three or four (often more) subtypes, with quite characteristic distributions in different brain areas. In most cases, we are only beginning to discover what this diversity means at a functional level, through the study of transgenic 'gene knockout' and 'gene knock-in' animals. From the pharmacological standpoint, the molecular diversity of such targets raises the possibility of developing drugs with improved selectivity of action, e.g. interacting with one kind of $GABA_A$ receptor without affecting others (see Ch. 43). The potential of these new approaches in terms of improved drugs for neurological and psychiatric diseases is large but as yet relatively untapped.
- The pathophysiology of neurodegeneration in conditions such as Alzheimer's disease and stroke is beginning to be understood (see Ch. 39), and progress is being made in understanding the mechanisms underlying drug dependence (see Ch. 48). These advances are suggesting new strategies for treating these disabling conditions. Other areas of brain research (e.g. the neurobiology of epilepsy, schizophrenia and depressive illnesses) are advancing less rapidly, but there is still progress to report.

DRUG ACTION IN THE CENTRAL NERVOUS SYSTEM

As we have already emphasised, the molecular and cellular mechanisms underlying drug action in the CNS and in the periphery are essentially similar. Understanding how drugs affect brain function is, however, made difficult by several factors. One is the complexity of neuronal

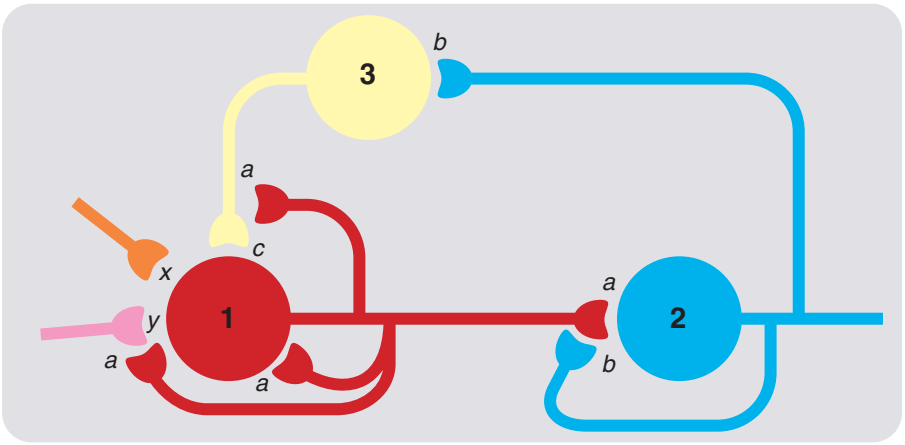

Fig. 36.2 Simplified scheme of neuronal interconnections in the central nervous system. Neurons 1, 2 and 3 are shown releasing transmitters *a*, *b* and *c*, respectively, which may be excitatory or inhibitory. Boutons of neuron 1 terminate on neuron 2, but also on neuron 1 itself, and on presynaptic terminals of other neurons that make synaptic connections with neuron 1. Neuron 2 also feeds back on neuron 1 via interneuron 3. Transmitters (*x* and *y*) released by other neurons are also shown impinging on neuron 1. Even with such a simple network, the effects of drug-induced interference with specific transmitter systems can be difficult to predict.

interconnections in the brain—the wiring diagram. Figure 36.2 illustrates in a schematic way the kind of interconnections that typically exist for, say, a noradrenergic neuron in the *locus coeruleus* (see Ch. 38), shown as **neuron 1** in the diagram, releasing **transmitter *a*** at its terminals. Release of *a* affects **neuron 2** (which releases **transmitter *b***), and also affects neuron 1 by direct feedback and, indirectly, by affecting presynaptic inputs impinging on neuron 1. The firing pattern of neuron 2 also affects the system, partly through interneuronal connections (**neuron 3**, releasing **transmitter *c***). Even at this grossly oversimplified level, the effects on the system of blocking or enhancing the release or actions of one or other of the transmitters are difficult to predict, and will depend greatly on the relative strength of the various excitatory and inhibitory synaptic connections, and on external inputs (*x* and *y* in the diagram). Added to this complexity at the level of neuronal interconnections is the influence of glial cells, mentioned above.

A further important complicating factor is that a range of secondary, adaptive responses is generally set in train by any drug-induced perturbation of the system. Typically, an increase in transmitter release, or interference with transmitter reuptake, is countered by inhibition of transmitter synthesis, enhanced transporter expression or decreased receptor expression. These changes, which involve altered gene expression, generally take time (hours, days or weeks) to develop and are not evident in acute pharmacological experiments.

In the clinical situation, the effects of psychotropic drugs often take weeks to develop, so it is likely that they reflect the adaptive responses rather than the immediate pharmacodynamic effects of the drug. This is well documented for antidepressant drugs (Ch. 46) and some antipsychotic drugs (Ch. 45). The development of dependence on drugs such as opioids, benzodiazepines and psychostimulants is similarly gradual (Ch. 48). Thus, one has to take into account not only the primary interaction of the drug with its target, but also the secondary response of the brain to this primary effect; it is often the secondary response, rather than the primary effect, which leads to clinical benefit.

BLOOD–BRAIN BARRIER

▼ A further important factor in CNS pharmacology is the existence of the blood–brain barrier (see Ch. 8), penetration of which requires molecules to traverse the vascular endothelial cells rather than going between them. In general, only small non-polar molecules can diffuse passively across cell membranes. Some neuroactive drugs penetrate the blood–brain barrier in this way, but many do so via transporters, which either facilitate entry into the brain or diminish it by pumping the compound from the endothelial cell interior back into the bloodstream. Drugs that gain entry in this way include **L-dopa** (Ch. 39), **valproate** (Ch. 44) and various sedative histamine antagonists (Ch. 17). Active extrusion of drugs from the brain occurs via P-glycoprotein, an ATP-driven drug efflux transporter, and related transporter proteins (see Ch. 8). Drugs that are excluded from the brain include many antibacterial and anticancer drugs while the brain concentrations of some CNS-acting drugs—including certain opioid, antidepressant,

Drug action in the central nervous system

- The basic types of drug target (ion channels, receptors, enzymes and transporter proteins) described in Chapter 3 apply in the central nervous system, as elsewhere.
- Most of these targets occur in several different molecular isoforms, giving rise to subtle differences in function and pharmacology.
- Many of the currently available neuroactive drugs are relatively non-specific, affecting several different targets, the principal ones being receptors, ion channels and transporters.
- The relationship between the pharmacological profile and the therapeutic effect of neuroactive drugs is often unclear.
- Slowly developing secondary responses to the primary interaction of the drug with its target are often important (e.g. the delayed efficacy of antidepressant drugs, and tolerance and dependence with opioids).

Table 36.2 General classification of drugs acting on the central nervous system

Class	Definition	Examples	See Chapter
General anaesthetic agents	Drugs used to produce surgical anaesthesia	Isoflurane, propofol	40
Analgesic drugs	Drugs used clinically for controlling pain	Opiates, carbamazepine, gabapentin	41
Anxiolytics and sedatives Synonyms: hypnotics, sedatives, minor tranquillisers	Drugs that reduce anxiety and cause sleep	Benzodiazepines	43
Antiepileptic drugs Synonym: anticonvulsants	Drugs used to reduce seizures	Carbamazepine, valproate, lamotrigine	44
Antipsychotic drugs Synonyms: neuroleptic drugs,[a] antischizophrenic drugs, major tranquillisers	Drugs used to relieve the symptoms of schizophrenic illness	Clozapine, haloperidol, risperidone	45
Antidepressant drugs	Drugs that alleviate the symptoms of depressive illness	Selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors	46
Psychomotor stimulants Synonym: psychostimulants	Drugs that cause wakefulness and euphoria	Amphetamine, cocaine, methylphenidate, caffeine	47
Psychotomimetic drugs Synonyms: hallucinogens, psychodysleptics[a]	Drugs that cause disturbance of perception (particularly visual hallucinations) and of behaviour in ways that cannot be simply characterised as sedative or stimulant effects	Lysergic acid diethylamide, mescaline, phencyclidine	47
Cognition enhancers[b] Synonyms: nootropic drugs	Drugs that improve memory and cognitive performance	Acetylcholinesterase inhibitors: donepezil, galantamine, rivastigmine NMDA receptor antagonists: memantine Others: piracetam	39 37

[a] These strange terms are the remnants of a classification proposed by Javet in 1903, who distinguished psycholeptics (depressants of mental function), psychoanaleptics (stimulants of mental function) and psychodysleptics (drugs that produce disturbed mental function). The term neuroleptic (literally 'nerve seizing') was coined 50 years later to describe chlorpromazine-like drugs. It gained favour, presumably by virtue of its brevity rather than its literal meaning.
[b] This is something of a wishful category, in that several classes of drugs that improve learning and memory in animal tests have not been shown to do so in humans.

antipsychotic and antiepileptic drugs—may be limited by active extrusion from the brain see (Linnet & Ejsing, 2008). In addition, variations in the activity of efflux transporters between individuals due to levels of expression or genetic variations and the potential for drug interactions at the level of these transporters due to inhibition or induction of activity by other drugs is becoming an important consideration.

THE CLASSIFICATION OF PSYCHOTROPIC DRUGS

Psychotropic drugs are defined as those that affect mood and behaviour. Because these indices of brain function are difficult to define and measure, there is no consistent basis for classifying psychotropic drugs. Instead, we find a confusing mêlée of terms relating to chemical structure (*benzodiazepines*, *butyrophenones*, etc.), biochemical target (*monoamine oxidase inhibitors*, *serotonin reuptake inhibitors*, etc.), behavioural effect (*hallucinogens*, *psychomotor stimulants*) or clinical use (*antidepressants*, *antipsychotic agents*, *antiepileptic drugs*, etc.), together with a number of indefinable rogue categories (*atypical antipsychotic drugs*, *nootropic drugs*) thrown in for good measure.

However, grumbling about terminology is fruitless. The general classification in Table 36.2 is based on that suggested in 1967 by the World Health Organization; although flawed, it provides a basis for the material presented later (Chs 37–48).

Also, some drugs defy classification in this scheme, for example **lithium** (see Ch. 46), which is used in the treatment of manic depressive psychosis, and **ketamine** (see Ch. 40), which is classed as a dissociative anaesthetic but produces psychotropic effects rather similar to those produced by phencyclidine.

In practice, the use of drugs in psychiatric illness frequently cuts across specific therapeutic categories. For example, it is common for antipsychotic drugs to be used as 'tranquillisers' to control extremely anxious or unruly patients, or to treat severe depression. Antidepressant drugs are often used to treat anxiety (Ch. 43) and neuropathic pain (Ch. 41), and certain psychostimulants are of proven efficacy for treating hyperactive children. Here we will adhere to the conventional pharmacological categories, but it needs to be emphasised that in clinical use these distinctions are often disregarded.

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37 Amino acid transmitters

OVERVIEW

In this chapter, we discuss the major neurotransmitters in the central nervous system (CNS), namely the excitatory transmitter, glutamate, and the inhibitory transmitters, GABA and glycine. It is an area in which scientific interest has been intense in recent years. Unravelling the complexities of amino acid receptors and signalling mechanisms has thrown considerable light on their role in brain function and their likely involvement in CNS disease. Drugs that target specific receptors and transporters have been developed, but translating this knowledge into drugs for therapeutic use is only now beginning to happen. Here, we present the pharmacological principles and include recent references for those seeking more detail.

EXCITATORY AMINO ACIDS

EXCITATORY AMINO ACIDS AS CNS TRANSMITTERS

L-Glutamate is the principal and ubiquitous excitatory transmitter in the CNS (see Cotman et al., 1995, for general review). **Aspartate** plays a similar role in certain brain regions, and possibly also **homocysteate**, but this is controversial.

▼ The realisation of glutamate's importance came slowly (see Watkins & Jane, 2006). By the 1950s, work on the peripheral nervous system had highlighted the transmitter roles of acetylcholine and catecholamines, and as the brain also contained these substances, there seemed little reason to look further. The presence of **γ-aminobutyric acid** (GABA; see below) in the brain, and its powerful inhibitory effect on neurons, were discovered in the 1950s, and its transmitter role was postulated. At the same time, work by Curtis's group in Canberra showed that glutamate and various other acidic amino acids produced a strong excitatory effect, but it seemed inconceivable that such workaday metabolites could actually be transmitters. Through the 1960s, GABA and excitatory amino acids (EAAs) were thought, even by their discoverers, to be mere pharmacological curiosities. In the 1970s, the humblest amino acid, glycine, was established as an inhibitory transmitter in the spinal cord, giving the lie to the idea that transmitters had to be exotic molecules, too beautiful for any role but to sink into the arms of a receptor. Once glycine had been accepted, the rest quickly followed. A major advance was the discovery of EAA antagonists, based on the work of Watkins in Bristol, which enabled the physiological role of glutamate to be established unequivocally, and also led to the realisation that EAA receptors are heterogeneous.

To do justice to the wealth of discovery in this field in the past two decades is beyond the range of this book; for more detail see Gereau & Swanson (2008). Here we concentrate on pharmacological aspects. After several beautiful but false dawns, a number of new drugs are in development on the basis of EAA mechanisms.[1] The major problem has been that EAA-mediated neurotransmission is ubiquitous in the brain and so agonist and antagonist drugs exert effects at many sites, giving rise not only to therapeutically beneficial effects, but also to other, unwanted harmful effects.

[1]**Memantine**, an N-methyl-D-aspartate (NMDA) antagonist, licensed for the treatment of moderate to severe Alzheimer's disease (Ch. 39), has been around for some time as has the dissociative anaesthetic, **ketamine**, an NMDA channel blocker.

METABOLISM AND RELEASE OF AMINO ACIDS

Glutamate is widely and fairly uniformly distributed in the CNS, where its concentration is much higher than in other tissues. It has an important metabolic role, the metabolic and neurotransmitter pools being linked by transaminase enzymes that catalyse the interconversion of glutamate and α-oxoglutarate (Fig. 37.1). Glutamate in the CNS comes mainly from either glucose, via the Krebs cycle, or glutamine, which is synthesised by glial cells and taken up by the neurons; very little comes from the periphery. The interconnection between the pathways for the synthesis of EAAs and inhibitory amino acids (GABA and glycine), shown in Figure 37.1, makes it difficult to use experimental manipulations of transmitter synthesis to study the functional role of individual amino acids, because disturbance of any one step will affect both excitatory and inhibitory mediators.

In common with other fast neurotransmitters, glutamate is stored in synaptic vesicles and released by Ca^{2+}-dependent exocytosis; specific transporter proteins account for its uptake by neurons and other cells, and for its accumulation by synaptic vesicles (see Ch. 12). Released glutamate is taken up into cells by $Na^+/H^+/K^+$ dependent transporters (cf. monoamine transporters—Chs 12 & 14), and transported into synaptic vesicles, by a different transporter driven by the proton gradient across the vesicle membrane. Several EAA transporters have been cloned and characterised in detail (see Shigeri et al., 2004). There may be value in developing enhancers and inhibitors of glutamate uptake (see Bunch et al., 2009) for the treatment of CNS disorders in which the level of extracellular glutamate may be abnormal, e.g. neurodegeneration (see Ch. 39), schizophrenia (see Ch. 45) and depression (see Ch. 46). In contrast to the situation with monoamine synthesis and transport (Chs 14 and 38), few drugs (none in clinical use) are known that interfere specifically with glutamate metabolism.

The action of glutamate is terminated mainly by carrier-mediated reuptake into the nerve terminals and neighbouring astrocytes (Fig. 37.2). This transport can, under some circumstances (e.g. depolarisation by increased extracellular $[K^+]$), operate in reverse and constitute a source of glutamate release (see Takahashi et al., 1997), a process that may occur under pathological conditions such as brain ischaemia (see Ch. 39). Glutamate taken up by astrocytes is converted to glutamine and recycled, via transporters, back to the neurons, which convert the glutamine back to glutamate. Glutamine, which lacks the pharmacological activity of glutamate, thus serves as a pool of inactive transmitter under the regulatory control of the astrocytes, which act as ball boys, returning the ammunition in harmless form in order to rearm the neurons.

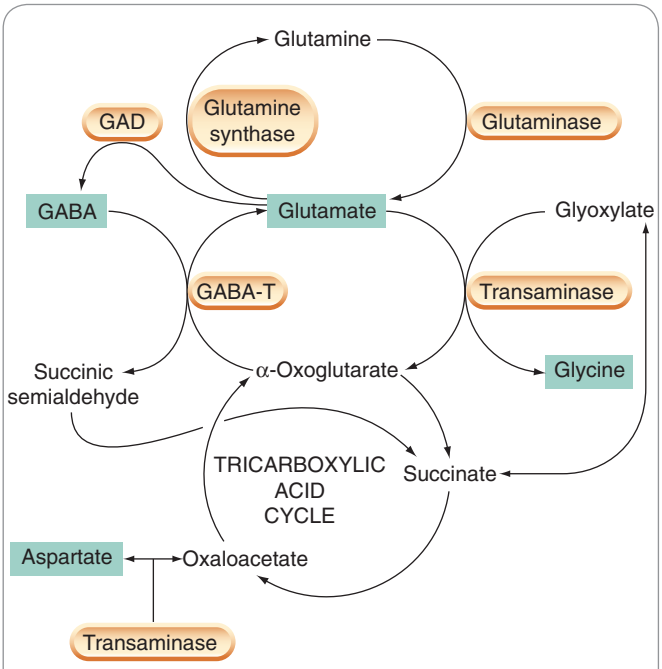

Fig. 37.1 Metabolism of transmitter amino acids in the brain. Transmitter substances are marked with green boxes. GABA-T, GABA transaminase; GAD, glutamic acid decarboxylase.

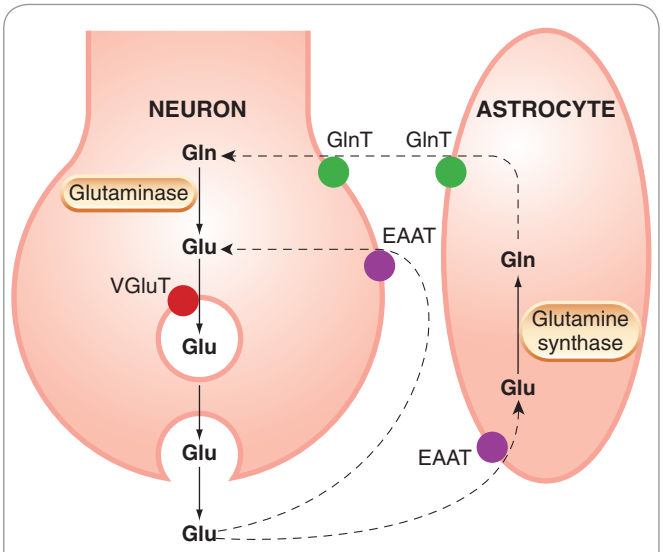

Fig. 37.2 Transport of glutamate (Glu) and glutamine (Gln) by neurons and astrocytes. Released glutamate is captured partly by neurons and partly by astrocytes, which convert most of it to glutamine. EAAT, excitatory amino acid transporter; GlnT, glutamine transporter; VGluT, vesicular glutamate transporter.

GLUTAMATE

GLUTAMATE RECEPTOR SUBTYPES

Glutamate and related excitatory amino acids activate both ionotropic (ligand-gated cation channels) and metabotropic (G-protein-coupled) receptors (see Ch. 3 for a general description of ionotropic and metabotropic receptors).

IONOTROPIC GLUTAMATE RECEPTORS

On the basis of studies with selective agonists and antagonists (Fig. 37.3), three main subtypes of ionotropic receptors for glutamate can be distinguished: **NMDA**, **AMPA** and **kainate**[2] receptors, named originally according to their specific agonists (Table 37.1). These ligand-gated channels can be homomeric or heteromeric assemblies of four subunits, each with the 'pore loop' structure shown in Figure 3.18. There are some 16 different receptor subunits and their nomenclature has, until recently, been somewhat confusing.[3] Here, in this brief, general description, we use the new International Union of Basic and Clinical Pharmacology (IUPHAR) recommended terminology because it simplifies the subject considerably, but beware confusion when reading older papers. NMDA receptors are assembled from seven types of subunit (GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, GluN3B). The subunits comprising AMPA receptors (GluA1–4)[4] and kainate receptors (GluK1–5), are closely related to, but distinct from, GluN subunits. Receptors comprising different subunits can have different pharmacological and physiological characteristics, e.g. AMPA receptors lacking the GluA2 subunit have much higher permeability to Ca^{2+} than the others, which has important functional consequences (see Ch. 4).

AMPA receptors, and in certain brain regions kainate receptors (see Bleakman & Lodge, 1998), serve to mediate fast excitatory synaptic transmission in the CNS—absolutely essential for our brains to function. Kainate and NMDA receptors are also expressed on nerve terminals where they can enhance or reduce transmitter release (see Corlew et al., 2008; Jane et al., 2009).[5] AMPA receptors occur on astrocytes as well as on neurons, and these cells play an important role in communication in the brain. Postsynaptic NMDA receptors (which often coexist with AMPA receptors) contribute a slow component to the excitatory synaptic potential (Fig. 37.4), the magnitude of which varies in different pathways.

Binding studies show that ionotropic glutamate receptors are most abundant in the cortex, basal ganglia and sensory pathways. NMDA and AMPA receptors are generally co-localised, but kainate receptors have a much more restricted distribution. Expression of the many different receptor subtypes in the brain also shows distinct regional differences, but we have hardly begun to understand the significance of this extreme organisational complexity.

[2]In the past, AMPA and kainate receptors were often lumped together as AMPA/kainate or non-NMDA receptors, but nowadays it is realised that they each have distinct subunit compositions and should not be grouped together.

[3]An international committee has sought to bring order to the area but, despite the logic of their recommendations, how generally accepted they will be remains to be seen (see Collingridge et al., 2009). Scientists can get very stuck in their ways.

[4]AMPA receptor subunits are also subject to other kinds of variation, namely alternative splicing, giving rise to the engagingly named *flip* and *flop* variants, and RNA editing at the single amino acid level, both of which contribute yet more functional diversity to this diverse family.

[5]In the CNS, presynaptic ligand-gated ion channels such as kainate and NMDA receptors as well as nicotinic and P2X receptors (see Ch. 38) control neurotransmitter release. An explanation of how this control can be either facilitatory or inhibitory is given in Khahk & Henderson (2000).

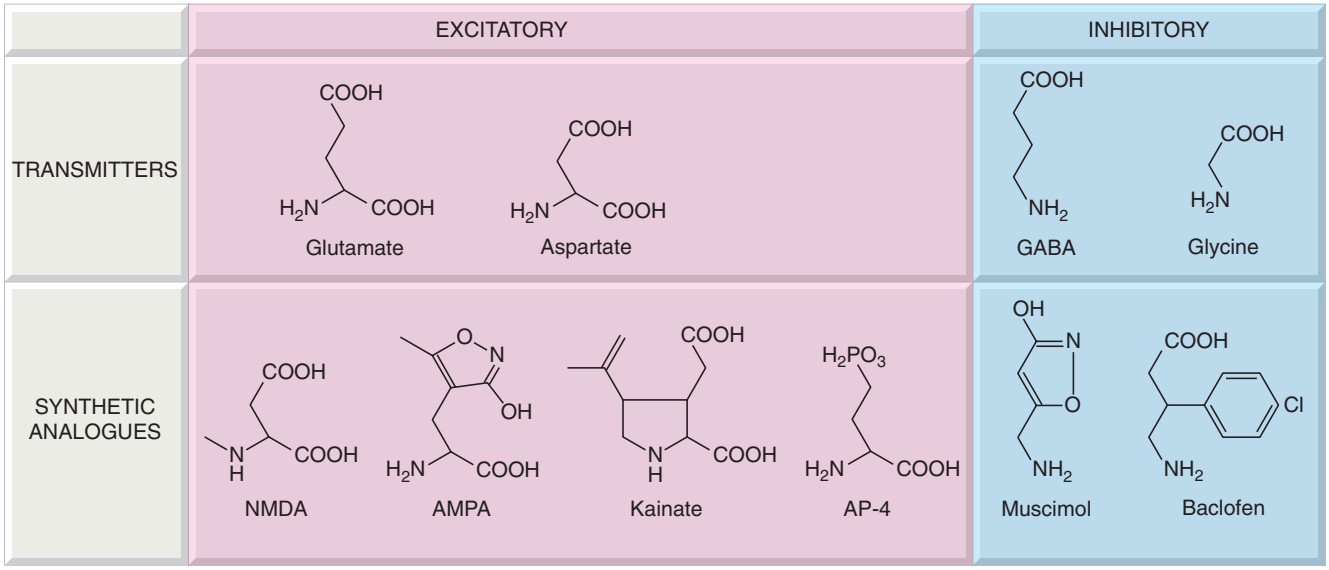

Fig. 37.3 Structures of agonists acting on glutamate, GABA and glycine receptors. The receptor specificity of these compounds is shown in Tables 37.1 and 37.2. AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propianic acid; AP-4, 3-amino-4-phosphonopentanoic acid; NMDA, N-methyl-D-asparatic acid.

Special features of NMDA receptors

NMDA receptors and their associated channels have been studied in more detail than the other types and show special pharmacological properties, summarised in Fig. 37.5, which are postulated to play a role in pathophysiological mechanisms.

- They are highly permeable to Ca^{2+}, as well as to other cations, so activation of NMDA receptors is particularly effective in promoting Ca^{2+} entry.
- They are readily blocked by Mg^{2+}, and this block shows marked voltage dependence. It occurs at physiological Mg^{2+} concentrations when the cell is normally polarised, but disappears if the cell is depolarised.
- Activation of NMDA receptors requires glycine as well as glutamate (Fig. 37.6). The binding site for glycine is distinct from the glutamate binding site, and both have to be occupied for the channel to open. This discovery by Johnson and Ascher caused a stir, because glycine had hitherto been recognised as an inhibitory transmitter (see below), so to find it facilitating excitation ran counter to the prevailing doctrine. The concentration of glycine required depends on the subunit composition of the NMDA receptor: for some NMDA receptor subtypes, physiological variation of the glycine concentration may serve as a regulatory mechanism, whereas others are fully activated at all physiological glycine concentrations. Competitive antagonists at the glycine site (see Table 37.1) indirectly inhibit the action of glutamate. **D-serine**, somewhat surprisingly,[6] has been found to activate the NMDA receptor via the glycine site and to be released from astrocytes.
- Certain well-known anaesthetic and psychotomimetic agents, such as **ketamine** (Ch. 40) and **phencyclidine** (Ch. 47), are selective blocking agents for NMDA-operated channels. The experimental compound **dizocilpine** (codename MK801) shares this property.
- Certain endogenous polyamines (e.g. **spermine**, **spermidine**) act on a different accessory site to facilitate channel opening. The experimental drugs **ifenprodil** and **eliprodil** block their action.

METABOTROPIC GLUTAMATE RECEPTORS

There are eight different metabotropic glutamate receptors ($mGlu_{1-8}$) which are unusual in showing no sequence homology with other G-protein-coupled receptors (Ferraguti & Shigemoto, 2006). They function as homodimers (see Ch. 3) cross-linked by a disulfide bridge across the extracellular domain of each protein (see Goudet et al., 2009). They are members of class C G-protein-coupled receptors, possessing a large extracellular N terminus domain that forms a venus fly trap-like structure into which glutamate binds. They can be divided into three groups on the basis of their sequence homology, G-protein coupling and pharmacology (see Table 37.2). Alternatively spliced receptor variants have been reported.

mGlu receptors are widely distributed throughout the central nervous system (see Ferraguti & Shigemoto, 2006) on neurons, where they regulate cell excitability and synaptic transmission, and on glia. Neuronal group 1 mGlu receptors are located postsynaptically and are largely excitatory. By raising intracellular $[Ca^{2+}]$, they modify responses through ionotropic glutamate receptors (see Fig. 37.7). Group 2 and 3 mGlu receptors are mostly presynaptic receptors and their activation tends to reduce synaptic transmission and neuronal excitability. They can be autoreceptors, involved in reducing glutamate release or heteroreceptors, e.g. when present on GABA-containing terminals.

[6]Surprising, because it is the 'wrong' enantiomer for amino acids of higher organisms. Nevertheless, vertebrates possess specific enzymes and transporters for this D-amino acid, which is abundant in the brain.

Table 37.1 Properties of ionotropic glutamate receptors

	NMDA		AMPA	Kainate
Subunit composition	Tetramers consisting of GluN1–3 subunits		Tetramers consisting of GluA1–4 subunits (variants splicing and RNA editing)	Tetramers consisting of GluK1–5 subunits
	Receptor site	**Modulatory site (glycine)**		
Endogenous agonist(s)	Glutamate Aspartate	Glycine D-Serine	Glutamate	Glutamate
Other agonist(s)[a]	NMDA	Cycloserine	AMPA Quisqualate	Kainate Domoate[b]
Antagonist(s)[a]	AP-5, CPP	7-Chloro-kynurenic acid, HA-466	NBQX	NBQX ACET
Other modulators	Polyamines (e.g. spermine, spermidine) Mg^{2+}, Zn^{2+}		Cyclothiazide Piracetam CX-516	—
Channel blockers	Dizocilpine (MK801) Phencyclidine Ketamine Remacemide Memantine Mg^{2+}		—	—
Effector mechanism	Ligand-gated cation channel (slow kinetics, high Ca^{2+} permeability)		Ligand-gated cation channel (fast kinetics; channels possessing GluR2A subunits show low Ca^{2+} permeability)	Ligand-gated cation channel (fast kinetics, low Ca^{2+} permeability)
Location	Postsynaptic (some presynaptic, also glial) Wide distribution		Postsynaptic (also glial)	Pre- and postsynaptic
Function	Slow epsp Synaptic plasticity (long-term potentiation, long-term depression) Excitotoxicity		Fast epsp Wide distribution	Fast epsp Presynaptic inhibition Limited distribution

[a] Structures of experimental compounds can be found in Brauner-Osborne et al. 2002 (J Med Chem 43: 2609–2645).
[b] A neurotoxin from mussels (see Ch. 39).
ACET, -(*S*)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione; AP-5, 2-amino-5-phosphonopentanoic acid; CPP, 3-(2-carboxypirazin-4-yl)-propyl-1-phosphonic acid; CX-516, 1-(quinoxalin-6-ylcarbonyl)-piperidine; epsp, excitatory postsynaptic potential; NBQX, 2,3-dihydro-6-nitro-7-sulfamoyl-benzoquinoxaline. (Other structures are shown in Figure 37.3.)

SYNAPTIC PLASTICITY AND LONG-TERM POTENTIATION

▼ In general, it appears that NMDA and mGlu receptors play a particular role in long-term adaptive and pathological changes in the brain, and are of particular interest as potential drug targets. AMPA receptors, on the other hand, are mainly responsible for fast excitatory transmission, and if they are fully blocked, brain function shuts down entirely; nevertheless, they too are involved in synaptic plasticity.

Two aspects of glutamate receptor function are of particular pathophysiological importance, namely *synaptic plasticity*, discussed here, and *excitotoxicity* (discussed in Ch. 39).

Synaptic plasticity is a general term used to describe long-term changes in synaptic connectivity and efficacy, either following physiological alterations in neuronal activity (as in learning and memory), or resulting from pathological disturbances (as in epilepsy, chronic pain or drug dependence). Synaptic plasticity underlies much of what we call 'brain function'. Needless to say, no single mechanism is responsible; however, one significant and much-studied component is *long-term potentiation* (LTP), a phenomenon in which AMPA and NMDA receptors play a central role.

Long-term potentiation (LTP; see Bennett, 2000; Bear et al., 2006) is a prolonged (hours in vitro, days or weeks in vivo) enhancement of synaptic transmission that occurs at various CNS synapses following a short (conditioning) burst of high-frequency presynaptic stimulation. Its counterpart is *long-term depression* (LTD), which is produced at some synapses by a longer train of stimuli at lower frequency (see Massey & Bashir, 2007). These phenomena have been studied at various synapses in the CNS, most especially in the hippocampus which plays a central role in learning and memory (Fig. 37.4). It has been argued that 'learning', in the synaptic sense, can occur if synaptic strength is enhanced following simultaneous activity in both pre- and postsynaptic neurons. LTP shows this characteristic; it does not occur if presynaptic activity fails to excite the postsynaptic neuron, or if the latter is activated independently, for instance by a different presynaptic input. The mechanisms underlying both LTP and LTD differ somewhat at different synapses in the brain (see Bear et al., 2006). Here only a brief, generic view of the underlying events is given. LTP initiation may involve both presynaptic and postsynaptic components, and results from enhanced activation of postsynaptic AMPA receptors at EAA synapses and (probably) to enhanced glutamate release (although the argument rumbles on about whether increased transmitter release does or does not occur in LTP; see Blundon & Zakharenko, 2008). The response of postsynaptic AMPA

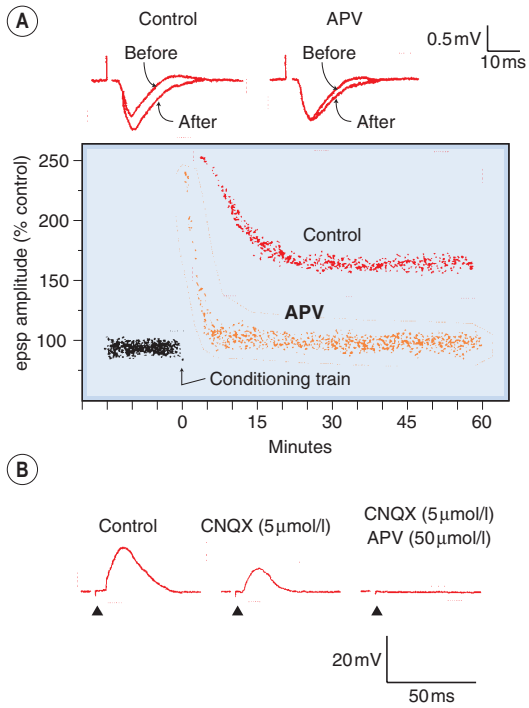

Fig. 37.4 Effects of excitatory amino acid receptor antagonists on synaptic transmission. [**A**] APV (NMDA antagonist) prevents long-term potentiation (LTP) in the rat hippocampus without affecting the fast excitatory postsynaptic potential (epsp). Top records show the extracellularly recorded fast epsp (downward deflection) before, and 50 min after, a conditioning train of stimuli (100 Hz for 2 s). The presence of LTP in the control preparation is indicated by the increase in epsp amplitude. In the presence of APV (50 µmol/l), the normal epsp is unchanged, but LTP does not occur. Lower trace shows epsp amplitude as a function of time. The conditioning train produces a short-lasting increase in epsp amplitude, which still occurs in the presence of APV, but the long-lasting effect is prevented. [**B**] Block of fast and slow components of epsp by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; AMPA receptor antagonist) and APV (NMDA receptor antagonist). The epsp (upward deflection) in a hippocampal neuron recorded with intracellular electrode is partly blocked by CNQX (5 µmol/l), leaving behind a slow component, which is blocked by APV (50 µmol/l). (From: [**A**] Malinow R, Madison D, Tsien R W 1988 Nature 335: 821; [**B**] Andreasen M, Lambert J D, Jensen M S 1989 J Physiol 414: 317–336.)

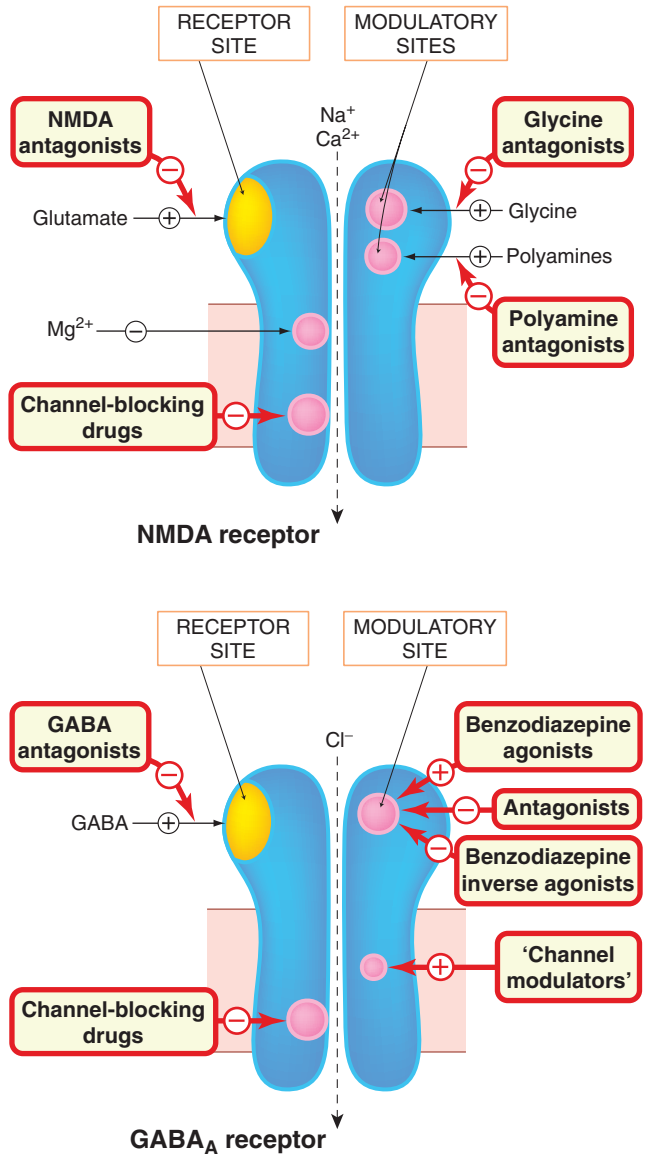

Fig. 37.5 Main sites of drug action on NMDA and $GABA_A$ receptors. Both receptors are multimeric ligand-gated ion channels. Drugs can act as agonists or antagonists at the neurotransmitter receptor site or at modulatory sites associated with the receptor. They can also act to block the ion channel at one or more distinct sites. In the case of the $GABA_A$ receptor, the mechanism by which 'channel modulators' (e.g. ethanol, anaesthetic agents) facilitate channel opening is uncertain; they may affect both ligand binding and channel sites. The location of the different binding sites shown in the figure is largely imaginary, although study of mutated receptors is beginning to reveal where they actually reside. Examples of the different drug classes are given in Tables 37.1 and 37.3.

receptors to glutamate is increased due to phosphorylation of the AMPA receptor subunits by kinases such as Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC), thus enhancing their conductance, as well as to increased expression and trafficking of AMPA receptors to synaptic sites. LTD, on the other hand, results from modest Ca^{2+} entry into the cell through AMPA receptors (NMDA receptors remain blocked by Mg^{2+}) activating phosphatases that reduce AMPA receptor phosphorylation and insertion into the plasma membrane.

LTP is reduced by agents that block the synthesis or effects of nitric oxide or arachidonic acid. These mediators (see Chs 17 and 20) may act as retrograde messengers through which events in the postsynaptic cell are able to influence the presynaptic nerve terminal. *Anandamide*, released by the postsynaptic cell, may also play a role by reducing the release of GABA from inhibitory nerve endings (see Ch. 18).

Two special properties of the NMDA receptor underlie its involvement in LTP, namely voltage-dependent channel block by Mg^{2+} and its high Ca^{2+} permeability. At normal membrane potentials, the NMDA channel is blocked by Mg^{2+}; a sustained postsynaptic depolarisation produced by glutamate acting repeatedly on AMPA receptors, however, removes the Mg^{2+} block, and NMDA receptor activation then allows Ca^{2+} to enter the cell. Activation of group 1 mGlu receptors also contributes to the increase in $[Ca^{2+}]_i$. This rise in $[Ca^{2+}]_i$ in

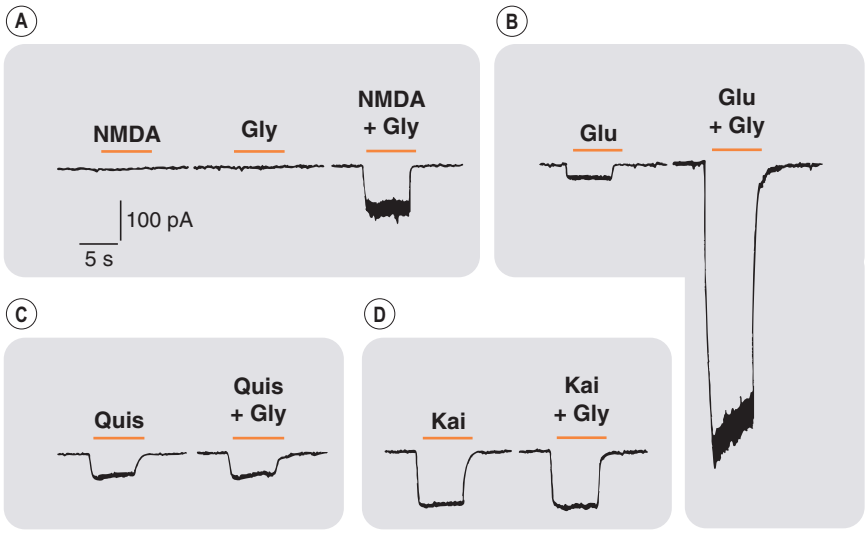

Fig. 37.6 Facilitation of NMDA by glycine. Recordings from mouse brain neurons in culture (whole-cell patch clamp technique). Downward deflections represent inward current through excitatory amino acid-activated ion channels. [**A**] NMDA (10 μmol/l) or glycine (1 μmol/l) applied separately had little or no effect, but together produced a response. [**B**] The response to glutamate (Glu,10 μmol/l) was strongly potentiated by glycine (Gly, 1 μmol/l). [**C**] and [**D**] Responses of AMPA and kainate receptors to quisqualate (Quis) and kainate (Kai) were unaffected by glycine. (From Johnson J W, Ascher P 1987 Nature 325: 529–531.)

Table 37.2 Metabotropic glutamate receptors

	Group 1	Group 2	Group 3
Members	$mGlu_1$, $mGlu_5$	$mGlu_2$, $mGlu_3$	$mGlu_4$, $mGlu_6$,[a] $mGlu_7$, $mGlu_8$
G-protein coupling	G_q	G_i/G_o	G_i/G_o
Agonist	DHPG CHPG[b]	LY354740	L-AP4 (S)-3,4-DCPG[c]
Antagonist	LY367385[d] S-4-CPG	LY341495	CPPG
Neuronal location	Somatodendritic	Somatodendritic and nerve terminals	Nerve terminals

[a] $mGlu_6$ is found only in the retina. [b] $mGlu_5$ selective. [c] $mGlu_8$ selective. [d] $mGlu_1$ selective.
CHPG, (RS)-2-chloro-5-hydroxyphenylglycine; CPPG, (RS)-α-cyclopropyl-4-phosphonophenylglycine; DHPG, 3,5-dihydroxyphenylglycine; L-AP4, 2-amino-4-phosphonobutyrate; (S)-3,4-DCPG, (S)-3,4-dicarboxyphenylglycine; S-4-CPG, (S)-4-carboxy-3-hydroxyphenylglycine.

the postsynaptic cell activates protein kinases, phospholipases and nitric oxide synthase, which act jointly with other cellular proceses (by mechanisms that are not yet fully understood) to facilitate transmission via AMPA receptors. Initially, during the induction phase of LTP, phosphorylation of AMPA receptors increases their responsiveness to glutamate. Later, during the maintenance phase, more AMPA receptors are recruited to the membrane of postsynaptic dendritic spines as a result of altered receptor trafficking; later still, various other mediators and signalling pathways are activated, causing structural changes and leading to a permanent increase in the number of synaptic contacts.

The general description of LTP given above is intended to provide the uninitiated reader with an overview of the topic. There are subtle differences in its forms and in the mechanisms underlying it at different synapses in the CNS. How LTP, in all of its guises, relates to different forms of memory is slowly being worked out (see Bear et al., 2006; Kessels & Malinow, 2009). Thus there is hope that drugs capable of enhancing LTP may improve learning and memory.

DRUGS ACTING ON GLUTAMATE RECEPTORS

ANTAGONISTS AND NEGATIVE MODULATORS

Inotropic glutamate receptor antagonists

The main types and examples of ionotropic glutamate antagonists are shown in Table 37.1. They are selective for the main receptor types but generally not for specific subtypes. Many of these compounds, although very useful as experimental tools in vitro, are unable to penetrate the blood–brain barrier, so they are not effective when given systemically.

NMDA receptors, as discussed above, require glycine as well as NMDA to activate them, so blocking of the glycine site is an alternative way to produce antagonism. **Kynurenic acid** and the more potent analogue **7-chloro-kynurenic acid** act in this way, as do various compounds currently in

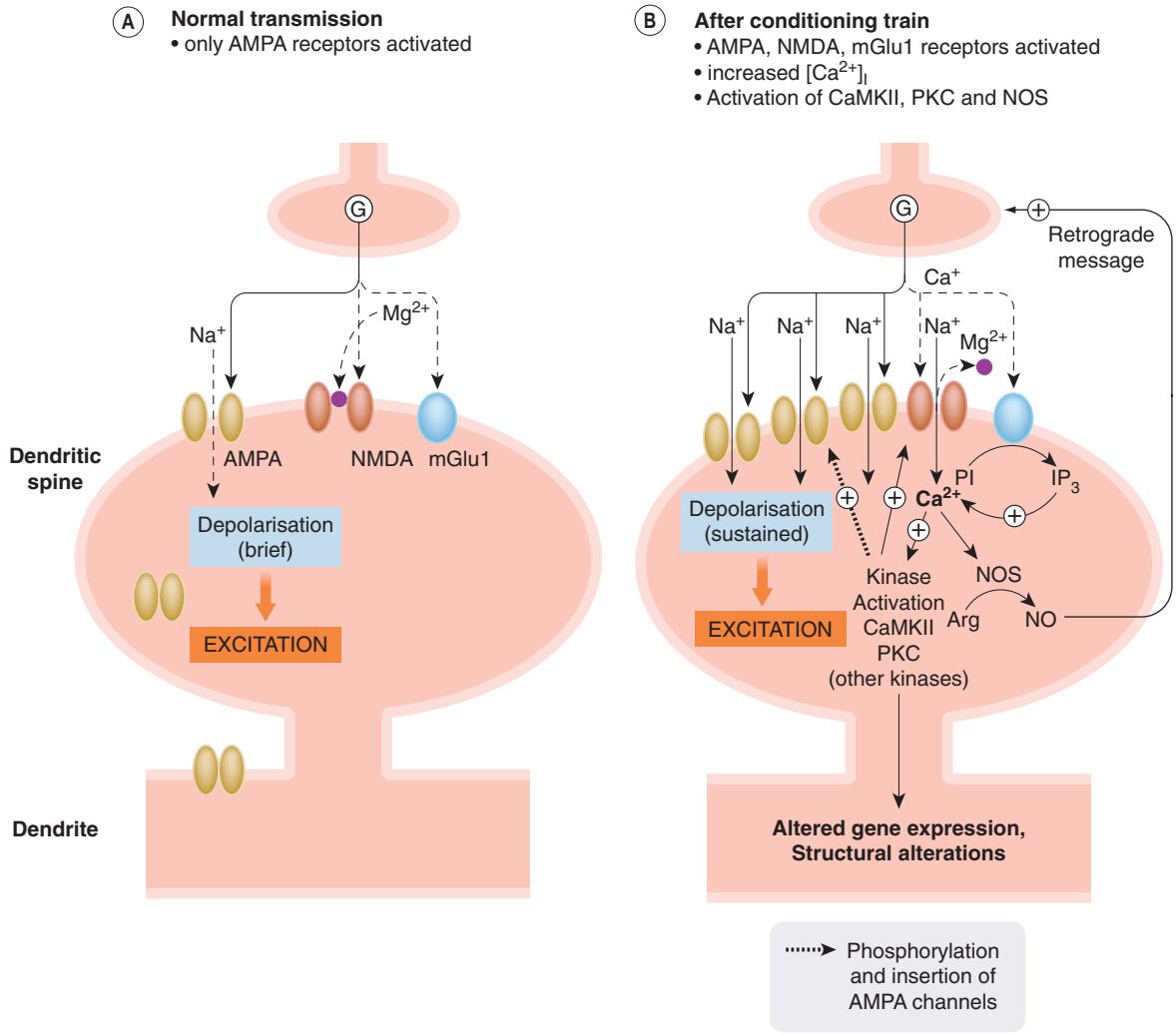

Fig. 37.7 Mechanisms of long-term potentiation. [**A**] With infrequent synaptic activity, glutamate (G) activates mainly AMPA receptors. There is insufficient glutamate to activate metabotropic receptors, and NMDA receptor channels are blocked by Mg^{2+}. [**B**] After a conditioning train of stimuli, enough glutamate is released to activate metabotropic receptors, and NMDA channels are unblocked by the sustained depolarisation. The resulting increase in $[Ca^{2+}]_i$ activates various enzymes, including the following:

- Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC) phosphorylates various proteins, including AMPA receptors (causing them to be trafficked to areas of synaptic contact on dendritic spines and facilitation of transmitter action) and other signal transduction molecules controlling gene transcription (not shown) in the postsynaptic cell.
- Nitric oxide synthase (NOS). Release of nitric oxide (NO) facilitates glutamate release (retrograde signalling, otherwise known as NO turning back).
- Phospholipase A_2 (not shown) catalyses the formation of arachidonic acid (Ch. 17), a retrograde messenger that increases presynaptic glutamate release.
- A phospholipase (NAPE-PLD, not shown) that catalyses production of the endocannabinoid, anandamide (Ch. 18). Anandamide appears to act on GABAergic inhibitory nerve terminals, enhancing transmission by suppressing GABA release.

Arg, arginine; IP_3, inositol (1,4,5) trisphosphate; NO, nitric oxide; PI, phosphatidylinositol.

development. Another site of block is the channel itself, where various substances act, for example **ketamine** and **phencyclidine**. **Dizocilpine**, **remacemide** and **memantine** are more recent examples. These agents are lipid soluble and thus able to cross the blood–brain barrier.

The potential therapeutic interest in ionotropic glutamate receptor antagonists lies mainly in the reduction of brain damage following strokes and head injury (Ch. 39), as well as in the treatment of epilepsy (Ch. 44) and Alzheimer's disease (Ch. 39). They have also been considered for indications such as drug dependence (Ch. 48) and schizophrenia (Ch. 45). Trials with NMDA antagonists and channel blockers have so far proved disappointing, and a serious drawback of these agents is their tendency to cause hallucinatory and other disturbances (also a feature of phencyclidine; Ch. 47). Only two NMDA receptor antagonists, **ketamine** (anaesthesia and analgesia; see Chs 40 and 41) and **memantine** (Alzheimer's disease; Ch. 39), are in

clinical use. It is possible that antagonists selective for NMDA receptors containing the GluN2B subunit, which is highly Ca^{2+} permeable, may be effective for treating neurodegeneration and have fewer CNS side effects. Glycine site antagonists may also have fewer unwanted effects, and experimental compounds have been tested in clinical trials for conditions such as stroke and epilepsy (see Jansen & Dannhart, 2003); the results have been inconclusive. AMPA receptor antagonists seem unpromising as therapeutic agents, because the available agents (as might be expected) produce overall CNS depression, including respiratory depression, cognition impairment and motor incoordination, with little margin of safety. Only if subtype selectivity can be achieved is this approach likely to succeed. The prospects for kainate receptor antagonists appear more promising—antagonists for GluK1 have shown potential for the treatment of pain, migraine, epilepsy, stroke and anxiety (see Jane et al., 2009).

Overall, the promise foreseen for ionotropic glutamate receptor antagonists in the clinic has simply not, so far, been fulfilled. The problem may be that glutamate is such a ubiquitous and multifunctional mediator—involved, it seems, in almost every aspect of brain function—that attempting to improve a specific malfunction by flooding the brain with a compound that affects the glutamate system in some way is just too crude a strategy.

Metabotropic glutamate receptor antagonists

While antagonists that discriminate between the different groups of mGlu receptors are available (see Table 37.2), it has proven more difficult to develop selective antagonists for the subtypes within the groups. mGlu receptors, like many G-protein-coupled receptors, possess allosteric modulatory sites (see Ch. 3). Allosteric modulation can be either inhibitory or facilitatory. Antagonists or negative modulators acting at group 1 mGlu receptors have potential for the treatment of various pain states, Parkinson's disease, neuroprotection, epilepsy and drug abuse; whereas antagonists or negative modulators of group 2 mGlu receptors have potential as cognition enhancers (Kew, 2004).

AGONISTS AND POSITIVE MODULATORS

Ionotropic glutamate receptors

Various agonists at ionotropic glutamate receptors that are used experimentally are shown in Table 37.1. From the clinical perspective, interest centres on the theory that positive AMPA receptor modulators may improve memory and cognitive performance. Examples include **cyclothiazide**, **piracetam** and CX-516 (**Ampalex**). These positive modulators, known as *ampakines*, are allosteric modulators and can act in subtly different ways to increase response amplitude, slow deactivation and attenuate desensitisation of AMPA receptor-mediated currents. They therefore increase AMPA-mediated synaptic responses and enhance long-term potentiation as well as upregulating the production of nerve growth factors such as *brain-derived neurotrophic factor* (BDNF). They are thought to have therapeutic potential as cognition enhancers and in the treatment of schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and Parkinson's disease (see Lynch, 2006).

Metabotropic glutamate receptors

Agonists at group 2 and 3 mGlu receptors decrease glutamate release. They therefore have therapeutic potential to decrease neuronal cell death in stroke and in the treatment of epilepsy and anxiety as well as in controlling the positive symptoms of schizophrenia. As with antagonists (see above), developing selective agonists of mGlu receptors has proven to be quite difficult; the hope is that it will be easier to develop highly selective positive allosteric modulators (see Kew, 2004).

Excitatory amino acids

- Excitatory amino acids (EAAs), namely glutamate, aspartate and possibly homocysteate, are the main fast excitatory transmitters in the central nervous system.
- Glutamate is formed mainly from the Krebs cycle intermediate α-oxoglutarate by the action of GABA transaminase.
- There are three main ionotropic glutamate receptors and eight metabotropic receptors.
- NMDA, AMPA and kainate receptors are ionotropic receptors regulating cation channels.
- The channels controlled by NMDA receptors are highly permeable to Ca^{2+} and are blocked by Mg^{2+}.
- AMPA and kainate receptors are involved in fast excitatory transmission; NMDA receptors mediate slower excitatory responses and, through their effect in controlling Ca^{2+} entry, play a more complex role in controlling synaptic plasticity (e.g. long-term potentiation).
- Competitive NMDA receptor antagonists include **AP-5** (2-amino-5-phosphonopentanoic acid) and CPP (3-(2-carboxypirazin-4-yl)-propyl-1-phosphonic acid); the NMDA-operated ion channel is blocked by **dizocilpine**, as well as by the psychotomimetic drugs **ketamine** and **phencyclidine**.
- **NBQX** (2,3-dihydro-6-nitro-7-sulfamoyl-benzoquinoxaline) is an AMPA and kainate receptor antagonist.
- NMDA receptors require low concentrations of glycine as a co-agonist, in addition to glutamate; 7-chlorokynurenic acid blocks this action of glycine.
- NMDA receptor activation is increased by endogenous polyamines, such as **spermine**, acting on a modulatory site that is blocked by **ifenprodil**.
- The entry of excessive amounts of Ca^{2+} produced by NMDA receptor activation can result in cell death—excitotoxicity (see Ch. 39).
- Metabotropic glutamate receptors ($mGlu_{1-8}$) are dimeric G-protein-coupled receptors. $mGlu_1$ and $mGlu_5$ receptors couple through G_q to inositol trisphosphate formation and intracellular Ca^{2+} release. They play a part in glutamate-mediated synaptic plasticity and excitotoxicity. The other mGlu receptors couple to G_i/G_o and inhibit neurotransmitter release, most importantly glutamate release.
- Specific metabotropic glutamate receptor agonists and antagonists are available as are positive and negative allosteric modulators.
- Glutamate receptor antagonists have yet to be developed for clinical use.

γ-AMINOBUTYRIC ACID

GABA is the main inhibitory transmitter in the brain. In the spinal cord and brain stem, glycine is also important.

SYNTHESIS, STORAGE AND FUNCTION

GABA occurs in brain tissue but not in other mammalian tissues, except in trace amounts. It is particularly abundant (about 10 μmol/g tissue) in the nigrostriatal system, but occurs at lower concentrations (2–5 μmol/g) throughout the grey matter.

GABA is formed from glutamate (Fig. 37.1) by the action of glutamic acid decarboxylase (GAD), an enzyme found only in GABA-synthesising neurons in the brain. Immunohistochemical labelling of GAD is used to map the GABA pathways in the brain. GABAergic neurons and astrocytes take up GABA via specific transporters, thus removing GABA after it has been released. GABA transport is inhibited by **guvacine**, **nipecotic acid** and **tiagabine**. Tiagabine is used to treat epilepsy (Ch. 44). GABA can be destroyed by a transamination reaction in which the amino group is transferred to α-oxoglutaric acid (to yield glutamate), with the production of succinic semialdehyde and then succinic acid. This reaction is catalysed by GABA transaminase, an enzyme located primarily in astrocytes. It is inhibited by **vigabatrine**, another compound used to treat epilepsy (Ch. 44).

GABA functions as an inhibitory transmitter in many different CNS pathways. About 20% of CNS neurons are GABAergic; most are short interneurons, but there are some long GABAergic tracts, e.g. from the striatum to the substantia nigra and globus pallidus (see Ch. 39 and Fig. 39.4). The widespread distribution of GABA—GABA serves as a transmitter at about 30% of all the synapses in the CNS—and the fact that virtually all neurons are sensitive to its inhibitory effect suggests that its function is ubiquitous in the brain. That antagonists such as **bicuculline** (see below) induce seizures illustrates the important, ongoing inhibitory role of GABA in the brain.

GABA RECEPTORS: STRUCTURE AND PHARMACOLOGY

GABA acts on two distinct types of receptor: $GABA_A$ receptors are ligand-gated ion channels whereas the other, $GABA_B$ receptors, are G-protein coupled.

$GABA_A$ RECEPTORS

$GABA_A$ receptors[7] (see Barnard, 2000) are members of the *Cys* loop family of receptors that also includes the glycine, nicotinic, and 5-HT_3 receptors (see Fig. 3.18). The $GABA_A$ receptors are pentamers made up of different subunits. The reader should not despair when informed that nineteen $GABA_A$ receptor subunits have been cloned (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) and that splice variants of some subunits also exist. Although the number of possible combinations is large, only a few dozen have been shown to exist (Mody & Pearce, 2004). The most common are α1β2γ2 (by far the most abundant), α2β3γ2 and α3β1–3γ2 subunits. To make up the pentamer, each receptor contains 2 α, 2 β and 1 γ subunit arranged in a circle in the sequence α–β–α–β–γ around the pore when viewed from the extracellular side of the membrane. GABA binds at the interface between the α and β subunits whereas benzodiazepines (see Ch. 43) bind at the α/γ interface. Receptors containing different α and γ subunits exhibit differential sensitivity to benzodiazepines and mediate different behavioural responses to these drugs. This raises the tantalising prospect of developing new agents with greater selectivity and potentially fewer side effects. The $GABA_A$ receptor should therefore be thought of as a group of receptors exhibiting subtle differences in their physiological and pharmacological properties.

$GABA_A$ receptors are primarily located postsynaptically and mediate fast postsynaptic inhibition, the channel being selectively permeable to Cl^-. Because the equilibrium membrane potential for Cl^- is usually negative to the resting potential, increasing Cl^- permeability hyperpolarises the cell as Cl^- ions enter, thereby reducing its excitability.[8] $GABA_A$ receptors are located both at areas of synaptic contact and extrasynaptically (Farrant & Nusser, 2005). Thus GABA produces inhibition by acting both as a fast 'point-to-point' transmitter and as an 'action-at-a-distance' neuromodulator, as the extrasynaptic $GABA_A$ receptors can be tonically activated by GABA that has diffused away from its site of release. Extrasynaptic $GABA_A$ receptors contain α4, α5 and α6 subunits as well as the δ subunit, and are highly sensitive to general anaesthetic agents (see Ch. 40) and ethanol (see Ch. 48), have higher affinities for GABA and show less desensitisation. **Gaboxadol** (previously known as THIP from its chemical structure) is a selective $GABA_A$ receptor agonist with a preference for δ subunit-containing $GABA_A$ receptors.

$GABA_B$ RECEPTORS

$GABA_B$ receptors (see Bettler et al., 2004) are located pre- and postsynaptically. They are class C G-protein-coupled receptors that couple through G_i/G_o to inhibit voltage-gated Ca^{2+} channels (thus reducing transmitter release), to open potassium channels (thus reducing postsynaptic excitability) and to inhibit adenylyl cyclase.

▼ For $GABA_B$ receptors, the functional receptor is a dimer (see Ch. 3) consisting of two different seven-transmembrane subunits, B1 and B2, held together by a coil/coil interaction between their C-terminal tails (Kubo & Tateyama, 2005). In the absence of B2, the B1 subunit does not traffic to the plasma membrane as it possesses an endoplasmic reticulum retention signal. Interaction of B1 with B2 masks the retention signal and facilitates trafficking to the membrane. Activation of the dimer results from GABA binding to the extracellular, venus fly trap-like domain of B1 (even although the B2 subunit possesses a similar domain) whereas it is the B2 subunit that interacts with and activates the G-protein (Fig. 37.8).

[7]The IUPHAR Nomenclature Committee has recommended (see Olsen & Sieghart, 2008) that the receptors previously referred to as '$GABA_C$' receptors, because they were insensitive to bicuculline or baclofen, should be subtypes of the $GABA_A$ receptor family as they are pentameric Cl^--permeable ligand-gated channels that contain ρ subunits. Their functional significance is slowly being worked out (see Chebib, 2004).

[8]During early brain development (in which GABA plays an important role), and also in some regions of the adult brain, GABA has an excitatory rather than an inhibitory effect, because the intracellular Cl^- concentration is relatively high, so that the equilibrium potential is positive to the resting membrane potential.

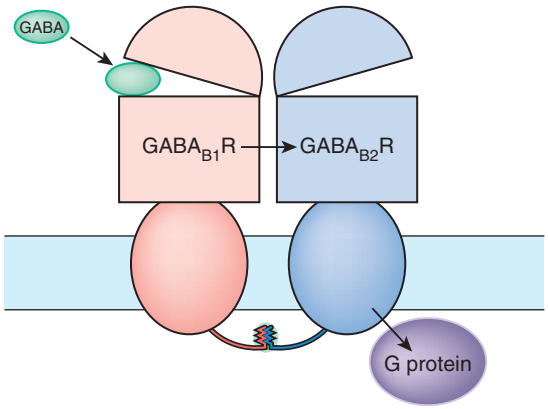

Fig 37.8 Dimeric structure of the GABA$_B$ receptor. The receptor is made up of two seven-transmembrane domain subunits held together by a coil/coil interaction between their C-terminal tails. Activation of the receptor occurs when GABA binds to the extracellular domain of the B1 subunit. This produces an allosteric change in the B2 subunit which is coupled to the G-protein. (Adapted from Kubo & Tateyama, 2005 Current Opinion in Neurobiology. 15: 289–295.)

DRUGS ACTING ON GABA RECEPTORS

GABA$_A$ RECEPTORS

GABA$_A$ receptors resemble NMDA receptors in that drugs may act at several different sites (Fig. 37.5; see Johnston, 1996). These include:

- the GABA-binding site
- several modulatory sites
- the ion channel.

There is growing evidence that the different receptor subtypes differ in their pharmacological properties.

GABA$_A$ receptors are the target for several important centrally acting drugs, notably benzodiazepines, barbiturates, neurosteroids (see below) and several general anaesthetics. The main agonists, antagonists and modulatory substances that act on GABA receptors are shown in Table 37.3.

Muscimol, derived from a hallucinogenic mushroom, resembles GABA chemically and is a powerful GABA$_A$ receptor agonist. A synthetic analogue, **gaboxadol** is a partial agonist that was developed as a hypnotic drug (Ch. 43) but has now been withdrawn. **Bicuculline**, a naturally occurring convulsant compound, is a specific antagonist

Table 37.3 Properties of inhibitory amino acid receptors

	GABA$_A$			GABA$_B$	Glycine
	Receptor site	**Modulatory site (benzodiazepine)**	**Modulatory site (others)**		
Endogenous agonists	GABA	Unknown, several postulated (see text)	Various neurosteroids (e.g. progesterone metabolites)	GABA	Glycine β-Alanine Taurine
Other agonist(s)	Muscimol Gaboxadol (THIP,[a] partial agonist)	Anxiolytic benzodiazepines (e.g. diazepam)	Barbiturates Steroid anaesthetics (e.g. alphaxolone)	Baclofen	—
Antagonist(s)	Bicuculline Gabazine	Flumazenil (inverse agonist?)	—	2-Hydroxy-saclofen CGP 35348 and others	Strychnine
Channel blocker	Picrotoxin[b]			Not applicable	—
Effector mechanism(s)	Ligand-gated chloride channel			G-protein-coupled receptor; inhibition of adenylyl cyclase, inhibition of Ca^{2+} channels, activation of K^+ channels	Ligand-gated chloride channel
Location	Widespread; mainly GABAergic interneurons			Pre- and postsynaptic Widespread	Postsynaptic Mainly in brain stem and spinal cord
Function	Postsynaptic inhibition (fast ipsp and tonic inhibition)			Presynaptic inhibition (decreased Ca^{2+} entry) Postsynaptic inhibition (increased K^+ permeability)	Postsynaptic inhibition (fast ipsp)

[a] THIP is an abbreviation of the chemical name of gaboxadol. It is reported to have preference for δ subunit-containing extrasynaptic GABA$_A$ receptors.
[b] Picrotoxin also blocks homomeric α subunit-containing glycine receptors but not heteromeric glycine receptors.
ipsp, inhibitory postsynaptic potential.

that blocks the fast inhibitory synaptic potential in most CNS synapses. **Gabazine**, a synthetic GABA analogue, is similar. These compounds are useful experimental tools but have no therapeutic uses.

Benzodiazepines, which have powerful sedative, anxiolytic and anticonvulsant effects (see Ch. 43), selectively potentiate the effects of GABA on some $GABA_A$ receptors depending upon the subunit composition of the receptor. They bind with high affinity to an accessory site (the 'benzodiazepine receptor') on the $GABA_A$ receptor, in such a way that the binding of GABA is facilitated and its agonist effect is enhanced. Conversely, inverse agonists at the benzodiazepine receptor (e.g. Ro15-4513) reduce GABA binding and are anxiogenic and proconvulsant—they are unlikely to be therapeutically useful!

Modulators that also enhance the action of GABA, but whose site of action is less well defined than that of benzodiazepines (shown as 'channel modulators' in Fig. 37.5), include other CNS depressants such as barbiturates (Ch. 43), anaesthetic agents (Ch. 40) and neurosteroids. Neurosteroids (see Lambert et al., 2003) are compounds that are related to steroid hormones but that act (like benzodiazepines) to enhance activation of $GABA_A$ receptors as well as on conventional intracellular steroid receptors. Interestingly, they include metabolites of progesterone and androgens that are formed in the nervous system, and are believed to have a physiological role. Synthetic neurosteroids include **alphaxolone**, developed as an anaesthetic agent (Ch. 40).

Picrotoxin is a convulsant that acts by blocking the chloride channel associated with the $GABA_A$ receptor, thus blocking the postsynaptic inhibitory effect of GABA. It has no therapeutic uses.

$GABA_B$ RECEPTORS

When the importance of GABA as an inhibitory transmitter was recognised, it was thought that a GABA-like substance might prove to be effective in controlling epilepsy and other convulsive states; because GABA itself fails to penetrate the blood–brain barrier, more lipophilic GABA analogues were sought, one of which, **baclofen** (see Fig. 37.3), was introduced in 1972. Unlike GABA, its actions are not blocked by bicuculline. These findings led to the recognition of the $GABA_B$ receptor, for which baclofen is a selective agonist (see Bowery, 1993). Baclofen is used to treat spasticity and related motor disorders (Ch. 44) and may also be useful in the treatment of drug dependence (see Ch. 48).

Competitive antagonists for the $GABA_B$ receptor include a number of experimental compounds (e.g. **2-hydroxysaclofen** and more potent compounds with improved brain penetration, such as CGP 35348). Tests in animals have shown that these compounds produce only slight effects on CNS function (in contrast to the powerful convulsant effects of $GABA_A$ antagonists). The main effect observed, paradoxically, was an antiepileptic action, specifically in an animal model of absence seizures (see Ch. 44), together with enhanced cognitive performance. Whether such compounds will prove to have therapeutic uses remains to be seen.

γ-HYDROXYBUTYRATE

γ-Hydroxybutyrate (GHB; see Wong et al., 2004) occurs naturally in the brain as a side product of GABA synthesis. As a synthetic drug from 1960 onwards, it has found favour with bodybuilders, based on its ability to evoke the release of growth hormone, and with party-goers, based on its euphoric and disinhibitory effects. In common with many abused drugs (see Ch. 48), it activates 'reward pathways' in the brain, and its use is now illegal in most countries. The pharmacological properties of GHB are not well understood, although it is believed to be a weak partial agonist at $GABA_B$ receptors and to bind to specific GHB receptor sites (see Wu et al., 2004), of which little is known.

GLYCINE

Glycine is present in particularly high concentration (5 μmol/g) in the grey matter of the spinal cord. Applied ionophoretically to motor neurons or interneurons, it produces an inhibitory hyperpolarisation that is indistinguishable from the inhibitory synaptic response. **Strychnine**, a convulsant poison that acts mainly on the spinal cord, blocks both the synaptic inhibitory response and the response to glycine. This, together with direct measurements of glycine release in response to nerve stimulation, provides strong evidence for its physiological transmitter role. **β-Alanine** has pharmacological effects and a pattern of distribution very similar to those of glycine, but its action is not blocked by strychnine.

The inhibitory effect of glycine is quite distinct from its role in facilitating activation of NMDA receptors (see p. 450).

▼ The glycine receptor (see Lynch, 2009) resembles the $GABA_A$ receptor in that it is a *Cys* loop, pentameric ligand-gated chloride channel. There are no specific metabotropic receptors for glycine. Five glycine receptor subunits have been cloned (α1–4, β) and it appears that in the adult brain the main form of receptor is made up of α1 and β subunits, although debate is ongoing about the exact stoichiometry. The situation for glycine is therefore much simpler than for GABA (see above). Mutations of the receptor have been identified in some inherited neurological disorders associated with muscle spasm and reflex hyperexcitability. There are no therapeutic drugs that act specifically by modifying glycine receptors, although it turns out that many of the compounds (such as benzodiazepines and anaesthetic agents) that enhance $GABA_A$ receptor activation act similarly on glycine receptors.

Tetanus toxin, a bacterial toxin resembling **botulinum toxin** (Ch. 13), acts selectively to prevent glycine release from inhibitory interneurons in the spinal cord, causing excessive reflex hyperexcitability and violent muscle spasms (lockjaw).

Glycine is removed from the extracellular space by two transporters Gly_{T1} and Gly_{T2} (Eulenburg et al., 2005). Gly_{T1} is located primarily on astrocytes and expressed throughout most regions of the CNS. Gly_{T2} on the other hand is expressed on glycinergic neurons in the spinal cord, brain stem and cerebellum. As described above, in addition to its function as an inhibitory transmitter, glycine also functions as a co-agonist with glutamate at NMDA receptors. Inhibition of glycine uptake by Gly_{T1} leads to an elevation of extracellular glycine levels throughout the brain and, through potentiation of NMDA receptor-mediated responses, could be beneficial in the treatment of schizophrenia (see Ch. 45). Another potential use of glycine transporter inhibitors could be as analgesics.

Inhibitory amino acids: GABA and glycine

- GABA is the main inhibitory transmitter in the brain.
- It is present fairly uniformly throughout the brain; there is very little in peripheral tissues.
- GABA is formed from glutamate by the action of glutamic acid decarboxylase. Its action is terminated mainly by reuptake, but also by deamination, catalysed by GABA transaminase.
- There are two main types of GABA receptor: $GABA_A$ and $GABA_B$.
- $GABA_A$ receptors, which occur mainly postsynaptically, are directly coupled to chloride channels, the opening of which reduces membrane excitability.
- **Muscimol** is a specific $GABA_A$ agonist, and the convulsant **bicuculline** is an antagonist.
- Other drugs that interact with $GABA_A$ receptors and channels include:
  - benzodiazepines, which act at an accessory binding site to facilitate the action of GABA
  - convulsants such as **picrotoxin**, which block the anion channel
  - neurosteroids, including endogenous progesterone metabolites, and other CNS depressants, such as barbiturates and some general anaesthetic agents, which facilitate the action of GABA.
- $GABA_B$ receptors are heterodimeric G-protein-coupled receptors. They cause pre- and postsynaptic inhibition by inhibiting Ca^{2+} channel opening and increasing K^+ conductance. **Baclofen** is a $GABA_B$ receptor agonist used to treat spasticity. $GABA_B$ antagonists are not yet in clinical use.
- Glycine is an inhibitory transmitter mainly in the spinal cord, acting on its own receptor, structurally and functionally similar to the $GABA_A$ receptor.
- The convulsant drug **strychnine** is a competitive glycine antagonist. Tetanus toxin acts mainly by interfering with glycine release.

CONCLUDING REMARKS

The study of amino acids and their receptors in the brain has been one of the most active fields of research in the past two decades, and the amount of information available is prodigious. These signalling systems have been speculatively implicated in almost every kind of neurological and psychiatric disorder, and the pharmaceutical industry has put a great deal of effort into identifying specific ligands—agonists, antagonists, modulators, enzyme inhibitors, transport inhibitors—designed to influence them. However, while a large number of pharmacologically unimpeachable compounds have emerged, and many clinical trials undertaken, there have been no major therapeutic breakthroughs. The optimistic view is that a better understanding of the particular functions of the many molecular subtypes of these targets, and the design of more subtype-specific ligands, will lead to future breakthroughs. Expectations have, however, undoubtedly dimmed in recent years.

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38 Other transmitters and modulators

OVERVIEW

The principal 'amine' transmitters in the central nervous system (CNS), namely noradrenaline, dopamine, 5-hydroxytryptamine (5-HT, serotonin) and acetylcholine (ACh), are described in this chapter, with briefer coverage of other mediators, including histamine, melatonin and purines. The monoamines were the first CNS transmitters to be identified, and during the 1960s a combination of neurochemistry and neuropharmacology led to many important discoveries about their role, and about the ability of drugs to influence these systems. Amine mediators differ from the amino acid transmitters discussed in Chapter 37 in being localised to small populations of neurons with cell bodies in the brain stem and basal forebrain, which project diffusely both rostrally to cortical and other areas, and in some cases caudally to the spinal cord. These amine-containing neurons are broadly associated with high-level behaviours (e.g. emotion, cognition and awareness), rather than with localised synaptic excitation or inhibition.[1] More recently, some 'atypical' chemical mediators, such as nitric oxide (NO; Ch. 20) and endocannabinoids (Ch. 18) have come on the scene, and they are discussed at the end of the chapter. The other major class of CNS mediators, the neuropeptides, are described in Chapter 19, and information on specific neuropeptides (e.g. endorphins and neurokinins) appears in later chapters in this section.

INTRODUCTION

Although we know much about the many different mediators, their cognate receptors and signalling mechanisms at the cellular level, when describing their effects on brain function and behaviour we fall back on relatively crude terms—psychopharmacologists will be at our throats for so under-rating the sophistication of their measurements—such as 'motor coordination', 'arousal', 'cognitive impairment' and 'exploratory behaviour'. The gap between these two levels of understanding still frustrates the best efforts to link drug action at the molecular level to drug action at the therapeutic level. Modern approaches, such as the use of transgenic animal technology (see Ch. 7) and non-invasive imaging techniques, are helping to forge links, but there is still a long way to go.

More detail on the content of this chapter can be found in Davis et al. (2002), Nestler et al. (2008) and Iversen et al. (2009).

[1]They are, if you like, voices from the nether regions, which make you happy or sad, sleepy or alert, cautious or adventurous, energetic or lazy, although you do not quite know why—very much the stuff of mental illness.

NORADRENALINE

The basic processes responsible for the synthesis, storage and release of noradrenaline are the same in the CNS as in the periphery (Ch. 14). In the CNS, inactivation of released noradrenaline is by neuronal reuptake or by metabolism, largely through the *monamine oxidase*, *aldehyde reductase* and *catechol-O-methyl transferase* mediated pathway to 3-hydroxy-4-methoxyphenylglycol (MHPG) (see Fig. 14.4).

NORADRENERGIC PATHWAYS IN THE CNS

Although the transmitter role of noradrenaline in the brain was suspected in the 1950s, detailed analysis of its neuronal distribution became possible only when a technique, based on the formation of fluorescent catecholamine derivatives when tissues are exposed to formaldehyde, was devised by Falck and Hillarp. Detailed maps of the pathway of noradrenergic, dopaminergic and serotonergic neurons in laboratory animals were produced and later confirmed in human brains. The cell bodies of noradrenergic neurons occur in small clusters in the *pons* and *medulla*, and they send extensively branching axons to many other parts of the brain and spinal cord (Fig. 38.1). The most prominent cluster is the locus coeruleus (LC), located in the pons. Although it contains only about 10 000 neurons in humans, the axons, running in a discrete *medial forebrain bundle*, give rise to many millions of noradrenergic nerve terminals throughout the cortex, hippocampus, thalamus, hypothalamus and cerebellum. These nerve terminals do not form distinct synaptic contacts but appear to release transmitter somewhat diffusely. The LC also projects to the spinal cord and is involved in the descending control of pain (Ch. 41).

Other noradrenergic neurons lie close to the LC in the pons and project to the amygdala, hypothalamus, hippocampus and other parts of the forebrain, as well as to the spinal cord. A small cluster of adrenergic neurons, which release adrenaline rather than noradrenaline, lies more ventrally in the brain stem. These cells contain phenylethanolamine *N*-methyl transferase, the enzyme that converts noradrenaline to adrenaline (see Ch. 14), and project mainly to the pons, medulla and hypothalamus. Rather little is known about them, but they are believed to be important in cardiovascular control.

FUNCTIONAL ASPECTS

With the exception of the β_3 adrenoceptor, all of the adrenoceptors (α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B}, α_{2C}, β_1 and β_2) are expressed in the CNS (see Bylund, 2007). They are G-protein-coupled receptors that interact with a variety of effector mechanisms (see Table 14.1). The role of α_1 receptors in the CNS is poorly understood. They are widely distributed, located both on postsynaptic neurons and on glial cells, and may be involved in motor control, cognition and fear.

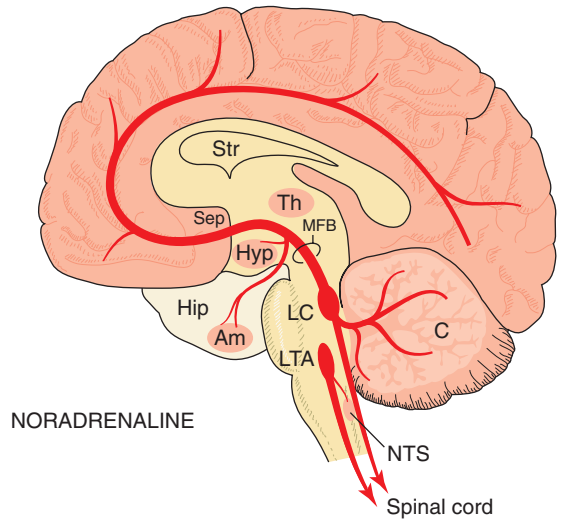

Fig. 38.1 Simplified diagram of the noradrenaline pathways in the brain. The location of the main groups of cell bodies and fibre tracts is in solid colour. Light-shaded areas show the location of noradrenergic terminals. Am, amygdaloid nucleus; C, cerebellum; Hip, hippocampus; Hyp, hypothalamus; LC, locus coeruleus; LTA, lateral tegmental area, part of the reticular formation; MFB, medial forebrain bundle; NTS, nucleus of the tractus solitarius (vagal sensory nucleus); Sep, septum; Str, corpus striatum; Th, thalamus.

α_2 Adrenoceptors are located on noradrenergic neurons (in both somatodendritic and nerve terminal regions where they function as inhibitory autoreceptors) as well as on postsynaptic non-noradrenergic neurons. They are involved in blood pressure control (see below), sedation (α_2 agonists such as **medetomidine** are used as anaesthetics in veterinary practice) and analgesia. β_1 Receptors are found in the cortex, striatum and hippocampus whereas β_2 receptors are largely found in the cerebellum. They have been implicated in the long-term effects of antidepressant drugs but quite how remains a mystery (see Ch. 46).

Research on the α_2 adrenoceptor antagonist, **idazoxan**, has led to the identification of other putative imidazoline 'receptors' (see Head & Mayorov, 2006). These are the I_1 receptor, which plays a role in the central control of blood pressure; the I_2 receptor, an allosteric binding site on monoamine oxidase, and the I_3 receptor, present in the pancreas with a role in regulating insulin secretion.

Arousal and mood

Attention has focused mainly on the LC, which is the source of most of the noradrenaline released in the brain, and from which neuronal activity can be measured by implanted electrodes. LC neurons are silent during sleep, and their activity increases with behavioural arousal. 'Wake-up' stimuli of an unfamiliar or threatening kind excite these neurons much more effectively than familiar stimuli. Amphetamine-like drugs, which release catecholamines in the brain, increase wakefulness, alertness and exploratory activity (although, in this case, firing of LC neurons is actually reduced by feedback mechanisms; see Ch. 47).

There is a close relationship between mood and state of arousal; depressed individuals are usually lethargic and unresponsive to external stimuli. The catecholamine hypothesis of depression (see Ch. 46) suggested that it results from a functional deficiency of noradrenaline in certain parts of the brain, while mania results from an excess. This remains controversial, and subsequent findings suggest that 5-HT may be more important than noradrenaline in relation to mood.

Blood pressure regulation

The role of central, as well as peripheral, noradrenergic synapses in blood pressure control is shown by the action of hypotensive drugs such as **clonidine** and **methyldopa** (see Chs 14 and 22) which decrease the discharge of sympathetic nerves emerging from the CNS. They cause hypotension when injected locally into the medulla or fourth ventricle, in much smaller amounts than are required when the drugs are given systemically. Noradrenaline and other α_2 adrenoceptor agonists have the same effect when injected locally. Noradrenergic synapses in the medulla probably form part of the baroreceptor reflex pathway, because stimulation or antagonism of α_2 adrenoceptors in this part of the brain has a powerful effect on the activity of baroreceptor reflexes.

Ascending noradrenergic fibres run to the hypothalamus, and descending fibres run to the lateral horn region of the spinal cord, acting to increase sympathetic discharge in the periphery. It has been suggested that these regulatory neurons may release adrenaline rather than noradrenaline as inhibition of phenylethanolamine *N*-methyl transferase, the enzyme that converts noradrenaline to adrenaline, interferes with the baroreceptor reflex.

Moxonidine, reported to be an I_1 receptor agonist with less activity at α_2 adrenoceptors, acts centrally to reduce peripheral sympathetic activity, thus decreasing peripheral vascular resistance.

Noradrenaline in the CNS

- Mechanisms for synthesis, storage, release and reuptake of noradrenaline in the central nervous system (CNS) are essentially the same as in the periphery, as are the receptors (Ch. 14).
- Noradrenergic cell bodies occur in discrete clusters, mainly in the pons and medulla, one important such cell group being the locus coeruleus.
- Noradrenergic pathways, running mainly in the medial forebrain bundle and descending spinal tracts, terminate diffusely in the cortex, hippocampus, hypothalamus, cerebellum and spinal cord.
- The actions of noradrenaline are mediated through α_1, α_2, β_1 and β_2 receptors.
- Noradrenergic transmission is believed to be important in:
  - the 'arousal' system, controlling wakefulness and alertness
  - blood pressure regulation
  - control of mood (functional deficiency contributing to depression).
- Psychotropic drugs that act partly or mainly on noradrenergic transmission in the CNS include antidepressants, **cocaine** and **amphetamine**. Some antihypertensive drugs (e.g. **clonidine**, **methyldopa**) act mainly on noradrenergic transmission in the CNS.

DOPAMINE

Dopamine is particularly important in relation to neuropharmacology, because it is involved in several common disorders of brain function, notably Parkinson's disease, schizophrenia and attention deficit disorder, as well as in drug dependence and certain endocrine disorders. Many of the drugs used clinically to treat these conditions work by influencing dopamine transmission.

The distribution of dopamine in the brain is more restricted than that of noradrenaline. Dopamine is most abundant in the *corpus striatum*, a part of the extrapyramidal motor system concerned with the coordination of movement (see Ch. 39), and high concentrations also occur in certain parts of the frontal cortex, limbic system and hypothalamus (where its release into the pituitary blood supply inhibits secretion of prolactin; Ch. 32).

The synthesis of dopamine follows the same route as that of noradrenaline (see Fig. 14.2), namely conversion of tyrosine to dopa (the rate-limiting step), followed by decarboxylation to form dopamine. Dopaminergic neurons lack dopamine β-hydroxylase, and thus do not convert dopamine to noradrenaline.

Dopamine is largely recaptured, following its release from nerve terminals, by a specific dopamine transporter, one of the large family of monoamine transporters (see Ch. 14). It is metabolised by monoamine oxidase and catechol-*O*-methyl transferase (Fig. 38.2), the main products being *dihydroxyphenylacetic acid* (DOPAC) and *homovanillic acid* (HVA, the methoxy derivative of DOPAC). The brain content of HVA is often used as an index of dopamine turnover. Drugs that cause the release of dopamine increase HVA, often without changing the concentration of dopamine. DOPAC and HVA, and their sulfate conjugates, are excreted in the urine, which provides an index of dopamine release in human subjects.

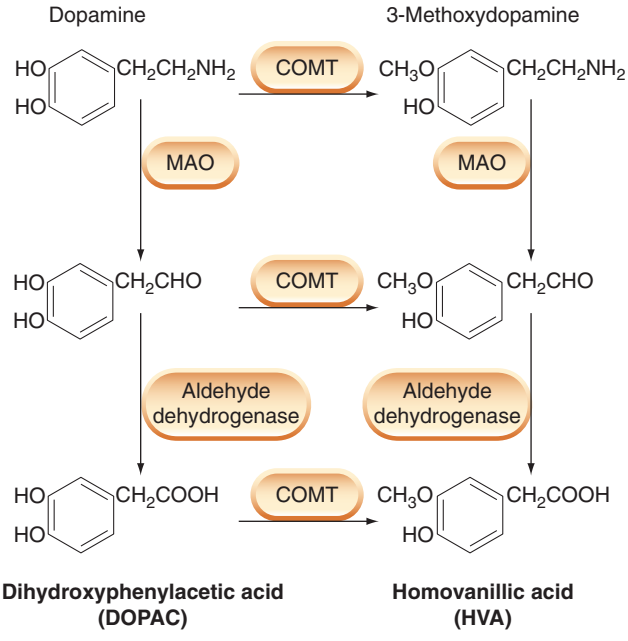

Fig. 38.2 The main pathways for dopamine metabolism in the brain. COMT, catechol-*O*-methyl transferase; MAO, monoamine oxidase.

6-Hydroxydopamine, which selectively destroys dopaminergic nerve terminals, is commonly used as a research tool. It is taken up by the dopamine transporter and converted to a reactive metabolite that causes oxidative cytotoxicity.

DOPAMINERGIC PATHWAYS IN THE CNS

There are four main dopaminergic pathways in the brain (Fig. 38.3):

1. The **nigrostriatal pathway**, accounting for about 75% of the dopamine in the brain, consists of cell bodies largely in the substantia nigra whose axons terminate in the corpus striatum. These fibres run in the medial forebrain bundle along with other monoamine-containing fibres. The abundance of dopamine-containing neurons in the human striatum can be appreciated from the image shown in Figure 38.4, which was obtained by injecting a dopa derivative containing radioactive fluorine, and scanning for radioactivity 3 h later by positron emission tomography.
2. The **mesolimbic pathway**, whose cell bodies occur in the midbrain ventral tegmental area (VTA), adjacent to the substantia nigra, and whose fibres project via the medial forebrain bundle to parts of the limbic system, especially the *nucleus accumbens* and the *amygdaloid nucleus*.
3. The **mesocortical pathway**, whose cell bodies also lie in the VTA and which project via the medial forebrain bundle to the frontal cortex.
4. The **tuberohypophyseal** (or **tuberoinfundibular**) system is a group of short neurons running from the ventral hypothalamus to the median eminence and pituitary gland, the secretions of which they regulate.

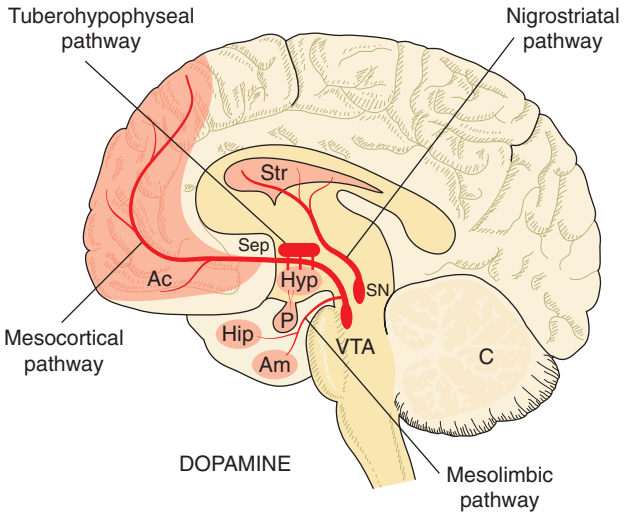

Fig. 38.3 Simplified diagram of the dopamine pathways in the brain, drawn as in Figure 38.1. The pituitary gland (P) is shown, innervated with dopaminergic fibres from the hypothalamus. Ac, nucleus accumbens; SN, substantia nigra; VTA, ventral tegmental area; other abbreviations as in Figure 38.1.

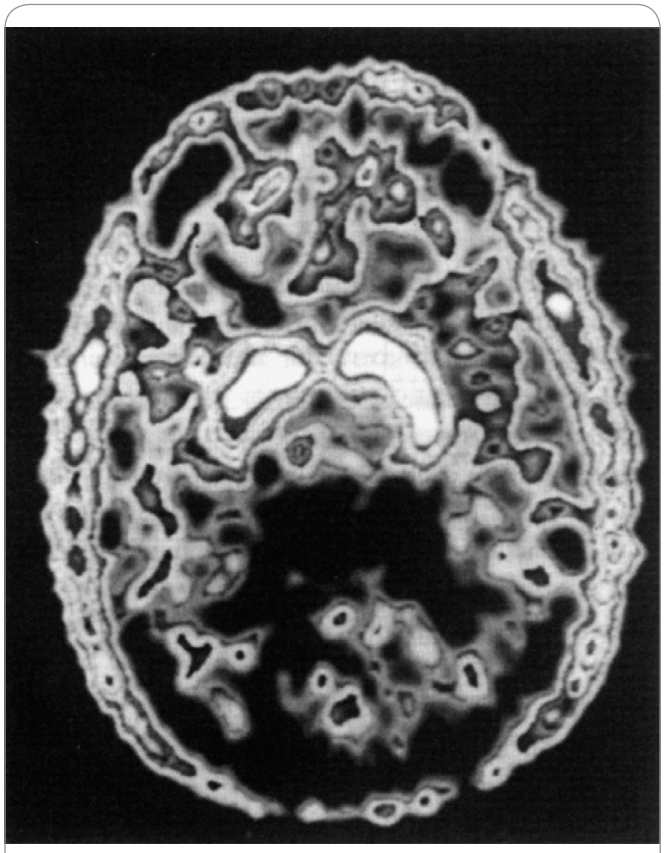

Fig. 38.4 Dopamine in the basal ganglia of a human subject. The subject was injected with 5-fluoro-dopa labelled with the positron-emitting isotope ^{18}F, which was localised 3 h later by the technique of positron emission tomography. The isotope is accumulated (white areas) by the dopa uptake system of the neurons of the basal ganglia, and to a smaller extent in the frontal cortex. It is also seen in the scalp and temporalis muscles. (From Garnett E S et al. Nature 305: 137.)

There are also dopaminergic neurons in other brain regions and in the retina. For a more complete description, see Björklund & Dunnett (2007). The functions of the main dopaminergic pathways are discussed below.

DOPAMINE RECEPTORS

Two types of receptor, D_1 and D_2, were originally distinguished on pharmacological and biochemical grounds. Gene cloning revealed further subgroups, D_1 to D_5 (for review, see Missale et al., 1998). The original D_1 family now includes D_1 and D_5, while the D_2 family, which is pharmacologically more important in the CNS, consists of D_2, D_3 and D_4 (see Table 38.1). Splice variants, leading to long and short forms of D_2, and genetic polymorphisms, particularly of D_4 (see below), have subsequently been identified.

▼ All belong to the family of G-protein-coupled transmembrane receptors described in Chapter 3—D_1 and D_5 link through G_s to stimulate adenylyl cyclase; D_2, D_3, and D_4 link through G_i/G_o and activate potassium channels as well as inhibiting calcium channels and adenylyl cyclase. In addition they can also affect other cellular second messenger cascades (see Ch. 3). A key component in the signal transduction pathway is the protein DARPP-32 (*32-kDa dopamine- and cAMP-regulated phosphoprotein*; see Girault & Greengard, 2004). When intracellular cAMP is increased through activation of D_1 receptors, activating protein kinase A, DARPP-32 is phosphorylated (Fig. 38.5). Phosphorylated DARPP-32 acts as an inhibitor of protein phosphatases such as protein phosphatase-1 and calcineurin, thus acting in concert with protein kinases and favouring protein phosphorylation—effectively an amplifying mechanism. In general, activation of D_2 receptors opposes the effect of D_1 receptor activation.

Dopamine receptors are expressed in the brain in distinct but overlapping areas. D_1 receptors are the most abundant and widespread in areas receiving a dopaminergic innervation (namely the striatum, limbic system, thalamus and hypothalamus; Fig. 38.3), as are D_2 receptors, which also occur in the pituitary gland. D_2 receptors are found not only on dopaminergic neurons (cell bodies, dendrites and nerve terminals), where they function as inhibitory autoreceptors, but also on non-dopaminergic neurons (see De Mei et al., 2009). D_3 receptors occur in the limbic system but not in the striatum. The D_4 receptor is much more weakly expressed, mainly in the cortex and limbic systems.

▼ The D_4 receptor displays an unexpected polymorphism in humans, with a varying number (from 2 to 10) of 16 amino acid repeat sequences being expressed in the third intracellular loop, which participates in G-protein coupling (Ch. 3). Expectations that D_4 receptor polymorphism might be related to the occurrence of schizophrenia in humans were disappointed after several studies failed to find any correlation (Tarazi et al., 2004). There may be a connection with attention deficit hyperactivity disorder (see Thapar et al., 2007).

Dopamine, like many other transmitters and modulators, acts presynaptically as well as postsynaptically. Presynaptic D_2 receptors occur mainly on dopaminergic neurons, for example those in the striatum and limbic system, where they act to inhibit dopamine synthesis and release. Dopamine antagonists, by blocking these receptors, increase dopamine synthesis and release, and cause accumulation of dopamine metabolites in these parts of the brain. They also cause an increase in the rate of firing of dopaminergic neurons, probably by blocking feedback at the somatodendritic level mediated by locally released dopamine.

Dopamine receptors also mediate various effects in the periphery (mediated by D_1 receptors), notably renal vasodilatation and increased myocardial contractility (dopamine itself has been used clinically in the treatment of circulatory shock; see Ch. 21).

FUNCTIONAL ASPECTS

The functions of dopaminergic pathways divide broadly into:

- motor control (nigrostriatal system)
- behavioural effects (mesolimbic and mesocortical systems)
- endocrine control (tuberohypophyseal system).

Dopamine and motor systems

Ungerstedt showed, in 1968, that bilateral ablation of the substantia nigra in rats, which destroys the nigrostriatal neurons, causes profound catalepsy, the animals becoming so inactive that they die of starvation unless artificially fed. Parkinson's disease (Ch. 39) is a disorder of motor control, associated with a deficiency of dopamine in the nigrostriatal pathway.

In treating CNS disorders, it is often desired that a certain receptor type be activated or inhibited only in one part of the brain but the problem is that drugs are rarely brain region selective and will affect all of a receptor type throughout the brain. For example, many antipsychotic

Table 38.1 Dopamine receptors

	Functional role	D_1 type		D_2 type		
		D_1	D_5	D_2	D_3	D_4
Distribution						
Cortex	Arousal, mood	+++	–	++	–	+
Limbic system	Emotion, stereotypic behaviour	+++	+	++	+	+
Striatum	Prolactin secretion	+++	+	++	+	+
Ventral hypothalamus and anterior pituitary	Prolactin secretion	–	–	++	+	–
Agonists						
Dopamine		+ (Low potency)		+ (High potency)		
Apomorphine		PA (Low potency)		+ (High potency)		
Bromocriptine		PA (Low potency)		+ (High potency)		
Quinpirole		Inactive		Active		
Antagonists						
Chlorpromazine		+	+	+++	++	+
Haloperidol		+	+	+++	++	++
Spiperone		+?	–	+++	+++	+++
Sulpiride		–	–	++	++	+
Clozapine		+	+	+	+	++
Aripiprazole		–	–	+++(PA)	++	–
Raclopride		–	?	+++	++	–
Signal transduction		G_s coupled—activates adenylyl cyclase		G_i/G_o coupled—inhibits adenylyl cyclase, activates K^+ channels, inhibits Ca^{2+} channels, may also activate phospholipase C		
Effect		Mainly postsynaptic inhibition		Pre- and postsynaptic inhibition Stimulation/inhibition of hormone release		

PA, partial agonist.
Affinity data based on data contained at NIMH Psychoactive Drug Screening Program database (http://pdsp.med.unc.edu/).

drugs (see Ch. 45) are D_2 receptor antagonists, exerting a beneficial effect by blocking D_2 receptors in the mesolimbic pathway. However, their D_2 antagonist property also gives rise to their major side effect, which is to cause movement disorders, by simultaneously blocking D_2 receptors in the nigrostriatal pathway.

Transgenic mice lacking D_2 receptors show greatly reduced spontaneous movement, resembling Parkinson's disease.

Behavioural effects

Administration of **amphetamine** to rats, which releases both dopamine and noradrenaline, causes a cessation of normal 'ratty' behaviour (exploration and grooming), and the appearance of repeated 'stereotyped' behaviour (rearing, gnawing and so on) unrelated to external stimuli. These amphetamine-induced motor disturbances in rats probably reflect hyperactivity in the nigrostriatal dopaminergic system, and are prevented by dopamine antagonists and by destruction of dopamine-containing cell bodies in the midbrain, but not by drugs that inhibit the noradrenergic system.

Amphetamine and **cocaine** (which act by inhibiting the dopamine transporter) and also other drugs of abuse (Ch. 48) activate mesolimbic dopaminergic 'reward' pathways to produce feelings of euphoria in humans. The main receptor involved appears to be D_1, and transgenic mice lacking D_1 receptors behave as though generally demotivated, with reduced food intake and insensitivity to amphetamine and cocaine (see Sibley, 1999).

Neuroendocrine function

The tuberohypophyseal dopaminergic pathway (see Fig. 38.3) is involved in the control of prolactin secretion. The hypothalamus secretes various mediators (mostly small peptides; see Ch. 32), which control the secretion of different hormones from the pituitary gland. One of these mediators, which has an inhibitory effect on prolactin release, is dopamine. This system is of clinical importance. Many antipsychotic drugs (see Ch. 45), by blocking D_2 receptors, increase prolactin secretion and can cause breast development and lactation, even in males. **Bromocriptine**, a dopamine receptor agonist derived from ergot, is used clinically to suppress prolactin secretion by tumours of the pituitary gland.

Growth hormone production is increased in normal subjects by dopamine, but bromocriptine paradoxically inhibits the excessive secretion responsible for acromegaly

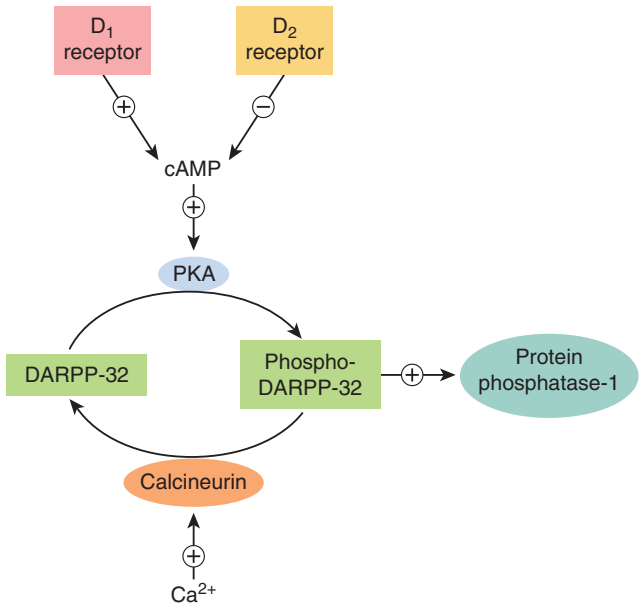

Fig. 38.5 The role of the neuron-specific phosphoprotein DARPP-32 in signalling by dopamine receptors (see text). PKA, protein kinase A.

(probably because it desensitises dopamine receptors, in contrast to the physiological release of dopamine, which is pulsatile) and has a useful therapeutic effect, provided it is given before excessive growth has taken place. It is now rarely used, as other agents are more effective (see Ch. 32). Bromocriptine and other dopamine agonists, such as **cabergoline**, enhance libido and sexual performance.

Vomiting

Pharmacological evidence strongly suggests that dopaminergic neurons have a role in the production of nausea and vomiting. Thus nearly all dopamine receptor agonists (e.g. bromocriptine) and other drugs that increase dopamine release in the brain (e.g. **levodopa**; Ch. 39) cause nausea and vomiting as side effects, while many dopamine antagonists (e.g. phenothiazines, **metoclopramide**; Ch. 29) have antiemetic activity. D_2 receptors occur in the area of the medulla (chemoreceptor trigger zone) associated with the initiation of vomiting (Ch. 29), and are assumed to mediate this effect.

5-HYDROXYTRYPTAMINE

The occurrence and functions of 5-HT (serotonin) in the periphery are described in Chapter 15. Interest in 5-HT as a possible CNS transmitter dates from 1953, when Gaddum found that **lysergic acid diethylamide** (LSD), a drug known to be a powerful hallucinogen (see Ch. 47), acted as a 5-HT antagonist on peripheral tissues, and suggested that its central effects might also be related to this action. The presence of 5-HT in the brain was demonstrated a few years later. Even though brain accounts for only about 1% of the total body content, 5-HT is an important CNS transmitter (see Iversen et al., 2009; Muller & Jacobs, 2009). 5-HT is involved in various physiological processes including sleep, appetite, thermoregulation and pain perception as well as in disorders such as migraine, depression, anxiety, obsessive compulsive disorders, schizophrenia and drug abuse.

In its formation, storage and release, 5-HT resembles noradrenaline. Its precursor is tryptophan, an amino acid derived from dietary protein, the plasma content of which varies considerably according to food intake and time of day. Tryptophan is actively taken up into neurons, converted by tryptophan hydroxylase to 5-hydroxytryptophan (see Fig. 15.1), and then decarboxylated by a non-specific amino acid decarboxylase to 5-HT. Tryptophan hydroxylase can be selectively and irreversibly inhibited by ***p*-chlorophenylalanine** (PCPA). Availability of tryptophan and the activity of tryptophan hydroxylase are thought to be the main factors that regulate 5-HT synthesis. The decarboxylase is very similar, if not identical, to dopa decarboxylase, and does not play any role in regulating 5-HT synthesis. Following release, 5-HT is largely recovered by neuronal uptake, through a specific transporter (see Ch. 3) similar to, but not identical with, those that take up noradrenaline and dopamine. 5-HT reuptake is specifically inhibited by *selective serotonin reuptake inhibitors* (SSRIs) such as **fluoxetine** and by many of the drugs that inhibit catecholamine uptake (e.g. *tricyclic antidepressants*). SSRIs (see Ch. 46) constitute an important group of antidepres-

Dopamine in the CNS

- Dopamine is a neurotransmitter as well as being the precursor for noradrenaline. It is degraded in a similar fashion to noradrenaline, giving rise mainly to dihydroxyphenylacetic acid and homovanillic acid, which are excreted in the urine.
- There are four main dopaminergic pathways:
  - nigrostriatal pathway, important in motor control
  - mesolimbic pathway, running from groups of cells in the midbrain to parts of the limbic system, especially the nucleus accumbens, involved in emotion and drug-induced reward
  - mesocortical pathway, running from the midbrain to the cortex, involved in emotion
  - tuberohypophyseal neurons, running from the hypothalamus to the pituitary gland, whose secretions they regulate.
- There are five dopamine receptor subtypes. D_1 and D_5 receptors are linked to stimulation of adenylyl cyclase. D_2, D_3 and D_4 receptors are linked to activation of K^+ channels and inhibition of Ca^{2+} channels as well as to inhibition of adenylyl cyclase.
- D_2 receptors may be implicated in the positive symptoms and D_1 receptors in the negative symptoms of schizophrenia. The D_4 receptor shows marked polymorphism in humans, but no clear relationship with disease has been established.
- Parkinson's disease is associated with a deficiency of nigrostriatal dopaminergic neurons.
- Hormone release from the anterior pituitary gland is regulated by dopamine, especially prolactin release (inhibited) and growth hormone release (stimulated).
- Dopamine acts on the chemoreceptor trigger zone to cause nausea and vomiting.

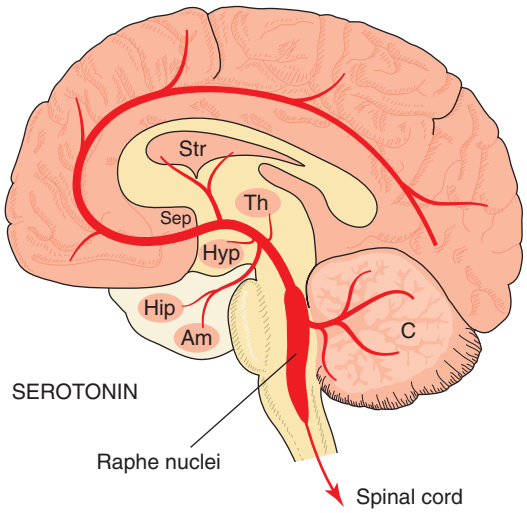

Fig. 38.6 Simplified diagram of the 5-hydroxytryptamine pathways in the brain, drawn as in Figure 38.1. Abbreviations as in Figure 38.1.

sant drugs. 5-HT is degraded almost entirely by monoamine oxidase (Fig. 15.1), which converts it to 5-hydroxyindole acetaldehyde, most of which is then dehydrogenated to form 5-hydroxyindole acetic acid (5-HIAA) and excreted in the urine.

5-HT PATHWAYS IN THE CNS

The distribution of 5-HT-containing neurons (Fig. 38.6) resembles that of noradrenergic neurons. The cell bodies are grouped in the pons and upper medulla, close to the midline (raphe), and are often referred to as raphe nuclei. The rostrally situated nuclei project, via the medial forebrain bundle, to many parts of the cortex, hippocampus, basal ganglia, limbic system and hypothalamus. The caudally situated cells project to the cerebellum, medulla and spinal cord.

5-HT RECEPTORS IN THE CNS

The main 5-HT receptor types are shown in Table 15.1. All are G-protein-coupled receptors except for 5-HT_3, which is a ligand-gated cation channel (see below). All are expressed in the CNS, and their functional roles have been extensively analysed. With some 14 identified subtypes plus numerous splice variants, and a large number of pharmacological tools of relatively low specificity, assigning clear-cut functions to 5-HT receptors is not simple. Detailed accounts of our present state of knowledge are given by Barnes & Sharp (1999) and Bockaert et al. (2006). Knowledge about the newer members of the family (5-$HT_{5\text{-}7}$ receptors) is summarised in reviews by Woolley et al. (2004) and Hedlund & Sutcliffe (2004).

Certain generalisations can be made:

- 5-HT_1 receptors are predominantly inhibitory in their effects. 5-HT_{1A} receptors are expressed as somatodendritic autoreceptors by the 5-HT neurons in the raphe nuclei, and their autoinhibitory effect tends to limit the rate of firing of these cells. They are also widely distributed in the limbic system, and are believed to be a major target for drugs used to treat anxiety and depression (see Chs 43 and 46). 5-HT_{1B} and 5-HT_{1D} receptors are found mainly as presynaptic inhibitory receptors on both 5-HT-containing and other nerve terminals in the basal ganglia and cortex. Agonists acting on peripheral 5-HT_{1D} receptors are used to treat migraine (see Ch. 15).
- 5-HT_2 receptors (5-HT_{2A} and 5-HT_{2C}) are abundant in the cortex and limbic system where they are located at both pre- and postsynaptic sites. They can exert excitatory or inhibitory effects by enhancing the release of glutamate and GABA. They are believed to be the target of some antidepressants (see Ch. 46) and various hallucinogenic drugs (see Ch. 47). The use of 5-HT_2 receptor antagonists such as **methysergide** in treating migraine is discussed in Chapter 15.
- 5-HT_3 receptors are pentameric ligand-gated cation channels that can be either homomeric or heteromeric complexes of different 5-HT_3 receptor subunits (see Peters et al., 2005). While 5-HT_{3A} and 5-HT_{3B} subunits are the most extensively studied, the roles of other subunits remain to be fully investigated (see Jensen et al., 2008). In the brain, 5-HT_3 receptors are found in the *area postrema* (a region of the medulla involved in vomiting; see Ch. 29) and other parts of the brain stem, extending to the dorsal horn of the spinal cord. They are also present in certain parts of the cortex, as well as in the peripheral nervous system. They are excitatory ionotropic receptors, and specific antagonists (e.g. **ondansetron**; see Chs 15 and 29) are used to treat nausea and vomiting.
- 5-HT_4 receptors are important in the gastrointestinal tract (see Chs 15 and 29), and are also expressed in the brain, particularly in the limbic system, basal ganglia, hippocampus and substantia nigra. They are located at both pre- and postsynaptic sites. They exert a presynaptic facilitatory effect, particularly on ACh release, thus enhancing cognitive performance (see Ch. 39). Activation of medullary 5-HT_4 receptors opposes the respiratory depressant actions of opioids (see Ch. 41).
- Little is known about 5-HT_5 receptors at present. Studies on CNS distribution and function have so far provided conflicting data (see Bockaert et al., 2006).
- 5-HT_6 receptors occur only in the CNS, particularly in the hippocampus, cortex and limbic system. They are considered potential targets for drugs to improve cognition or relieve symptoms of schizophrenia, although no such drugs are yet available.
- 5-HT_7 receptors occur in the hippocampus, cortex, amygdala, thalamus and hypothalamus. They are found on the soma and axon terminals of GABAergic neurons. They are also expressed in blood vessels and the gastrointestinal tract. Likely CNS functions include thermoregulation and endocrine regulation, as well as suspected involvement in mood, cognitive function and sleep. Selective antagonists are being developed for clinical use in a variety of potential indications.

FUNCTIONAL ASPECTS

The precise localisation of 5-HT neurons in the brain stem has allowed their electrical activity to be studied in detail and correlated with behavioural and other effects pro-

duced by drugs thought to affect 5-HT-mediated transmission. 5-HT cells show an unusual, highly regular, slow discharge pattern, and are strongly inhibited by 5-HT_1 receptor agonists, suggesting a local inhibitory feedback mechanism.

In vertebrates, certain physiological and behavioural functions relate particularly to 5-HT pathways (see Barnes & Sharp, 1999), namely:

- hallucinations and behavioural changes
- sleep, wakefulness and mood
- feeding behaviour
- control of sensory transmission (especially pain pathways; see Ch. 41).

Hallucinatory effects

Many hallucinogenic drugs (e.g. LSD; Ch. 47) are agonists at 5-HT_{2A} receptors. It is suggested that a loss of cortical inhibition underlies the hallucinogenic effect, as well as certain behavioural effects in experimental animals, such as the 'wet dog shakes' that occur in rats when the 5-HT precursor 5-hydroxytryptophan is administered. Many antipsychotic drugs (Ch. 45) are antagonists at 5-HT_{2A} receptors in addition to blocking dopamine D_2 receptors. The psychostimulant properties of MDMA ('ecstasy'; see Ch. 47) are due partly to its ability to release 5-HT. MDMA is taken up by the serotonin transporter, causing it to displace 5-HT from storage vesicles—a mechanism analogous to the action of amphetamine on noradrenergic nerve terminals (Ch. 14).

Sleep, wakefulness and mood

Lesions of the raphe nuclei, or depletion of 5-HT by PCPA administration, abolish sleep in experimental animals, whereas microinjection of 5-HT at specific points in the brain stem induces sleep. 5-HT_7 receptor antagonists inhibit 'rapid-eye-movement' (REM) sleep and increase the latency to onset of REM sleep. Attempts to cure insomnia in humans by giving 5-HT precursors (tryptophan or 5-hydroxytryptophan) have, however, proved unsuccessful. There is strong evidence that 5-HT, as well as noradrenaline, may be involved in the control of mood (see Ch. 46), and the use of tryptophan to enhance 5-HT synthesis has been tried in depression, with equivocal results.

Feeding and appetite

In experimental animals, 5-HT_{1A} agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) cause hyperphagia, leading to obesity. Antagonists acting on 5-HT_2 receptors, including several antipsychotic drugs used clinically, also increase appetite and cause weight gain. On the other hand, antidepressant drugs that inhibit 5-HT uptake (see Ch. 46) cause loss of appetite.

Sensory transmission

After lesions of the raphe nuclei or administration of PCPA, animals show exaggerated responses to many forms of sensory stimulus. They are startled much more easily, and also quickly develop avoidance responses to stimuli that would not normally bother them. It appears that the normal ability to disregard irrelevant forms of sensory input requires intact 5-HT pathways. The 'sensory enhancement' produced by hallucinogenic drugs may be partly due to loss of this gatekeeper function of 5-HT. 5-HT also exerts an inhibitory effect on transmission in the pain pathway, both in the spinal cord and in the brain, and there is a synergistic effect between 5-HT and analgesics such as **morphine** (see Ch. 41). Thus, depletion of 5-HT by PCPA, or selective lesions to the descending 5-HT-containing neurons that run to the dorsal horn, antagonise the analgesic effect of morphine, while inhibitors of 5-HT uptake have the opposite effect.

Other possible roles

Other putative roles of 5-HT include various autonomic and endocrine functions, such as the regulation of body temperature, blood pressure and sexual function. Further information can be found in Azmitia & Whitaker-Azmitia (1995) and Iversen et al. (2009).

CLINICALLY USED DRUGS

Several classes of drugs used clinically influence 5-HT-mediated transmission. They include:

- 5-HT reuptake inhibitors, such as **fluoxetine**, used as antidepressants (Ch. 46) and anxiolytic agents (Ch. 43)
- 5-HT_{1D} receptor agonists, such as **sumatriptan**, used to treat migraine (Ch. 15)
- **buspirone**, a 5-HT_{1A} receptor agonist used in treating anxiety (Ch. 43)
- 5-HT_3 receptor antagonists, such as **ondansetron**, used as antiemetic agents (see Ch. 29)
- antipsychotic drugs (e.g. **clozapine**, Ch. 45), which owe their efficacy partly to an action on 5-HT receptors.

ACETYLCHOLINE

There are numerous cholinergic neurons in the CNS, and the basic processes by which ACh is synthesised, stored and released are the same as in the periphery (see Ch. 13). Various biochemical markers have been used to locate cholinergic neurons in the brain, the most useful being choline acetyltransferase, the enzyme responsible for ACh synthesis, and the transporters that capture choline and package ACh, which can be labelled by immunofluorescence. Biochemical studies on ACh precursors and metabolites are generally more difficult than corresponding studies on other amine transmitters, because the relevant substances, choline and acetate, are involved in many processes other than ACh metabolism.

CHOLINERGIC PATHWAYS IN THE CNS

Acetylcholine is very widely distributed in the brain, occurring in all parts of the forebrain (including the cortex), midbrain and brain stem, although there is little in the cerebellum. Cholinergic neurons in the forebrain and brain stem send diffuse projections to many parts of the brain (see Fig. 38.7). Cholinergic neurons in the forebrain lie in a discrete area, forming the magnocellular forebrain nuclei (so called because the cell bodies are conspicuously large). Degeneration of one of these, the *nucleus basalis of Meynert*, which projects mainly to the cortex, is associated with Alzheimer's disease (Ch. 39). Another cluster, the *septohippocampal nucleus*, provides the main cholinergic input to the hippocampus, and is involved in memory. In addition, there are—in contrast to the monoamine pathways—many local cholinergic interneurons, particularly in the corpus

5-Hydroxytryptamine in the CNS

- The processes of synthesis, storage, release, reuptake and degradation of 5-hydroxytryptamine (5-HT) in the brain are very similar to events in the periphery (Ch. 15).
- Availability of tryptophan is the main factor regulating synthesis.
- Urinary excretion of 5-hydroxyindole acetic acid provides a measure of 5-HT turnover.
- 5-HT neurons are concentrated in the midline raphe nuclei in the brain stem projecting diffusely to the cortex, limbic system, hypothalamus and spinal cord, similar to the noradrenergic projections.
- Functions associated with 5-HT pathways include:
  - various behavioural responses (e.g. hallucinatory behaviour, 'wet dog shakes')
  - feeding behaviour
  - control of mood and emotion
  - control of sleep/wakefulness
  - control of sensory pathways, including nociception
  - control of body temperature
  - vomiting.
- 5-HT can exert inhibitory or excitatory effects on individual neurons, acting either presynaptically or postsynaptically.
- The main receptor subtypes (see Table 15.1) in the CNS are 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3. Associations of behavioural and physiological functions with these receptors have been partly worked out. Other receptor types (5-$HT_{4–7}$) also occur in the central nervous system, but less is known about their function.
- Drugs acting selectively on 5-HT receptors or transporters include:
  - **buspirone**, 5-HT_{1A} receptor agonist used to treat anxiety (see Ch. 43)
  - 'triptans' (e.g. **sumatriptan**), 5-HT_{1D} agonists used to treat migraine (see Ch. 15)
  - 5-HT_2 antagonists (e.g. **pizotifen**) used for migraine prophylaxis (see Ch. 15)
  - selective serotonin uptake inhibitors (e.g. **fluoxetine**) used to treat depression (see Ch. 46)
  - **ondansetron**, a 5-HT_3 antagonist, used to treat chemotherapy-induced emesis (see Chs 15 and 29)
  - **MDMA** (ecstasy), a substrate for the 5-HT transporter. It then displaces 5-HT from nerve terminals onto 5-HT receptors to produce its mood-altering effects (see Ch. 47).

striatum, these being important in relation to Parkinson's disease and Huntington's chorea (Ch. 39).

ACETYLCHOLINE RECEPTORS

Acetylcholine acts on both muscarinic (G-protein-coupled) and nicotinic (ionotropic) receptors in the CNS (see Ch. 13).

The muscarinic ACh receptors (mAChRs) in the brain are predominantly of the G_q-coupled M_1 class (i.e. M_1, M_3 and M_5 subtypes; see Ch. 13). Activation of these receptors

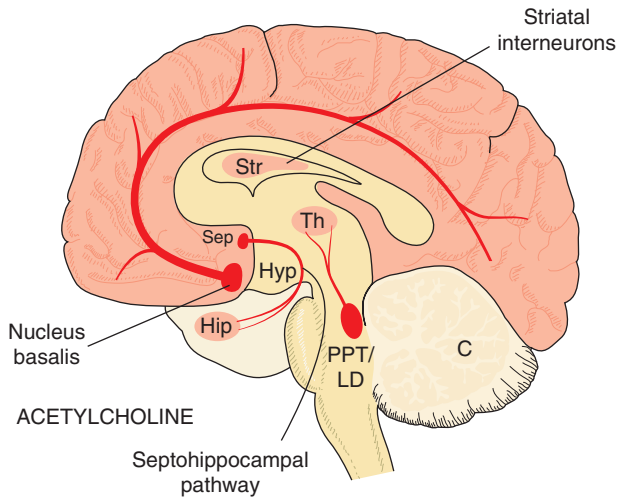

Fig. 38.7 Simplified diagram of the acetylcholine pathways in the brain, drawn as in Figure 38.1. PPT/LD, pedunculopontine and laterodorsal tegmental nuclei; other abbreviations as in Figure 38.1.

can result in excitation through blockade of M-type (KCNQ/Kv7) K^+ channels (see Delmas & Brown, 2005). G_i/G_o-coupled M_2 and M_4 receptors, on the other hand, are inhibitory through activation of inwardly rectifying K^+ channels and inhibition of voltage-sensitive Ca^{2+} channels. mAChRs on cholinergic terminals function to inhibit ACh release, and muscarinic antagonists, by blocking this inhibition, markedly increase ACh release. Many of the behavioural effects associated with cholinergic pathways seem to be produced by ACh acting on mAChRs.

Nicotinic ACh receptors (nAChRs) are ligand-gated cation channels permeable to Na^+, K^+ and Ca^{2+} ions (see Ch. 13). They are pentamers and can be formed as homomeric or heteromeric combinations of α (α2–7) and β (β2–4) subunits (Ch. 3; see Gotti et al., 2008) distributed widely throughout the brain (see Table 38.2). The heteromeric α4β2 and the homomeric α_7 subtypes are the most extensively characterised. The lack of subtype-specific ligands and the fact that some neurons express multiple subtypes has made the elucidation of the functions of each receptor subtype extremely difficult. Nicotine (see Ch. 48) exerts its central effects by agonist action on nAChRs.

For the most part, nAChRs are located presynaptically and act usually to facilitate the release of other transmitters such as glutamate, dopamine and GABA.[2] In a few situations, they function postsynaptically to mediate fast excitatory transmission, as in the periphery.

Many of the drugs that block nAChRs (e.g. **tubocurarine**; see Ch. 13) do not cross the blood–brain barrier, and even those that do (e.g. **mecamylamine**) produce only modest CNS effects. Various nAChR knockout mouse strains have been produced and studied. Deletion of the various CNS-specific nAChR subtypes generally has rather little effect, although some cognitive impairment can be detected. Mutations in nAChRs may be the cause of some forms of epilepsy and changes in nAChR expression may

[2]See Khahk & Henderson, 2000, for a description of how presynaptic cation-selective ligand-gated channels can, under different circumstances, facilitate or enhance neurotransmitter release.

Table 38.2 Presence of nicotinic receptors of different subunit composition in selected regions of the central nervous system

Brain region	Nicotinic receptors						
	α7	α3β2	α3β4	α4β2	α4α5β	α6β2β3	α6α4β2β3
Cortex	+			+	+		
Hippocampus	+		+	+	+		
Striatum				+	+	+	+
Amygdala	+			+			
Thalamus				+			
Hypothalamus	+			+			
Substantia nigra	+		+	+	+	+	
Cerebellum	+	+	+	+			
Spinal cord	+	+		+			

nAChRs comprising α2β2 and α3β3β4 are found in some other areas of the brain.
Data taken from Gotti et al., 2006.

occur in disorders such as schizophrenia, attention deficit hyperactivity disorder, depression and anxiety, as well as following neurodegeneration in Alzheimer's and Parkinson's diseases.

FUNCTIONAL ASPECTS

The functional roles of cholinergic pathways have been deduced mainly from studies of the action of drugs that mimic, accentuate or block the actions of ACh, and from studies of transgenic animals in which particular AChRs were deleted or mutated (see Cordero-Erausquin et al., 2000; Hogg et al., 2003).

The main functions ascribed to cholinergic pathways are related to arousal, learning and memory, and motor control. The cholinergic projection from the ventral forebrain to the cortex is thought to mediate arousal, whereas the septohippocampal pathway is involved in learning and short-term memory (see Hasselmo, 2006). Cholinergic interneurons in the striatum are involved in motor control (see Ch. 39).

Muscarinic agonists have been shown to restore partially learning and memory deficits induced in experimental animals by lesions of the septohippocampal cholinergic pathway. **Hyoscine**, a muscarinic antagonist, impairs memory in human subjects and causes amnesia when used as preanaesthetic medication. M_1 receptor knockout mice, however, show only slight impairment of learning and memory (see Wess, 2004).

Nicotine increases alertness and also enhances learning and memory, as do various synthetic agonists at neuronal nAChRs. Conversely, CNS-active nAChR antagonists such as **mecamylamine** cause detectable, although slight, impairment of learning and memory. Transgenic mice with disruption of brain nAChRs are only slightly impaired in spatial learning tasks.

In conclusion, both nAChRs and mAChRs may play a role in learning and memory, while nAChRs also mediate behavioural arousal. Receptor knockout mice are surprisingly little affected, suggesting that alternative mechanisms may be able to compensate for the loss of ACh receptor signalling.

Acetylcholine in the CNS

- Synthesis, storage and release of acetylcholine (ACh) in the central nervous system (CNS) are essentially the same as in the periphery (Ch. 13).
- ACh is widely distributed in the CNS, important pathways being:
  - basal forebrain (magnocellular) nuclei, which send a diffuse projection to most forebrain structures, including the cortex
  - septohippocampal projection
  - short interneurons in the striatum and nucleus accumbens.
- Certain neurodegenerative diseases, especially dementia and Parkinson's disease (see Ch. 39), are associated with abnormalities in cholinergic pathways.
- Both nicotinic and muscarinic (predominantly M_1) ACh receptors occur in the CNS. The former mediate the central effects of nicotine. Nicotinic receptors are mainly located presynaptically; there are few examples of transmission mediated by postsynaptic nicotinic receptors.
- Muscarinic receptors appear to mediate the main behavioural effects associated with ACh, namely effects on arousal, and on learning and short-term memory.
- Muscarinic antagonists (e.g. **hyoscine**) cause amnesia.

The importance of cholinergic neurons in neurodegenerative conditions such as dementia and Parkinson's disease is discussed in Chapter 39. The role of nAChRs in modulating pain transmission in the CNS is described in Chapter 41.

PURINES

Both adenosine and ATP act as transmitters and/or modulators in the CNS (for review, see Fredholm et al., 2005;

Khahk & North, 2006) as they do in the periphery (Ch. 16). Mapping the pathways is difficult, because purinergic neurons are not easily identifiable histochemically, but it is likely that adenosine serves as a very widespread neuromodulator, while ATP has more specific synaptic functions as a fast transmitter and as a local modulator.

Adenosine is produced intracellularly from ATP. It is not packaged into vesicles but is released mainly by carrier-mediated transport. Because the intracellular concentration of ATP (several mmol/l) greatly exceeds that of adenosine, conversion of a small proportion of ATP results in a large increase in adenosine. ATP is packaged into vesicles and released by exocytosis as a conventional transmitter, but can also leak out of cells in large amounts under conditions of tissue damage. In high concentrations, ATP can act as an excitotoxin (like glutamate; see Ch. 39) and cause further neuronal damage. It is also quickly converted to adenosine, which exerts a protective effect. These special characteristics of adenosine metabolism suggest that it serves mainly as a safety mechanism, protecting the neurons from damage when their viability is threatened, for example by ischaemia or seizure activity.

Adenosine produces its effects through G-protein-coupled adenosine A receptors (see Ch. 16). For ATP there are two forms of receptor—P2X and P2Y receptors (see Ch. 16 also). P2X receptors are trimeric ligand-gated cation channels that can be homomeric or heteromeric in composition whereas P2Y receptors are G-protein coupled.

There are four adenosine receptors—A_1, A_{2A}, A_{2B} and A_3—distributed throughout the CNS. The overall effect of adenosine, or of various adenosine receptor agonists, is inhibitory, leading to effects such as drowsiness and sedation, motor incoordination, analgesia and anticonvulsant activity. Xanthines, such as **caffeine** (Ch. 47), which are antagonists at A_2 receptors, produce arousal and alertness.

While there is little doubt that purinergic signalling plays a major role in CNS function, our understanding is still very limited. There is optimism that purinergic receptor ligands—both agonists and antagonists—will prove useful in a wide range of CNS disorders (see Burnstock, 2008).

HISTAMINE

▼ Histamine is present in the brain in much smaller amounts than in other tissues, such as skin and lung, but undoubtedly serves a neurotransmitter role (see Brown et al., 2001). The cell bodies of histaminergic neurons, which also synthesise and release a variety of other transmitters, are restricted to a small part of the hypothalamus, and their axons run to virtually all parts of the brain. Unusually, no uptake mechanism for histamine is present, its action being terminated instead by enzymic methylation.

Histamine acts on at least three types of receptor (H_{1-3}; Ch. 17) in the brain (the evidence for H_4 receptors in brain is still rather flimsy). They occur in most brain regions and are all G-protein coupled—H_1 receptors to G_q, H_2 to G_s and H_3 to G_i/G_o. H_3 receptors are inhibitory autoreceptors on histamine-releasing neurons.

Like other monoamine transmitters, histamine is involved in many different CNS functions. Histamine release follows a distinct circadian pattern, the neurons being active by day and silent by night. H_1 receptors in the cortex and reticular activating system contribute to arousal and wakefulness, and H_1 receptor antagonists produce sedation (see Ch. 43). Other functions ascribed to histamine include control of food and water intake, and thermoregulation, but these are less well characterised. Antihistamines are widely used to control nausea and vomiting, for example in motion sickness and middle ear disorders, as well as to induce sleep.

OTHER CNS MEDIATORS

We now move from the familiar neuropharmacological territory of the 'classic' monoamines to some of the frontier towns, bordering on the Wild West. Useful drugs are still few and far between in this area, and if applied pharmacology is your main concern, you can safely skip the next part and wait a few years for law and order to be established.

MELATONIN

▼ Melatonin (*N*-acetyl-5-methoxytryptamine) (reviewed by Dubocovich et al., 2003) is synthesised exclusively in the pineal, an endocrine gland that plays a role in establishing circadian rhythms. The gland contains two enzymes, not found elsewhere, which convert 5-HT by acetylation and *O*-methylation to melatonin, its hormonal product.

There are two well-defined melatonin receptors (MT_1 and MT_2) which are G-protein-coupled receptors—both coupling to G_i/G_o—found mainly in the brain and retina but also in peripheral tissues (see Jockers et al., 2008). Another type (termed MT_3) has been suggested to be the enzyme quinone reductase 2 (QR2). The function of the interaction between melatonin and QR2 is still unclear.

Melatonin secretion (in all animals, whether diurnal or nocturnal in their habits) is high at night and low by day. This rhythm is controlled by input from the retina via a noradrenergic retinohypothalamic tract that terminates in the suprachiasmatic nucleus (SCN) in the hypothalamus, a structure often termed the 'biological clock', which generates the circadian rhythm. Activation of MT_1 receptors inhibits neuronal firing in the SCN and prolactin secretion from the pituitary. Activation of MT_2 receptors phase shifts circadian rhythms generated within the SCN.

Given orally, melatonin is well absorbed but quickly metabolised, its plasma half-life being a few minutes. It has been promoted as a means of controlling jet lag, or of improving the performance of night-shift workers, based on its ability to reset the circadian clock. A single dose appears to have the effect of resynchronising the physiological secretory cycle, although it is not clear how this occurs. **Ramelteon**, an agonist at MT_1 and MT_2 receptors, is used to treat insomnia (see Ch. 43) and **agomelatine**, which has agonist actions at MT_1 and MT_2 receptors as well as antagonist actions at 5-HT_{2c} receptors, is a novel antidepressant drug (see Ch. 46)

NITRIC OXIDE

Nitric oxide (NO) as a peripheral mediator is discussed in Chapter 20. Its significance as an important chemical mediator in the nervous system has demanded a considerable readjustment of our views about neurotransmission and neuromodulation (for review, see Garthwaite, 2008). The main defining criteria for transmitter substances—namely that neurons should possess machinery for synthesising and storing the substance, that it should be released from neurons by exocytosis, that it should interact with specific membrane receptors and that there should be mechanisms for its inactivation—do not apply to NO. Moreover, it is an inorganic gas, not at all like the kind of molecule we are used to. The mediator function of NO is now well established (Zhou & Zhu, 2009). NO diffuses rapidly through cell membranes, and its action is not highly localised. Its half-life depends greatly on the chemical environment, ranging from seconds in blood to several minutes in normal tissues. The rate of inactivation of NO (see Ch. 20, reaction 20.1) increases disproportionately with NO concentration, so low levels of NO are relatively stable. The presence of superoxide, with which NO reacts (see below), shortens its half-life considerably.

Nitric oxide in the nervous system is produced mainly by the constitutive neuronal form of *nitric oxide synthase* (nNOS; see Ch. 20), which can be detected either histochemically or by immunolabelling. This enzyme is present in roughly 2% of neurons, both short interneurons and long-tract neurons, in virtually all brain areas, with particular concentrations in the cerebellum and hippocampus. It occurs in cell bodies and dendrites, as well as in axon terminals, suggesting that NO may be produced both pre- and postsynaptically. nNOS is calmodulin dependent and is activated by a rise in intracellular Ca^{2+} concentration, which can occur by many mechanisms, including action potential conduction and neurotransmitter action, especially by glutamate activation of Ca^{2+}-permeable NMDA receptors. NO is not stored, but released as it is made. Many studies have shown that NO production is increased by activation of synaptic pathways, or by other events, such as brain ischaemia (see Ch. 39).

Nitric oxide exerts pre- and postsynaptic actions on neurons as well as acting on glial cells (Garthwaite, 2008). It produces its effects in two main ways:

1. By activation of soluble guanylyl cyclase, leading to the production of cGMP, which activates various phosphorylation cascades (Ch. 3). This 'physiological' control mechanism operates at low NO concentrations of about 0.1 µmol/l.
2. By reacting with the superoxide free radical to generate peroxynitrite, a highly toxic anion that acts by oxidising various intracellular proteins. This requires concentrations of 1–10 µmol/l, which are achieved in brain ischaemia.

There is good evidence that NO plays a role in synaptic plasticity (see Ch. 37), because long-term potentiation and depression are reduced or prevented by NOS inhibitors and are absent in transgenic mice in which the *nNOS* gene has been disrupted.

Based on the same kind of evidence, NO is also believed to play an important part in the mechanisms by which ischaemia causes neuronal death (see Ch. 39). There is also evidence that it may be involved in other processes, including neurodegeneration in Parkinson's disease, senile dementia and amyotrophic lateral sclerosis, and the local control of blood flow linked to neuronal activity.

▼ **Carbon monoxide** (CO) is best known as a poisonous gas present in vehicle exhaust, which binds strongly to haemoglobin, causing tissue anoxia. However, it is also formed endogenously and has many features in common with NO (see Barañano et al., 2001). Neurons and other cells contain a CO-generating enzyme, haem oxygenase, and CO, like NO, activates guanylyl cyclase.

The role of CO as a CNS mediator is not well established, but there is some evidence that it plays a role in memory mechanisms in the hippocampus (see Cutajar & Edwards, 2007).

LIPID MEDIATORS

▼ The formation of arachidonic acid, and its conversion to eicosanoids (mainly prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids (HETEs); see Ch. 17) and to endocannabinoids, anandamide and 2-arachidonoylglycerol (see Ch. 18), also take place in the CNS (for reviews, see Piomelli, 1995; Pertwee, 2008).

Phospholipid cleavage, leading to arachidonic acid production, occurs in neurons in response to receptor activation by many different mediators, including neurotransmitters. The arachidonic acid so formed can act directly as an intracellular messenger, controlling both ion channels and various parts of the protein kinase cascade (see Ch. 3), producing both rapid and delayed effects on neuronal function. Both arachidonic acid itself and its products escape readily from the cell of origin and can affect neighbouring structures, including presynaptic terminals (retrograde signalling) and adjacent cells (paracrine signalling), by acting on receptors or by acting directly as intracellular messengers. Figure 38.8 shows a schematic view of the variety of different roles these agents can play at the synapse.

Arachidonic acid can be metabolised to eicosanoids, some of which (principally the HETEs) can also act as intracellular messengers acting in the same cell. Eicosanoids can also exert an autocrine effect via membrane receptors expressed by the cell (see Ch. 17). The eicosa-

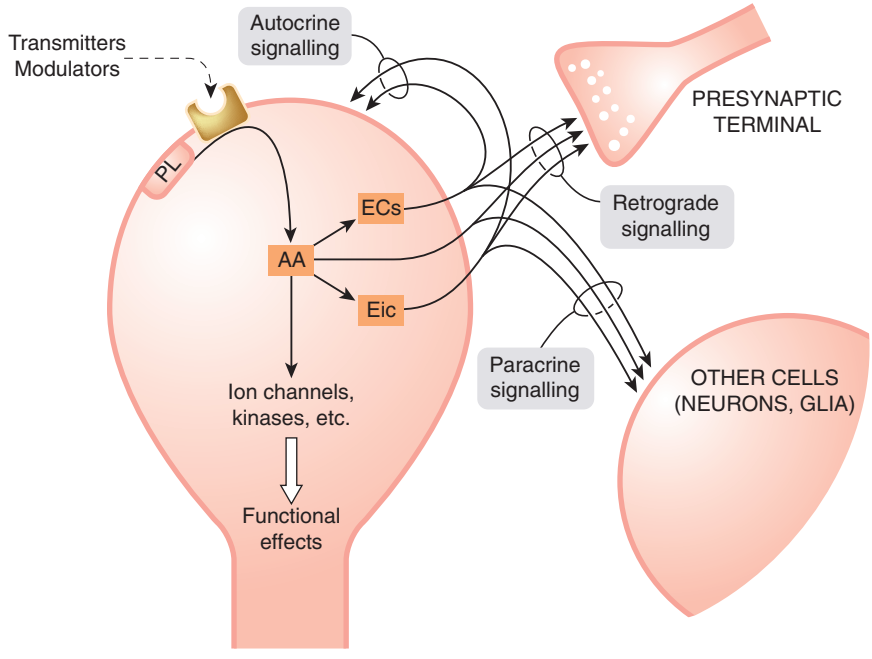

Fig. 38.8 Postulated modes of signalling by lipid mediators. Arachidonic acid (AA) is formed by receptor-mediated cleavage of membrane phospholipid. It can act directly as an intracellular messenger on ion channels or components of different kinase cascades, producing various long- and short-term effects. It can also be converted to eicosanoids (prostaglandins, leukotrienes or hydroxyeicosatetraenoic acids [HETEs]) or to the endocannabinoids (ECs), anandamide and 2-arachidonoylglycerol. HETEs can also act directly as intracellular messengers. All these mediators diffuse out of the cell, and exert effects on presynaptic terminals and neighbouring cells, acting either on extracellular receptors or intracellularly. There are examples of most of these modes of signalling but only limited information about their functional significance in the nervous system. Eic, eicosanoids; PL, membrane phospholipid.

noids play important roles in neural function including pain, temperature regulation, sleep induction, synaptic plasticity and spatial learning.

It is now generally accepted that the endocannabinoids act as retrograde synaptic messengers. They are synthesised and secreted in response to a rise in intracellular Ca^{2+} and activate presynaptic CB_1 receptors resulting in an inhibition of the release of neurotransmitters such as glutamate and GABA (see Vaughan & Christie, 2005). CB_1 receptors are widely distributed in the brain and spinal cord whereas CB_2 receptor expression is much less. Agonists at CB_1 receptors have therapeutic potential for the treatment of vomiting, pain (CB_2 receptor agonists may also be effective in some pain states), muscle spasms as occur in conditions such as multiple sclerosis and anxiety, as well as in other brain disorders including Alzheimer's disease and tardive dyskinesias (see Pertwee, 2008). The CB_1 receptor antagonist, **rimonabant**, was introduced as an antiobesity agent but subsequently had to be withdrawn because of negative effects on mood (see Ch. 18). One surprise in this field has been the discovery that anandamide, besides being an agonist at cannabinoid receptors, also activates TRPV1 channels (see Ch. 41) which are involved in the response of peripheral sensory nerve terminals to painful stimuli.

A FINAL MESSAGE

In the last two chapters, we have taken a long and tortuous tour through the brain and its chemistry, with two questions at the back of our minds. What mediators and what receptors play a key role in what brain functions? How does the information relate to existing and future drugs that aim to correct malfunctions? Through the efforts of a huge army of researchers deploying an arsenal of powerful new techniques, the answers to these questions are slowly being produced. The array of potential CNS targets—comprising multiple receptor subtypes, many with the added complexity of heteromeric assemblies, splice variants, etc., along with regulatory mechanisms that control their expression and localisation—continues to grow in complexity. Speculation about the best target to aim at in order to ameliorate the effect of a particular brain malfunction, such as stroke or schizophrenia, has become less focused, even if better informed, than

Other transmitters and modulators

Purines

- ATP functions as a neurotransmitter, being stored in vesicles and released by exocytosis. It acts, via ionotropic P2X receptors, as a fast excitatory transmitter in certain pathways and, via metabotropic P2Y receptors, as a neuromodulator.
- Cytosolic ATP is present at relatively high concentration and can be released directly if neuronal viability is compromised (e.g. in stroke). Excessive release may be neurotoxic.
- Released ATP is rapidly converted to ADP, AMP and adenosine.
- Adenosine is not stored in vesicles but is released by carrier mechanisms or generated from released ATP, mainly under pathological conditions.
- Adenosine exerts mainly inhibitory effects, through A_1 and A_2 receptors, resulting in sedative, anticonvulsant and neuroprotective effects, and acting as a safety mechanism.
- Methylxanthines (e.g. **caffeine**) are antagonists at A_2 receptors and increase wakefulness.

Histamine

- Histamine fulfils the criteria for a neurotransmitter. Histaminergic neurons originate in a small area of the hypothalamus and have a widespread distribution.
- H_1, H_2 and H_3 receptors are widespread in the brain.
- The functions of histamine are not well understood, the main clues being that histaminergic neurons are active during waking hours, and H_1 receptor antagonists are strongly sedative.
- H_1 receptor antagonists are antiemetic.

Melatonin

- Melatonin is synthesised from 5-hydroxytryptamine, mainly in the pineal gland, from which it is released as a circulating hormone.
- Secretion is controlled by light intensity, being low by day and high by night. Fibres from the retina run to the suprachiasmatic nucleus ('biological clock'), which controls the pineal gland via its sympathetic innervation.
- Melatonin acts on MT_1 and MT_2 receptors in the brain. Given orally, it causes sedation and also 'resets' the biological clock, being used for this purpose to counter jet lag.
- Agonists at melatonin receptors induce sleep and have antidepressant properties.

Nitric oxide (see Ch. 20)

- Neuronal nitric oxide synthase (nNOS) is present in many central nervous system neurons, and nitric oxide (NO) production is increased by mechanisms (e.g. transmitter action) that raise intracellular Ca^{2+}.
- NO affects neuronal function by increasing cGMP formation, producing both inhibitory and excitatory effects on neurons.
- In larger amounts, NO forms peroxynitrite, which contributes to neurotoxicity.
- Inhibition of nNOS reduces long-term potentiation and long-term depression, probably because NO functions as a retrograde messenger. Inhibition of nNOS also protects against ischaemic brain damage in animal models.
- Carbon monoxide shares many properties with NO and may also be a neural mediator.

Lipid mediators

- Arachidonic acid is produced in neurons by receptor-mediated hydrolysis of phospholipid. It is converted to various eicosanoids and endocannabinoids.
- Arachidonic acid itself, as well as its active products, can produce rapid and slow effects by regulation of ion channels and protein kinase cascades. Such effects can occur in the donor cell or in adjacent cells and nerve terminals.
- Anandamide and 2-arachidonoylglycerol are endogenous activators of cannabinoid CB_1 and CB_2 receptors (Ch. 18) and also of the TRPV1 receptor (Ch. 41).

it was two decades ago. In the ensuing chapters in this section, we shall find that most of the therapeutic successes have come from chance discoveries that were followed up empirically; few have followed a logical, mechanism-based route to success. The optimistic view is that this is changing, and that future therapeutic discoveries will depend less on luck and more on molecular logic. But the revolution is slow in coming. One of the key problems, perhaps, is that the brain puts cells, organelles and molecules exactly where they are needed, and uses the same molecules to perform different functions in different locations. Drug discovery scientists are getting quite good at devising molecule-specific ligands (see Ch. 60), but we lack delivery systems able to target them anatomically even to macroscopic brain regions, let alone to specific cells and subcellular structures.

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39 Neurodegenerative diseases

OVERVIEW

As a rule, dead neurons in the adult central nervous system (CNS) are not replaced,[1] nor can their terminals regenerate when their axons are interrupted. Therefore any pathological process causing neuronal death generally has irreversible consequences. At first sight, this appears to be very unpromising territory for pharmacological intervention, and indeed drug therapy is currently very limited, except in the case of Parkinson's disease (PD; see below). Nevertheless, the incidence and social impact of neurodegenerative brain disorders in ageing populations has resulted in a massive research effort in recent years.

In this chapter, we focus mainly on three common neurodegenerative conditions: Alzheimer's disease (AD), PD and ischaemic brain damage (stroke). AD and PD are the commonest examples of a group of chronic, slowly developing conditions that include various prion diseases (e.g. Creutzfeldt–Jakob disease, CJD). They have a common aetiology in that they are caused by the aggregation of misfolded variants of normal physiological proteins. The high hopes that the new pathophysiological understanding that has emerged over the last two decades would lead to significant therapeutic progress in this important area remain largely unrealised, and to date the available therapeutic interventions are aimed at compensating for, rather than preventing or reversing, the neuronal loss.

Stroke, which is a common disorder of enormous socioeconomic importance, results from acute ischaemic brain damage, quite different from the aetiology of chronic neurodegenerative diseases, requiring different but equally challenging therapeutic approaches.

The main topics discussed are:

- **mechanisms responsible for neuronal death, focusing on protein aggregation (e.g. amyloidosis), excitotoxicity, oxidative stress and apoptosis**
- **pharmacological approaches to neuroprotection, based on the above mechanisms**
- **pharmacological approaches to compensation for neuronal loss (applicable mainly to AD and PD).**

[1]It is recognised that new neurons are formed from progenitor cells (*neurogenesis*) in certain regions of the adult brain and can become functionally integrated, even in primates (see Rakic, 2002; Zhao et al., 2008). Neurogenesis in the hippocampus is thought to play a role in learning and memory, but plays little if any role in brain repair. However, learning how to harness the inherent ability of neuronal progenitors (stem cells) to form new neurons is seen as an obvious approach to treating neurodegenerative disorders.

PROTEIN MISFOLDING AND AGGREGATION IN CHRONIC NEURODEGENERATIVE DISEASES

Protein misfolding and aggregation is the first step in many neurodegenerative diseases (see Stefani & Dobson, 2003; Forman et al., 2004; Selkoe, 2004). Misfolding means the adoption of abnormal conformations, by certain normally expressed proteins, such that they tend to form large insoluble aggregates (Fig. 39.1). The conversion of the linear amino acid chain produced by the ribosome into a functional protein requires it to be folded correctly into a compact conformation with specific amino acids correctly located on its surface. This complicated stepwise sequence can easily go wrong and lead to misfolded variants that are unable to find a way back to the correct 'native' conformation. The misfolded molecules are non-functional with respect to the normal function of the protein, but can nonetheless make mischief within the cell. The misfolding often means that hydrophobic residues that would normally be buried in the core of the protein are exposed on its surface, which gives the molecules a strong tendency to stick to cell membranes and aggregate, initially as oligomers and then as insoluble microscopic aggregates (Fig. 39.1), leading to the death of neurons. The tendency to adopt such conformations may be favoured by specific mutations of the protein in question, or by infection with prions (see below).

Misfolded conformations can be generated spontaneously at a low rate throughout life, so that aggregates accumulate gradually with age. In the nervous system, the aggregates often form distinct structures, generally known as *amyloid deposits*, that are visible under the microscope and are characteristic of neurodegenerative disease. Although the mechanisms are not clear, such aggregates, or the misfolded protein precursors, lead to neuronal death. Examples of neurodegenerative diseases that are caused by such protein misfolding and aggregation are shown in Table 39.1.

The brain possesses a variety of protective mechanisms that limit the accumulation of such protein aggregates. The main ones involve the production of 'chaperone' proteins, which bind to newly synthesised or misfolded proteins and encourage them to fold correctly, and the 'ubiquitination' reaction, which prepares proteins for destruction within the cell. Accumulation of protein deposits occurs when these protective mechanisms are unable to cope.

MECHANISMS OF NEURONAL DEATH

Acute injury to cells causes them to undergo *necrosis*, recognised pathologically by cell swelling, vacuolisation and lysis, and associated with Ca^{2+} overload of the cells and membrane damage (see below). Necrotic cells typically spill their contents into the surrounding tissue, evoking an inflammatory response. Chronic inflammation is a feature

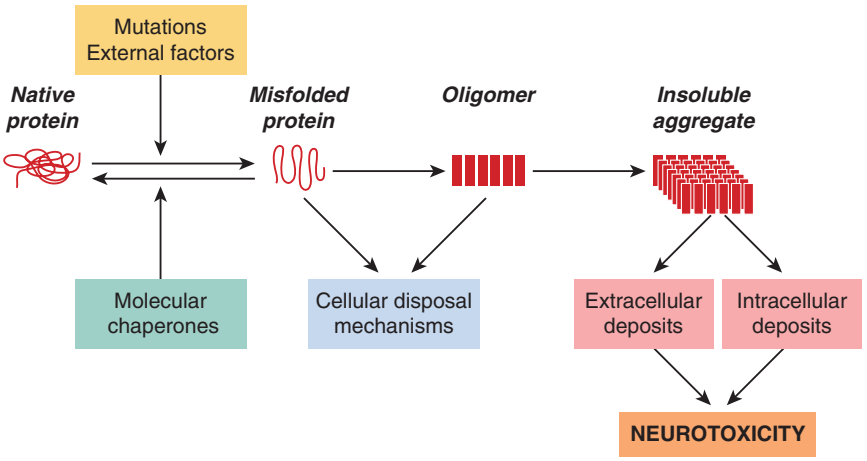

Fig. 39.1 Protein misfolding: a process involved in many chronic neurodegenerative diseases.

Protein misfolding

- Many chronic neurodegenerative diseases involve the misfolding of normal or mutated forms of physiological proteins. Examples include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and many less common diseases.
- Misfolded proteins are normally removed by intracellular degradation pathways, which may be altered in neurodegenerative disorders.
- Misfolded proteins tend to aggregate, initially as soluble oligomers, later as large insoluble aggregates that accumulate intracellularly or extracellularly as microscopic deposits, which are stable and resistant to proteolysis.
- Misfolded proteins often present hydrophobic surface residues that promote aggregation and association with membranes.
- The mechanisms responsible for neuronal death are unclear, but there is evidence that both the soluble aggregates and the microscopic deposits may be neurotoxic.

of most neurodegenerative disorders (see Schwab & McGeer, 2008), and a possible target for therapeutic intervention.

Cells can also die by *apoptosis* or programmed cell death (see Ch. 5), a mechanism that is essential for many processes throughout life, including development, immune regulation and tissue remodelling. Apoptosis, as well as necrosis, occurs in both acute neurodegenerative disorders (such as stroke and head injury) and chronic ones (such as Alzheimer's and Parkinson's disease; see Okouchi et al., 2007). The distinction between necrosis and apoptosis as processes leading to neurodegeneration is not absolute, for challenges such as excitotoxicity and oxidative stress may be enough to kill cells directly by necrosis or, if less intense, may induce them to undergo apoptosis. Both processes therefore represent possible targets for putative neuroprotective drug therapy. Pharmacological interference with the apoptotic pathway may become possible in the future, but for the present most efforts are directed at the processes involved in cell necrosis, and at compensating pharmacologically for the neuronal loss.

EXCITOTOXICITY

Despite its ubiquitous role as a neurotransmitter, **glutamate** is highly toxic to neurons, a phenomenon dubbed *excitotoxicity* (see Ch. 37). A low concentration of glutamate applied to neurons in culture kills the cells, and the finding in the 1970s that glutamate given orally produces neurodegeneration in vivo caused considerable alarm because of the widespread use of glutamate as a 'taste-enhancing' food additive. The 'Chinese restaurant syndrome'—an acute attack of neck stiffness and chest pain—is well known, but so far the possibility of more serious neurotoxicity is only hypothetical.

Local injection of the glutamate receptor agonist *kainic acid* is used experimentally to produce neurotoxic lesions. It acts by excitation of local glutamate-releasing neurons, and the release of glutamate, acting on NMDA and also metabotropic receptors (Ch. 37), leads to neuronal death.

Calcium overload is the essential factor in excitotoxicity. The mechanisms by which this occurs and leads to cell death are as follows (Fig. 39.2):

- Glutamate activates NMDA, AMPA and metabotropic receptors (sites 1, 2 and 3). Activation of AMPA receptors depolarises the cell, which unblocks the NMDA channels (see Ch. 37), permitting Ca^{2+} entry. Depolarisation also opens voltage-activated calcium channels (site 4), releasing more glutamate. Metabotropic receptors cause the release of intracellular Ca^{2+} from the endoplasmic reticulum. Na^+ entry further contributes to Ca^{2+} entry by stimulating Ca^{2+}/Na^+ exchange (site 5). Depolarisation inhibits or reverses glutamate uptake (site 6), thus increasing the extracellular glutamate concentration.
- The mechanisms that normally operate to counteract the rise in $[Ca^{2+}]_i$ include the Ca^{2+} efflux pump (site 7) and, indirectly, the Na^+ pump (site 8).
- The mitochondria and endoplasmic reticulum act as capacious sinks for Ca^{2+} and normally keep $[Ca^{2+}]_i$

Table 39.1 Examples of neurodegenerative diseases associated with protein misfolding and aggregation[a]

Disease	Protein	Characteristic pathology	Notes
Alzheimer's disease	β-Amyloid (Aβ) Tau	Amyloid plaques Neurofibrillary tangles	Aβ mutations occur in rare familial forms of Alzheimer's disease Implicated in other pathologies ('tauopathies') as well as Alzheimer's disease
Parkinson's disease	α-Synuclein	Lewy bodies	α-Synuclein mutations occur in some types of familial Parkinson's disease
Creutzfeldt–Jakob disease	Prion protein	Insoluble aggregates of prion protein	Transmitted by infection with prion protein in its misfolded state
Huntington's disease	Huntingtin	No gross lesions	One of several genetic 'polyglutamine repeat' disorders
Amyotrophic lateral sclerosis (motor neuron disease)	Superoxide dismutase	Loss of motor neurons	Mutated superoxide dismutase tends to form aggregates; loss of enzyme function increases susceptibility to oxidative stress

[a] Protein aggregation disorders are often collectively known as amyloidoses and commonly affect organs other than the brain.

under control. Loading of the mitochondrial stores beyond a certain point, however, disrupts mitochondrial function, reducing ATP synthesis, thus reducing the energy available for the membrane pumps and for Ca^{2+} accumulation by the endoplasmic reticulum. Formation of reactive oxygen species is also enhanced. This represents the danger point at which positive feedback exaggerates the process.

- Raised $[Ca^{2+}]_i$ affects many processes, the chief ones relevant to neurotoxicity being:
  - increased glutamate release
  - activation of proteases (calpains) and lipases, causing membrane damage
  - activation of nitric oxide synthase; while low concentrations of nitric oxide are neuroprotective, high concentrations in the presence of reactive oxygen species generate peroxynitrite and hydroxyl free radicals, which damage many important biomolecules, including membrane lipids, proteins and DNA
  - increased arachidonic acid release, which increases free radical production and also inhibits glutamate uptake (site 6).

Glutamate and Ca^{2+} are arguably the two most ubiquitous chemical signals, extracellular and intracellular, respectively, underlying brain function, so it is disconcerting that such cytotoxic mayhem can be unleashed when they get out of control. Both are stored in dangerous amounts in subcellular organelles, like hand grenades in an ammunition store. Defence against excitotoxicity is clearly essential if our brains are to have any chance of staying alive. Mitochondrial energy metabolism provides one line of defence (see above), and impaired mitochondrial function, by rendering neurons vulnerable to excitotoxic damage, may be a factor in various neurodegenerative conditions, including PD. Furthermore, impaired mitochondrial function can cause release of cytochrome c, which is an important initiator of apoptosis.

The role of excitotoxicity in ischaemic brain damage is well established (see below), and it is also believed to be a factor in other neurodegenerative diseases, such as those discussed below (see Lipton & Rosenberg, 1994).

▼ There are several examples of neurodegenerative conditions caused by environmental toxins acting as agonists on glutamate receptors. *Domoic acid* is a glutamate analogue produced by mussels, which was identified as the cause of an epidemic of severe mental and neurological deterioration in a group of Newfoundlanders in 1987. On the island of Guam, a syndrome combining the features of dementia, paralysis and PD was traced to an excitotoxic amino acid, β-methylamino-alanine, in the seeds of a local plant. Discouraging the consumption of these seeds has largely eliminated the disease.

Disappointingly, intense effort, based on the mechanisms described above, to find effective drugs for a range of neurodegenerative disorders in which excitotoxicity is believed to play a part has had very limited success. **Riluzole**, a compound that inhibits both the release and the postsynaptic action of glutamate, retards to some degree the deterioration of patients with amyotrophic lateral sclerosis. **Memantine**, a compound first described 40 years ago, is a weak NMDA receptor antagonist that produces slight improvement in moderate-to-severe cases of AD, but is not recommended for routine clinical use.

APOPTOSIS

Apoptosis can be initiated by various cell surface signals (see Ch. 5). The cell is systematically dismantled, and the shrunken remnants are removed by macrophages without causing inflammation. Apoptotic cells can be identified by a staining technique that detects the characteristic DNA breaks. Many different signalling pathways can result in apoptosis, but in all cases the final pathway resulting in cell death is the activation of a family of proteases (caspases), which inactivate various intracellular proteins. Neural apoptosis is normally prevented by neuronal growth factors, including *nerve growth factor* and *brain-derived neurotrophic factor*, secreted proteins that are required for the survival of different populations of neurons in the CNS. These growth factors regulate the expression of the two gene products Bax and Bcl-2, Bax being proapoptotic and Bcl-2 being antiapoptotic (see Ch. 5). Blocking apoptosis by interfering at specific points on these pathways represents an attractive strategy for developing neuroprotective drugs, but one that has yet to bear fruit.

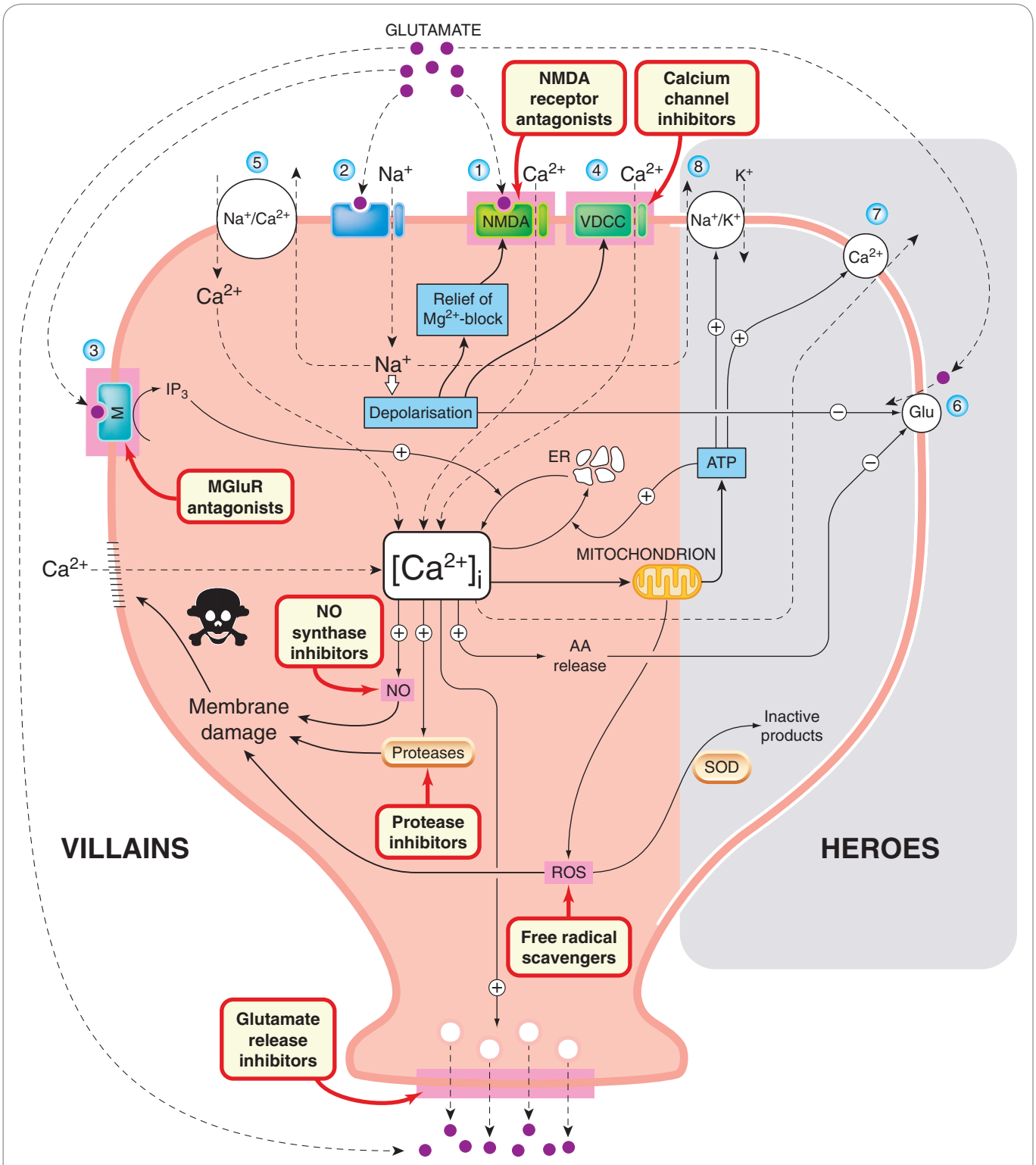

Fig. 39.2 Mechanisms of excitotoxicity. Membrane receptors, ion channels and transporters, identified by numbers 1–8, are discussed in the text. Possible sites of action of neuroprotective drugs (not yet of proven clinical value) are highlighted. Mechanisms on the left (villains) are those that favour cell death, while those on the right (heroes) are protective. See text for details. AA, arachidonic acid; ER, endoplasmic reticulum; Glu, glutamate uptake; IP_3, inositol trisphosphate; M, MGluR, metabotropic glutamate receptor; NO, nitric oxide; ROS, reactive oxygen species; SOD, superoxide dismutase; VDCC, voltage-dependent calcium channel.

OXIDATIVE STRESS

The brain has high energy needs, which are met almost entirely by mitochondrial oxidative phosphorylation, generating ATP at the same time as reducing molecular O_2 to H_2O. Under certain conditions, highly reactive oxygen species (ROS), for example oxygen and hydroxyl free radicals and H_2O_2, may be generated as side products of this process (see Coyle & Puttfarken, 1993; Barnham et al., 2004). Oxidative stress is the result of excessive production of these reactive species. They can also be produced as a byproduct of other biochemical pathways, including nitric oxide synthesis and arachidonic acid metabolism (which are implicated in excitotoxicity; see above), as well as the P450 mono-oxygenase system (see Ch. 9). Unchecked, reactive oxygen radicals attack many key molecules, including enzymes, membrane lipids and DNA. Not surprisingly, defence mechanisms are provided, in the form of enzymes such as *superoxide dismutase* (SOD) and *catalase*, as well as antioxidants such as ascorbic acid, glutathione and α-tocopherol (vitamin E), which normally keep these reactive species in check. Some cytokines, especially tumour necrosis factor (TNF)-α, which is produced in conditions of brain ischaemia or inflammation (Ch.17), exert a protective effect, partly by increasing the expression of SOD. Transgenic animals lacking TNF receptors show enhanced susceptibility to brain ischaemia. Mutations of the gene encoding SOD (Fig. 39.2) are associated with *amyotrophic lateral sclerosis* (ALS, also known as motor neuron disease), a fatal paralytic disease resulting from progressive degeneration of motor neurons, and transgenic mice expressing mutated SOD develop a similar condition.[2] Accumulation of aggregates of misfolded mutated SOD (see above) may also contribute to neurodegeneration.

Mitochondria play a central role in energy metabolism, failure of which leads to oxidative stress. Damage to mitochondria, leading to the release of cytochrome c into the cytosol, also initiates apoptosis. Mitochondrial integrity is therefore essential for neuronal survival, and mitochondrial dysfunction is seen as a major factor in many neurodegenerative disorders (see Petrozzi et al., 2007). It is possible that accumulated or inherited mutations in enzymes such as those of the mitochondrial respiratory chain lead to a congenital or age-related increase in susceptibility to oxidative stress, which is manifest in different kinds of inherited neurodegenerative disorders (such as Huntington's disease), and in age-related neurodegeneration.

Oxidative stress is both a cause and consequence of inflammation (Ch. 6), which is a general feature of neurodegenerative disease and is thought to contribute to neuronal damage (see Schwab & McGeer, 2008).

Several possible targets for therapeutic intervention with neuroprotective drugs are shown in Figure 39.2.

Excitotoxicity and oxidative stress

- Excitatory amino acids, especially glutamate, can cause neuronal death.
- Excitotoxicity is associated mainly with activation of NMDA receptors, but other types of excitatory amino acid receptors also contribute.
- Excitotoxicity results from a sustained rise in intracellular Ca^{2+} concentration (Ca^{2+} overload).
- Excitotoxicity can occur under pathological conditions (e.g. cerebral ischaemia, epilepsy) in which excessive glutamate release occurs. It can also occur when chemicals such as kainic acid are administered.
- Raised intracellular Ca^{2+} causes cell death by various mechanisms, including activation of proteases, formation of free radicals and lipid peroxidation. Formation of nitric oxide and arachidonic acid are also involved.
- Various mechanisms act normally to protect neurons against excitotoxicity, the main ones being Ca^{2+} transport systems, mitochondrial function and the production of free radical scavengers.
- Oxidative stress refers to conditions (e.g. hypoxia) in which the protective mechanisms are compromised, reactive oxygen species accumulate and neurons become more susceptible to excitotoxic damage.
- Excitotoxicity due to environmental chemicals may contribute to some neurodegenerative disorders.
- Measures designed to reduce excitotoxicity include the use of glutamate antagonists, calcium channel-blocking drugs and free radical scavengers; none is yet proven for clinical use.
- Mitochondrial dysfunction, associated with ageing, environmental toxins and genetic abnormalities, leads to oxidative stress and is a common feature of neurodegenerative diseases.

ISCHAEMIC BRAIN DAMAGE

After heart disease and cancer, strokes are the commonest cause of death in Europe and North America, and the 70% that are non-fatal are the commonest cause of disability. Approximately 85% of strokes are *ischaemic*, usually due to thrombosis of a major cerebral artery. The remainder are *haemorrhagic*, due to rupture of a cerebral artery. Atherosclerosis is the usual underlying cause of both types.

PATHOPHYSIOLOGY

Interruption of blood supply to the brain initiates the cascade of neuronal events shown in Figure 39.2, which lead in turn to later consequences, including cerebral oedema and inflammation, which can also contribute to brain damage (see Dirnagl et al., 1999). Further damage can occur following reperfusion,[3] because of the production of reactive oxygen species when the oxygenation is restored. Reperfusion injury may be an important component in stroke patients. These secondary processes often take hours to develop, providing a window of opportunity for therapeutic intervention. The lesion produced by occlusion of a major cerebral artery consists of a central core in which the neurons quickly undergo irreversible necrosis,

[2]Surprisingly, some SOD mutations associated with ALS are more, rather than less, active than the normal enzyme. The mechanism responsible for neurodegeneration probably involves abnormal accumulation of the enzyme in mitochondria.

[3]Nevertheless, early reperfusion (within 3 h of the thrombosis) is clearly beneficial, based on clinical evidence with fibrinolytic drugs.

surrounded by a penumbra of compromised tissue in which inflammation and apoptotic cell death develop over a period of several hours. It is assumed that neuroprotective therapies, given within a few hours, might inhibit this secondary penumbral damage.

Glutamate excitotoxicity plays a critical role in brain ischaemia. Ischaemia causes depolarisation of neurons, and the release of large amounts of glutamate. Ca^{2+} accumulation occurs, partly as a result of glutamate acting on NMDA receptors, for both Ca^{2+} entry and cell death following cerebral ischaemia are inhibited by drugs that block NMDA receptors or channels (see Ch. 37). Nitric oxide is also produced in amounts much greater than result from normal neuronal activity (i.e. to levels that are toxic rather than modulatory).

THERAPEUTIC APPROACHES

The only drug currently approved for treating strokes is recombinant tissue plasminogen activator, **alteplase**, given intravenously, which helps to restore blood flow by dispersing the thrombus (see Ch. 24). A controlled trial showed that it did not reduce mortality (about 8%), but gave significant functional benefit to patients who survive. To be effective, it must be given within about 3 h of the thrombotic episode. Also, it must not be given in the 15% of cases where the cause is haemorrhage rather than thrombosis, so preliminary computerised tomography (CT) scanning is essential. These stringent requirements seriously limit the use of fibrinolytic agents for treating stroke, except where specialised rapid response facilities are available.

A preferable approach would be to use neuroprotective agents aimed at rescuing cells in the penumbral region of the lesion, which are otherwise likely to die. In animal models involving cerebral artery occlusion, many drugs targeted at the mechanisms shown in Figure 39.2 (not to mention many others that have been tested on the basis of more far-flung theories) act in this way to reduce the size of the infarct. These include glutamate antagonists, calcium and sodium channel inhibitors, free radical scavengers, anti-inflammatory drugs, protease inhibitors and others (see Green, 2008). It seems that almost anything works. Altogether, Green et al. (2003) reported that more than 37 such agents had been tested in more than 114 clinical trials, and all had failed to show efficacy. The dispiriting list of failures includes calcium and sodium channel blockers (e.g. **nimodipine**, **fosphenytoin**), NMDA receptor antagonists (**selfotel**, **eliprodil**, **dextromethorphan**), drugs that inhibit glutamate release (adenosine analogues, **lobeluzole**), drugs that enhance GABA effects (e.g. **chlormethiazole**), 5-HT antagonists, metal chelators and various free radical scavengers (e.g. **tirilazad**). Green et al. (2003) argued, reasonably enough, that the animal models in use failed to replicate the clinical situation, and urged the use of more rigorous experimental protocols to make animal models more predictive, but 5 years later (see Green, 2008) the success rate was still zero, and the prospect for proven neuroprotective agents in clinical use remains bleak.[4] Controlled clinical trials on stroke patients are problematic and very expensive, partly because of the large variability of outcome in terms of functional recovery, which means that large groups of patients (typically thousands) need to be observed for several months. The need to start therapy within hours of the attack is an additional problem.

Stroke treatment is certainly not—so far at least—one of pharmacology's success stories, and medical hopes rest more on prevention (e.g. by controlling blood pressure, taking aspirin and preventing atherosclerosis) than on treatment.

Stroke

- Associated with intracerebral thrombosis or haemorrhage (less common), resulting in rapid death of neurons by necrosis in the centre of the lesion, followed by more gradual (hours) degeneration of cells in penumbra due to excitotoxicity and inflammation.
- Spontaneous functional recovery occurs to a highly variable degree.
- Although many types of drug that interfere with excitotoxicity are able to reduce infarct size in experimental animals, none of these has so far proved efficacious in humans.
- Recombinant tissue plasminogen activator (**alteplase**), which disperses blood clots, is beneficial if it is given within 3 h.
- None of the many neuroprotective drugs that are effective in animal models is efficacious in the clinical trials.

ALZHEIMER'S DISEASE

Loss of cognitive ability with age is considered to be a normal process whose rate and extent is very variable. AD was originally defined as presenile dementia, but it now appears that the same pathology underlies the dementia irrespective of the age of onset. AD refers to dementia that does not have an antecedent cause, such as stroke, brain trauma or alcohol. Its prevalence rises sharply with age, from about 5% at 65 to 90% or more at 95. Until recently, age-related dementia was considered to result from the steady loss of neurons that normally goes on throughout life, possibly accelerated by a failing blood supply associated with atherosclerosis. Studies over the past three decades have, however, revealed specific genetic and molecular mechanisms underlying AD (reviewed by Selkoe, 1997; Bossy-Wetzel et al., 2004). These advances have raised hopes of more effective treatments (see Yamada & Nabeshima, 2000), but success has proved elusive.

PATHOGENESIS OF ALZHEIMER'S DISEASE

Alzheimer's disease is associated with brain shrinkage and localised loss of neurons, mainly in the hippocampus and basal forebrain. The loss of cholinergic neurons in the hippocampus and frontal cortex is a feature of the disease, and is thought to underlie the cognitive deficit and loss of short-term memory that occur in AD. Two microscopic features are characteristic of the disease, namely

[4]Nevertheless, Besancon et al., 2008 retain their optimism that among the plethora of channels and transporters possessed by neurons and glia, there must be *something* that will prove to be a useful neuroprotective drug target.

extracellular *amyloid plaques*, consisting of amorphous extracellular deposits of β-amyloid protein (known as Aβ), and intraneuronal *neurofibrillary tangles*, comprising filaments of a phosphorylated form of a microtubule-associated protein (Tau). Both of these deposits are protein aggregates that result from misfolding of native proteins, as discussed above. They appear also in normal brains, although in smaller numbers. The early appearance of amyloid deposits presages the development of AD, although symptoms may not develop for many years. Altered processing of amyloid protein from its precursor (*amyloid precursor protein*, APP; see Bossy-Wetzel et al., 2004) is now recognised as the key to the pathogenesis of AD. This conclusion is based on several lines of evidence, particularly the genetic analysis of certain, relatively rare, types of familial AD, in which mutations of the APP gene, or of other genes that control amyloid processing, have been discovered.

The APP gene resides on chromosome 21, which is duplicated in Down's syndrome, in which early AD-like dementia occurs in association with overexpression of APP.

▼ Amyloid deposits consist of aggregates of Aβ (Fig. 39.3), a 40 or 42 residue segment of APP, generated by the action of specific proteases (*secretases*). Aβ40 is produced normally in small amounts, whereas Aβ42 is overproduced as a result of the genetic mutations mentioned above. Both proteins aggregate to form *amyloid plaques*, but Aβ42 shows a stronger tendency than Aβ40 to do so, and appears to be the main culprit in amyloid formation. APP is a 770-amino acid membrane protein normally expressed by many cells, including CNS neurons. Cleavage by α-secretase releases the large extracellular domain as *soluble APP*, which is believed to serve a physiological trophic function. Formation of Aβ involves cleavage at two different points, including one in the intramembrane domain of APP, by β- and γ-secretases (Fig. 39.3). γ-Secretase is a clumsy enzyme—actually a large intramembrane complex of several proteins—that lacks precision and cuts APP at different points in the transmembrane domain,

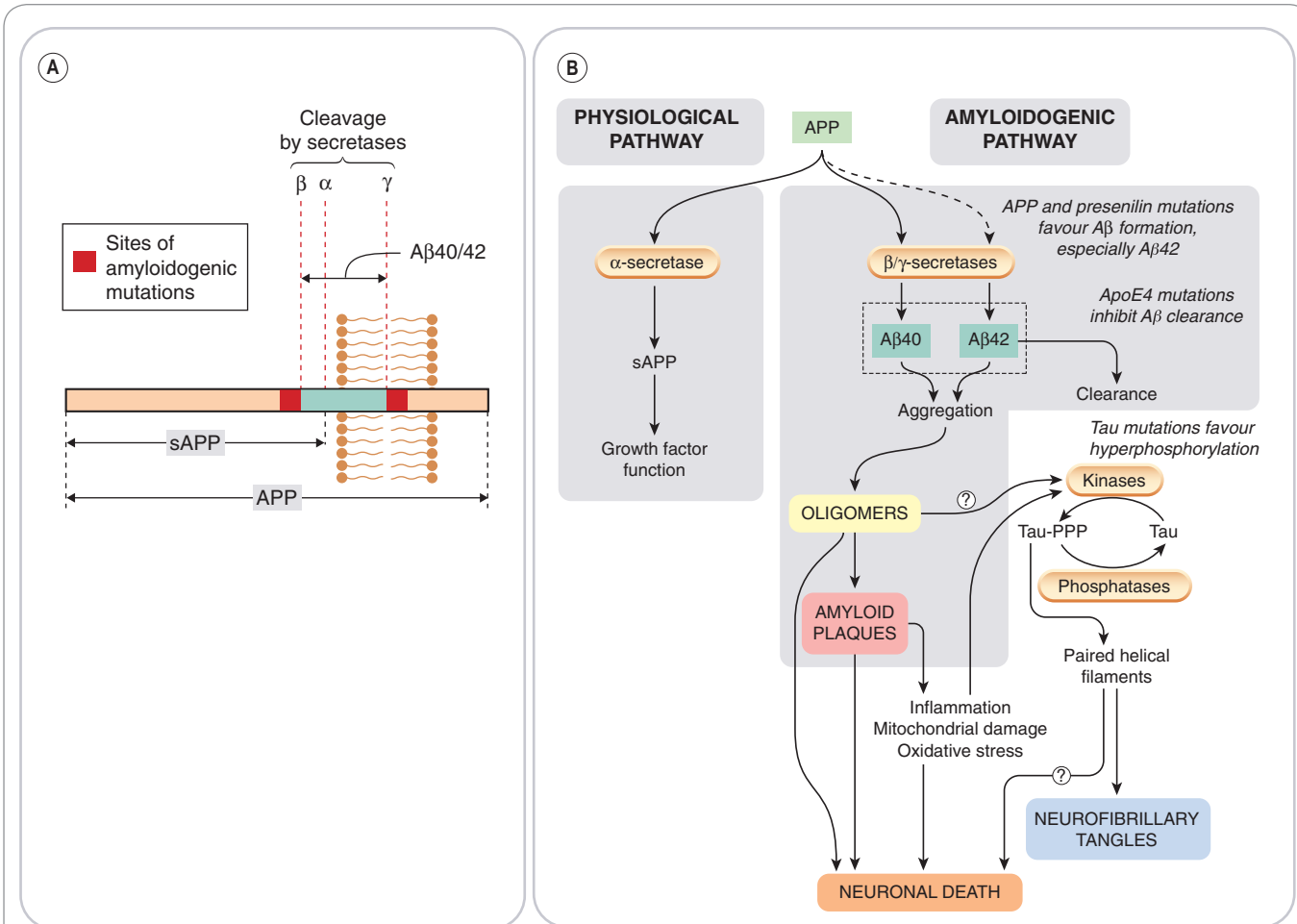

Fig. 39.3 Pathogenesis of Alzheimer's disease. [**A**] Structure of amyloid precursor protein (APP), showing origin of secreted APP (sAPP) and Aβ amyloid protein. The regions involved in amyloidogenic mutations discovered in some cases of familial Alzheimer's disease are shown flanking the Aβ sequence. APP cleavage involves three proteases: secretases α, β and γ. α-Secretase produces soluble APP, whereas β- and γ-secretases generate Aβ amyloid protein. γ-Secretase can cut at different points, generating Aβ peptides of varying lengths, including Aβ40 and Aβ42, the latter having a high tendency to aggregate as amyloid plaques. [**B**] Processing of APP. The main 'physiological' pathway gives rise to sAPP, which exerts a number of trophic functions. Cleavage of APP at different sites gives rise to Aβ, the predominant form normally being Aβ40, which is weakly amyloidogenic. Mutations in APP or presenilins increase the proportion of APP, which is degraded via the amyloidogenic pathway, and also increase the proportion converted to the much more strongly amyloidogenic form Aβ42. Clearance of Aβ is impaired by mutations in the *apoE4* gene. Hyperphosphorylated Tau results in dissociation of Tau from microtubules, misfolding and aggregation to form paired helical filaments, which enhance Aβ toxicity.

generating Aβ fragments of different lengths, including Aβ40 and 42. Mutations in this region of the APP gene affect the preferred cleavage point, tending to favour formation of Aβ42. Mutations of the unrelated presenilin genes result in increased activity of γ-secretase, because the presenilin proteins form part of the γ-secretase complex. These different AD-related mutations increase the ratio of Aβ42:Aβ40, which can be detected in plasma, serving as a marker for familial AD. Mutations in another gene, that for the lipid transport protein ApoE4 which facilitates the clearance of Aβ oligomers, also predispose to AD, probably because the mutant form of ApoE4 proteins are less effective in this function.

It is uncertain exactly how Aβ accumulation causes neurodegeneration, and whether the damage is done by soluble Aβ monomers or oligomers or by amyloid plaques. There is evidence that the cells die by apoptosis, although an inflammatory response is also evident. Expression of Alzheimer mutations in transgenic animals (see Götz & Ittner, 2008) causes plaque formation and neurodegeneration, and also increases the susceptibility of CNS neurons to other challenges, such as ischaemia, excitotoxicity and oxidative stress, and this increased vulnerability may be the cause of the progressive neurodegeneration in AD. These transgenic models are of great value in testing potential drug therapies aimed at retarding the neurodegenerative process.

The other main player on the biochemical stage is *Tau*, the protein of which the neurofibrillary tangles are composed (Fig. 39.3). Their role in neurodegeneration is unclear, although similar 'tauopathies' occur in many neurodegenerative conditions (see Brunden et al., 2009; Hanger et al., 2009). Tau is a normal constituent of neurons, being associated with the intracellular microtubules that serve as tracks for transporting materials along nerve axons. In AD and other tauopathies, Tau is abnormally phosphorylated by the action of various kinases, and dissociates from microtubules to be deposited intracellularly as *paired helical filaments* with a characteristic microscopic appearance. When the cells die, these filaments aggregate as extracellular *neurofibrillary tangles*. Tau phosphorylation is enhanced by the presence of Aβ, possibly by activation of kinases. Conversely, hyperphosphorylated Tau favours the formation of amyloid deposits. Whether hyperphosphorylation and intracellular deposition of Tau directly harms the cell is not certain, although it is known that it impairs fast axonal transport, a process that depends on microtubules.

Loss of cholinergic neurons

Although changes in many transmitter systems have been observed, mainly from measurements on postmortem AD brain tissue, a relatively selective loss of cholinergic neurons in the basal forebrain nuclei (Ch. 38) is characteristic. This discovery, made in 1976, implied that pharmacological approaches to restoring cholinergic function might be feasible, leading to the use of cholinesterase inhibitors to treat AD (see below).

Choline acetyl transferase activity, acetylcholine content, and acetylcholinesterase and choline transport in the cortex and hippocampus are all reduced considerably in AD but not in other disorders, such as depression or schizophrenia. Muscarinic receptor density, determined by binding studies, is not affected, but nicotinic receptors, particularly in the cortex, are reduced. The reason for the selective loss of cholinergic neurons resulting from Aβ formation is not known.

THERAPEUTIC APPROACHES

Unravelling the mechanism of neurodegeneration in AD has yet to result in therapies able to retard it. Currently, cholinesterase inhibitors (see Ch. 13) and **memantine** (see above) are the only drugs approved for treating AD. Many other approaches have been explored, based on the amyloid hypothesis as well as other ideas for neuroprotection (see Citron, 2004; Spencer et al., 2007), so far without success in clinical trials. The Web site http://www.alzforum.org keeps track of ongoing trials.[5]

Alzheimer's disease

- Alzheimer's disease (AD) is a common age-related dementia distinct from vascular dementia associated with brain infarction.
- The main pathological features of AD comprise amyloid plaques, neurofibrillary tangles and a loss of neurons (particularly cholinergic neurons of the basal forebrain).
- Amyloid plaques consist of aggregates of the Aβ fragment of amyloid precursor protein (APP), a normal neuronal membrane protein, produced by the action of β- and γ-secretases. AD is associated with excessive Aβ formation, resulting in neurotoxicity.
- Familial AD (rare) results from mutations in the APP gene, or in presenilin genes (involved in γ-secretase function), both of which cause increased Aβ formation.
- Mutations in the lipoprotein ApoE4 increase the risk of developing AD, probably by interfering with Aβ clearance
- Neurofibrillary tangles comprise intracellular aggregates of a highly phosphorylated form of a normal neuronal protein (Tau). Hyperphosphorylated Tau and Aβ act synergistically to cause neurodegeneration.
- Loss of cholinergic neurons is believed to account for much of the learning and memory deficit in AD.

CHOLINESTERASE INHIBITORS

Tacrine, the first drug approved for treating AD, was investigated on the basis that enhancement of cholinergic transmission might compensate for the cholinergic deficit. Trials showed modest improvements in tests of memory and cognition in about 40% of AD patients, but no improvement in other functional measures that affect quality of life. Tacrine has to be given four times daily and produces cholinergic side effects such as nausea and abdominal cramps, as well as hepatotoxicity in some patients, so it is far from an ideal drug. Later compounds, which also have limited efficacy but are more effective than tacrine in improving quality of life, include **donepezil**, **rivastigmine** and **galantamine** (Table 39.2). These drugs produce a measurable, although slight, improvement of cognitive function in AD patients, but this may be too small to be significant in terms of everyday life.

There is some evidence from laboratory studies that cholinesterase inhibitors may act somehow to reduce the formation or neurotoxicity of Aβ, and therefore retard the progression of AD as well as producing symptomatic benefit. Clinical trials, however, have shown only a small improvement in cognitive function, with no effect on disease progression.

Other drugs aimed at improving cholinergic function that are being investigated include other cholinesterase

[5]The authors admit to disappointment that, despite intense research efforts, no new drugs worthy of mention have emerged since the last edition of this book.

Table 39.2 Cholinesterase inhibitors used in the treatment of Alzheimer's disease[a]

Drug	Type of inhibition	Duration of action and dosage	Main side effects	Notes
Tacrine	Affects both AChE and BuChE Not CNS selective	~6 h 2–3 times daily oral dosage	Cholinergic side effects (abdominal pain, nausea, diarrhoea), hepatotoxicity	The first anticholinesterase shown to be efficacious in AD Monitoring for hepatotoxicity needed
Donepezil	CNS, AChE selective	~24 h Once-daily oral dosage	Slight cholinergic side effects	—
Rivastigmine	CNS selective	~8 h Twice-daily oral dosage	Cholinergic side effects that tend to subside with continuing treatment	Gradual dose escalation to minimise side effects
Galantamine	Affects both AChE and BuChE Also enhances nicotinic ACh receptor activation by allosteric action	~8 h Twice-daily oral dosage	Slight cholinergic side effects	—

[a] Similar level of limited clinical benefit for all drugs. No clinical evidence for retardation of disease process, although animal tests suggest diminution of Aβ and plaque formation by a mechanism not related to cholinesterase inhibition.
AChE, acetylcholinesterase; BuChE, butyryl cholinesterase.

inhibitors and a variety of muscarinic and nicotinic receptor agonists, none of which looks promising on the basis of early clinical results.

MEMANTINE

The other drug currently approved for the treatment of AD is **memantine**, an orally active antagonist at NMDA receptors, with weaker blocking actions on various other amine receptors. It was originally introduced as an antiviral drug, and resurrected as a potential inhibitor of excitotoxicity. It produces—surprisingly—a modest cognitive improvement in moderate or severe AD, but does not appear to be neuroprotective. It causes few side effects, and has a long plasma half-life.

Inhibiting neurodegeneration

▼ For most of the disorders discussed in this chapter, including AD, the Holy Grail, which so far eludes us, would be a drug that retards neurodegeneration. Now that we have several well-characterised targets, such as Aβ formation by the β- and γ-secretases, and Aβ neurotoxicity, together with a range of transgenic animal models of AD on which compounds can be tested, the prospects certainly look brighter than they did a decade ago. Particular developments are worth mentioning (see Selkoe & Schenk, 2003, and Citron, 2004, for more details).

Inhibitors of β- and γ-secretase have been identified and are undergoing clinical trials. Though they are effective in reducing Aβ formation, several have proved toxic to the immune system and gastrointestinal tract, and development has been halted.

Kinase inhibitors aimed at preventing Tau phosphorylation are also being investigated (see Brunden et al., 2009). The large number of phosphorylation sites and different kinases make this a difficult approach.

An ingenious new approach was taken by Schenk et al. (1999), who immunised AD transgenic mice with Aβ protein, and found that this not only prevented but also reversed plaque formation. Initial trials in humans had to be terminated because of neuroinflammatory complications, but monoclonal Aβ antibodies are undergoing clinical trials

Clinical use of drugs in dementia

- Acetylcholinesterase inhibitors and NMDA antagonists detectably improve cognitive impairment in clinical trials but have significant adverse effects and are of limited use clinically. They have not been shown to retard neurodegeneration.
- Efficacy is monitored periodically in individual patients, and administration continued only if the drugs are believed to be working and their effect in slowing functional and behavioural deterioration is judged to outweigh adverse effects.

Acetylcholinesterase inhibitors

- **Donepezil**, **galantamine**, **rivastigmine**. Tacrine is also effective, but may cause liver damage. Unwanted cholinergic effects may be troublesome.
- Used in mild to moderate Alzheimer's disease.

NMDA receptor antagonists

- For example, **memantine** (see Ch. 37).
- Used in moderate to severe Alzheimer's disease.

Epidemiological studies reveal that some non-steroidal anti-inflammatory drugs (NSAIDs; see Ch. 26) used routinely to treat arthritis reduce the likelihood of developing AD. **Ibuprofen** and **indometacin** have this effect, although other NSAIDs, such as **aspirin**, do not, nor do anti-inflammatory steroids such as **prednisolone**. There is some evidence that certain NSAIDs may affect Aβ-induced neurotoxicity by mechanisms other than cyclo-oxygenase inhibition (see Weggen et al., 2007). Disappointingly, however, clinical trials with various NSAIDs have so far failed to show evidence of benefit.

Aβ plaques bind copper and zinc, and removal of these metal ions promotes dissolution of the plaques. The amoebicidal drug **clioquinol** is a metal-chelating agent that causes regression of amyloid

deposits in animal models of AD, and showed some benefit in initial clinical trials. Clioquinol itself has known toxic effects in humans, which preclude its routine clinical use, but less toxic metal-chelating agents are under investigation.

Shortage of growth factors (particularly nerve growth factor) may contribute to the loss of forebrain cholinergic neurons in AD. Administering growth factors into the brain is not realistic for routine therapy, but alternative approaches, such as implanting cells engineered to secrete nerve growth factor, are under investigation.

PARKINSON'S DISEASE

FEATURES OF PARKINSON'S DISEASE

Parkinson's disease (see review by Schapira, 2009) is a progressive disorder of movement that occurs mainly in the elderly. The chief symptoms are:

- suppression of voluntary movements (*hypokinesia*), due partly to muscle rigidity and partly to an inherent inertia of the motor system, which means that motor activity is difficult to stop as well as to initiate
- tremor at rest, usually starting in the hands ('pill-rolling' tremor), which tends to diminish during voluntary activity
- muscle rigidity, detectable as an increased resistance in passive limb movement
- a variable degree of cognitive impairment.

Parkinsonian patients walk with a characteristic shuffling gait. They find it hard to start, and once in progress they cannot quickly stop or change direction. PD is commonly associated with dementia, depression and autonomic dysfunction, probably because the degenerative process is not confined to the basal ganglia but also affects other parts of the brain. In the later stages of the disease, the non-motor symptoms often predominate.

Parkinson's disease often occurs with no obvious underlying cause, but it may be the result of cerebral ischaemia, viral encephalitis or other types of pathological damage. The symptoms can also be drug induced, the main drugs involved being those that reduce the amount of dopamine in the brain (e.g. **reserpine**; see Ch. 14) or block dopamine receptors (e.g. antipsychotic drugs such as **chlorpromazine**; see Ch. 45). There are rare instances of familial early-onset PD, and several gene mutations have been identified, including those encoding *synuclein* and *parkin*. Study of these gene mutations has given some clues about the mechanism underlying the neurodegenerative process (see below).

Neurochemical changes

Parkinson's disease affects the basal ganglia, and its neurochemical origin was discovered in 1960 by Hornykiewicz, who showed that the dopamine content of the substantia nigra and corpus striatum (see Ch. 38) in post-mortem brains of PD patients was extremely low (usually less than 10% of normal), associated with a loss of dopaminergic neurons in the substantia nigra and degeneration of nerve terminals in the striatum. Other monoamines, such as noradrenaline and 5-hydroxytryptamine, were much less affected than dopamine. Gradual loss of dopamine occurs over several years, with symptoms of PD appearing only when the striatal dopamine content has fallen to 20–40% of normal. Lesions of the nigrostriatal tract or chemically induced depletion of dopamine in experimental animals also produce symptoms of PD. The symptom most clearly related to dopamine deficiency is *hypokinesia*, which occurs immediately and invariably in lesioned animals. Rigidity and tremor involve more complex neurochemical disturbances of other transmitters (particularly acetylcholine, noradrenaline, 5-hydroxytryptamine and GABA) as well as dopamine. In experimental lesions, two secondary consequences follow damage to the nigrostriatal tract, namely a hyperactivity of the remaining dopaminergic neurons, which show an increased rate of transmitter turnover, and an increase in the number of dopamine receptors, which produces a state of denervation hypersensitivity (see Ch. 12). The striatum expresses mainly D_1 (excitatory) and D_2 (inhibitory) receptors (see Ch. 38), but fewer D_3 and D_4 receptors. A simplified diagram of the neuronal circuitry involved, and the pathways primarily affected in PD and Huntington's disease, is shown in Figure 39.4.

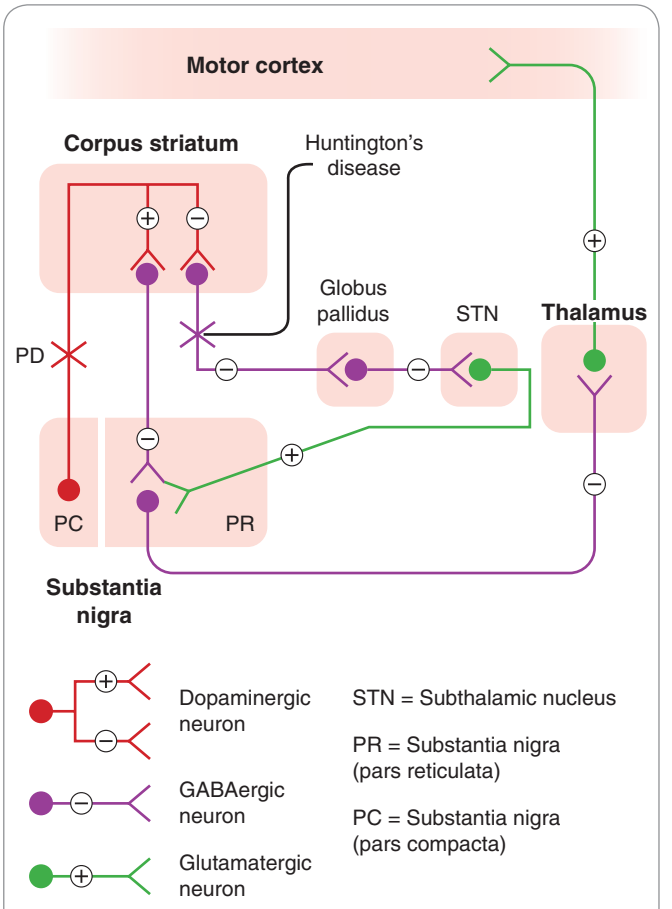

Fig. 39.4 Simplified diagram of the organisation of the extrapyramidal motor system and the defects that occur in Parkinson's disease (PD) and Huntington's disease. Normally, activity in nigrostriatal dopamine neurons causes excitation of striatonigral neurons and inhibition of striatal neurons that project to the globus pallidus. In either case, because of the different pathways involved, the activity of GABAergic neurons in the substantia nigra is suppressed, releasing the restraint on the thalamus and cortex, causing motor stimulation. In PD, the dopaminergic pathway from the substantia nigra (pars compacta) to the striatum is impaired. In Huntington's disease, the GABAergic striatopallidal pathway is impaired, producing effects opposite to the changes in PD.

Cholinergic interneurons of the corpus striatum (not shown in Fig. 39.4) are also involved in PD and Huntington's disease. Acetylcholine release from the striatum is strongly inhibited by dopamine, and it is suggested that hyperactivity of these cholinergic neurons contributes to the symptoms of PD. The opposite happens in Huntington's disease, and in both conditions therapies aimed at redressing the balance between the dopaminergic and cholinergic neurons are, up to a point, beneficial.

PATHOGENESIS OF PARKINSON'S DISEASE

Parkinson's disease is believed to be caused mainly by environmental factors, although the rare types of hereditary PD have provided some valuable clues about the mechanism. As with other neurodegenerative disorders, the damage is caused by protein misfolding and aggregation, aided and abetted by other familiar villains, namely excitotoxicity, mitochondrial dysfunction, oxidative stress, inflammation and apoptosis (see Lotharius & Brundin, 2002; Schapira, 2009). Aspects of the pathogenesis and animal models of PD are described by Meredith et al. (2008).

Neurotoxins

New light was thrown on the possible aetiology of PD by a chance event. In 1982, a group of young drug addicts in California suddenly developed an exceptionally severe form of PD (known as the 'frozen addict' syndrome), and the cause was traced to the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was a contaminant in a preparation used as a heroin substitute (see Langston, 1985). MPTP causes irreversible destruction of nigrostriatal dopaminergic neurons in various species, and produces a PD-like state in primates. MPTP acts by being converted to a toxic metabolite, MPP^+, by the enzyme monoamine oxidase (MAO, specifically by the MAO-B subtype; see Chs 14 and 46). MPP^+ is taken up by the dopamine transport system, and thus acts selectively on dopaminergic neurons; it inhibits mitochondrial oxidation reactions, producing oxidative stress. MPTP appears to be selective in destroying nigrostriatal neurons and does not affect dopaminergic neurons elsewhere—the reason for this is unknown. **Selegiline**, a selective MAO-B inhibitor, prevents MPTP-induced neurotoxicity by blocking its conversion to MPP^+. Selegiline is also used in treating PD (see below); as well as inhibiting dopamine breakdown, it might also work by blocking the metabolic activation of a putative endogenous, or environmental, MPTP-like substance, which is involved in the causation of PD. It is possible that dopamine itself could be the culprit, because oxidation of dopamine gives rise to potentially toxic metabolites. Whether or not the action of MPTP reflects the natural pathogenesis of PD, the MPTP model is a very useful experimental tool for testing possible therapies.

Impaired mitochondrial function is a feature of the disease in humans. Various herbicides, such as **rotenone**, that selectively inhibit mitochondrial function cause a PD-like syndrome in animals. PD in humans is more common in agricultural areas than in cities, suggesting that environmental toxins could be a factor in its causation.

Molecular aspects

▼ Parkinson's disease, as well as several other neurodegenerative disorders, is associated with the development of intracellular protein aggregates known as *Lewy bodies* in various parts of the brain. They consist largely of *α-synuclein*, a synaptic protein, present in large amounts in normal brains. Mutations occur in rare types of hereditary PD (see above), and it is believed that such mutations render the protein resistant to degradation within cells, causing it to pile up in Lewy bodies. It is possible (see Lotharius & Brundin, 2002) that the normal function of α-synuclein is related to synaptic vesicle recycling, and that the mutated form loses this functionality, with the result that vesicular storage of dopamine is impaired. This may lead to an increase in cytosolic dopamine, degradation of which produces reactive oxygen species and hence neurotoxicity. Consistent with the α-synuclein hypothesis, another mutation associated with PD (*parkin*) also involves a protein that participates in the intracellular degradation of rogue proteins. Other gene mutations that have been identified as risk factors for early-onset PD code for proteins involved in mitochondrial function, making cells more susceptible to oxidative stress. Thus, a picture similar to AD pathogenesis is slowly emerging. Misfolded α-synuclein, facilitated by genetic mutations or possibly by environmental factors, builds up in the cell as a result of impaired protein degradation (resulting from defective parkin) in the form of Lewy bodies, which, by unknown mechanisms, compromise cell survival. If oxidative stress is increased, as a result of ischaemia, mitochondrial poisons or mutations of certain mitochondrial proteins, the result is cell death.

Parkinson's disease

- Degenerative disease of the basal ganglia causing hypokinesia, tremor at rest and muscle rigidity, often with dementia and autonomic dysfunction.
- Associated with aggregation of α-synuclein (a protein normally involved in vesicle recycling) in the form of characteristic Lewy bodies.
- Often idiopathic but may follow stroke or virus infection; can be drug induced (antipsychotic drugs). Rare familial forms also occur, associated with various gene mutations, including α-synuclein.
- Associated with early degeneration of dopaminergic nigrostriatal neurons, followed by more general neurodegeneration.
- Can be induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin affecting dopamine neurons. Similar environmental neurotoxins, as well as genetic factors, may be involved in human Parkinson's disease.

DRUG TREATMENT OF PARKINSON'S DISEASE

Currently, the main drugs used (see Fig. 39.5) are:

- **levodopa** (often in combination with **carbidopa** and **entacopone**)
- dopamine agonists (e.g. **pramipexole**, **ropinirole**, **bromocriptine**)
- monoamine oxidase-B (MAO-B) inhibitors (e.g. **selegiline**, **rasagiline**).

Amantadine (thought to act by releasing dopamine), and muscarinic ACh receptor antagonists (e.g. **benztropine**) are occasionally used.

Despite past optimism, none of the drugs used to treat PD affects the progression of the disease. For general reviews of current and future approaches, see Olanow (2004) and Schapira (2009).

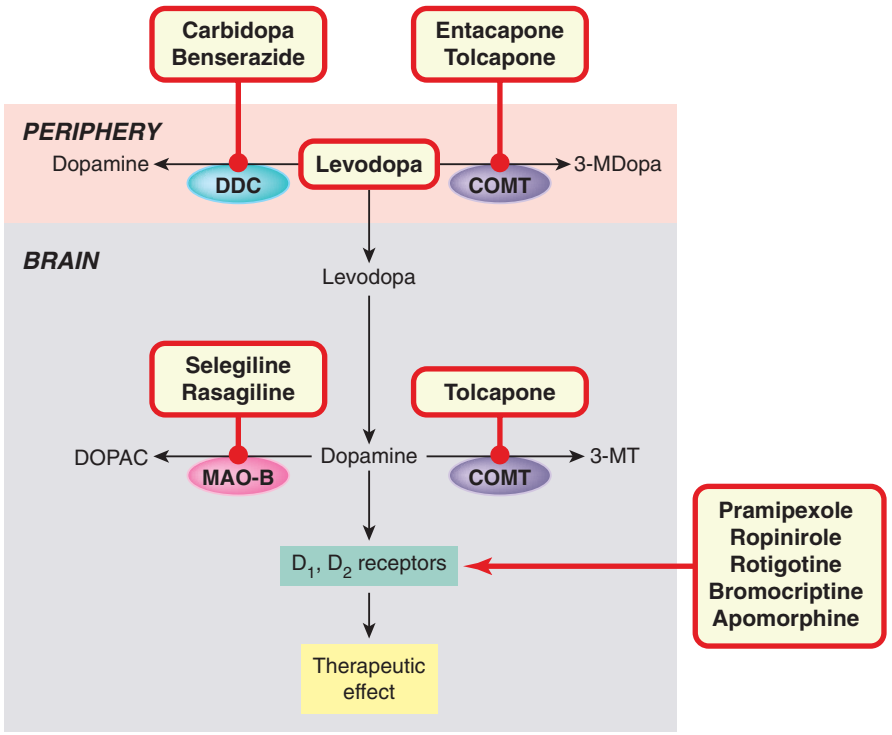

Fig. 39.5 Sites of action of drugs used to treat Parkinson's disease. Levodopa enters the brain and is converted to dopamine (the deficient neurotransmitter). Inactivation of levodopa in the periphery is prevented by inhibitors of DDC and COMT. Inactivation in the brain is prevented by inhibitors of COMT and MAO-B. Dopamine agonists act directly on striatal dopamine receptors. 3-MDopa, 3-methoxydopa; 3-MT, 3-methoxytyrosine; COMT, catechol-*O*-methyl transferase; DDC, DOPA decarboxylase; DOPAC, dihydroxyphenylacetic acid; MAO-B, monoamine oxidase B.

LEVODOPA

Levodopa is the first-line treatment for PD and is combined with a dopa decarboxylase inhibitor, either **carbidopa** or **benserazide**, which reduces the dose needed by about 10-fold and diminishes the peripheral side effects. It is well absorbed from the small intestine, a process that relies on active transport, although much of it is inactivated by MAO in the wall of the intestine. The plasma half-life is short (about 2 h). Conversion to dopamine in the periphery, which would otherwise account for about 95% of the levodopa dose and cause troublesome side effects, is largely prevented by the decarboxylase inhitor. Decarboxylation occurs rapidly within the brain, because the decarboxylase inhibitors do not penetrate the blood–brain barrier. It is not certain whether the effect depends on an increased release of dopamine from the few surviving dopaminergic neurons or on a 'flooding' of the synapse with dopamine formed elsewhere. Because synthetic dopamine agonists (see below) are equally effective, the latter explanation is more likely, and animal studies suggest that levodopa can act even when no dopaminergic nerve terminals are present. On the other hand, the therapeutic effectiveness of levodopa decreases as the disease advances, so part of its action may rely on the presence of functional dopaminergic neurons. Combination of levodopa plus dopa decarboxylase inhibitor with **entacapone**, a catechol-*O*-methyl transferase (COMT) inhibitor (see Ch. 14) to inhibit its degradation, is used in patients troubled by 'end of dose' motor fluctuations.

Therapeutic effectiveness

About 80% of patients show initial improvement with levodopa, particularly of rigidity and hypokinesia, and about 20% are restored virtually to normal motor function. As time progresses, the effectiveness of levodopa gradually declines (Fig. 39.6). In a typical study of 100 patients treated

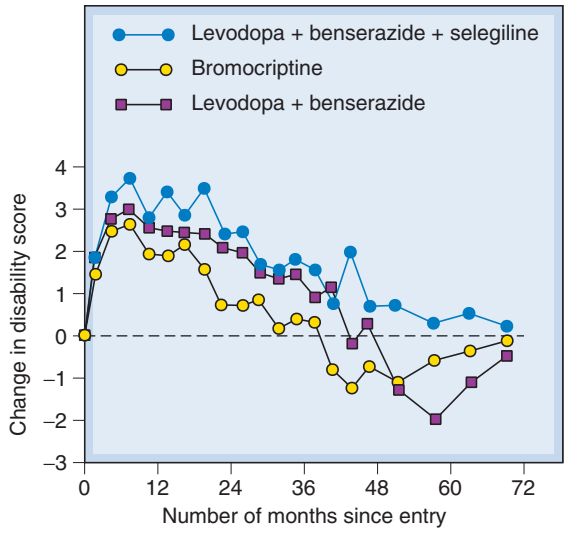

Fig. 39.6 Comparison of levodopa/benserazide, levodopa/benserazide/selegiline and bromocriptine on progression of Parkinson's disease symptoms. Patients (249–271 in each treatment group) were assessed on a standard disability rating score. Before treatment, the average rate of decline was 0.7 units/year. All three treatments produced improvement over the initial rating for 2–3 years, but the effect declined, either because of refractoriness to the drugs or disease progression. Bromocriptine appeared slightly less effective than levodopa regimens, and there was a higher drop-out rate due to side effects in this group. (From Parkinson's Disease Research Group 1993 Br Med J 307: 469–472.)

with levodopa for 5 years, only 34 were better than they had been at the beginning of the trial, 32 patients having died and 21 having withdrawn from the trial. It is likely that the loss of effectiveness of levodopa mainly reflects the natural progression of the disease, but receptor downregulation and other compensatory mechanisms may also contribute. There is no evidence that levodopa can actually accelerate the neurodegenerative process through overproduction of dopamine, as was suspected on theoretical grounds (see above). Overall, levodopa increases the life expectancy of PD patients, probably as a result of improved motor function, although some symptoms (e.g. dysphagia, cognitive decline) are not improved.

Unwanted effects

There are two main types of unwanted effect:

1. Involuntary writhing movements (dyskinesia), which do not appear initially but develop in the majority of patients within 2 years of starting levodopa therapy. These movements usually affect the face and limbs, and can become very severe. They occur at the time of the peak therapeutic effect, and the margin between the beneficial and the dyskinetic effect becomes progressively narrower. Levodopa is short acting, and the fluctuating plasma concentration of the drug may favour the development of dyskinesias, as longer-acting dopamine agonists are less problematic in this regard.
2. Rapid fluctuations in clinical state, where hypokinesia and rigidity may suddenly worsen for anything from a few minutes to a few hours, and then improve again. This 'on–off effect' is not seen in untreated PD patients or with other anti-PD drugs. The 'off effect' can be so sudden that the patient stops while walking and feels rooted to the spot, or is unable to rise from a chair, having sat down normally a few moments earlier. As with the dyskinesias, the problem seems to reflect the fluctuating plasma concentration of levodopa, and it is suggested that as the disease advances, the ability of neurons to store dopamine is lost, so the therapeutic benefit of levodopa depends increasingly on the continuous formation of extraneuronal dopamine, which requires a continuous supply of levodopa. The use of sustained-release preparations, or co-administration of COMT inhibitors such as entacapone (see above), may be used to counteract the fluctuations in plasma concentration of levodopa.

In addition to these slowly developing side effects, levodopa produces several acute effects, which are experienced by most patients at first but tend to disappear after a few weeks. The main ones are as follow:

- Nausea and anorexia. **Domperidone**, a dopamine antagonist that works in the chemoreceptor trigger zone (where the blood–brain barrier is leaky) but does not gain access to the basal ganglia, may be useful in preventing this effect.
- Hypotension. Postural hypotension is a problem in a few patients.
- Psychological effects. Levodopa, by increasing dopamine activity in the brain, can produce a schizophrenia-like syndrome (see Ch. 45) with delusions and hallucinations. More commonly, in about 20% of patients, it causes confusion, disorientation, insomnia or nightmares.

DOPAMINE AGONISTS

Two older drugs, **bromocriptine** and **pergolide**, are orally active ergot derivatives that act mainly on D_1 and D_2 receptors (see Ch. 38). Bromocriptine, which inhibits the release of prolactin from the anterior pituitary gland, was first introduced for the treatment of galactorrhoea and gynaecomastia (Ch. 32). Though effective in controlling the symptoms of PD, their usefulness is limited by side effects, mainly nausea and vomiting and somnolence. Pergolide is also believed to cause heart valve disease. These disadvantages have led to the replacement of these drugs by the non-ergot compounds **pramipexole** and **ropinirole**, which are $D_{2/3}$ selective and better tolerated, and do not show the fluctuations in efficacy associated with levodopa. They do, however, cause somnolence and sometimes hallucinations and recent evidence suggests that they may predispose to compulsive behaviours, such as excessive gambling,[6] over-eating and sexual excess, related to the 'reward' functions of dopamine (see Ch. 48).

A disadvantage of current dopamine agonists is their short plasma half-life (6–8 h), requiring three-times daily dosage, though slow-release once-daily formulations are now available.

Rotigotine is a newer agent, delivered as a transdermal patch, with similar efficacy and side effects.

Apomorphine, given by injection, is sometimes used to control the 'off effect' with levodopa. Because of its powerful emetic action, it must be combined with an oral antiemetic drug. It has other serious adverse effects (mood and behavioural changes, cardiac dysrhythmias, hypotension) and is a last resort if other drugs fail.

MAO-B INHIBITORS

Selegiline is a selective MAO-B[7] inhibitor, which lacks the unwanted peripheral effects of non-selective MAO inhibitors used to treat depression (Ch. 46) and, in contrast to them, does not provoke the 'cheese reaction' or interact so frequently with other drugs. Inhibition of MAO-B protects dopamine from extraneuronal degradation and was initially used as an adjunct to levodopa. Long-term trials showed that the combination of selegiline and levodopa was more effective than levodopa alone in relieving symptoms and prolonging life. Recognition of the role of MAO-B in neurotoxicity (see above) suggested that selegiline might be neuroprotective rather than merely enhancing the action of levodopa, but clinical studies do not support this. A large-scale trial (Fig. 39.6) showed no difference when selegiline was added to levodopa/benserazide treatment. Selegiline is metabolised to amphetamine, and sometimes causes excitement, anxiety and insomnia. **Rasagiline**, a very similar drug, does not have this unwanted effect, and a recent trial (Olanow et al., 2009) suggests that it may somewhat retard disease progression, as well alleviating symptoms.

[6]In 2008, a plaintiff was awarded $8.2m damages by a US court, having become a compulsive gambler (and losing a lot of money) after taking pramipexole for PD—a side effect of which the pharmaceutical company had been aware.

[7]MAO-B in the brain is located mainly in glial cells, and also in 5-HT neurons (though, surprisingly, it does not appear to be expressed in dopamine neurons).

OTHER DRUGS USED IN PARKINSON'S DISEASE

Amantadine

▼ Amantadine was introduced as an antiviral drug and discovered by accident in 1969 to be beneficial in PD. Many possible mechanisms for its action have been suggested based on neurochemical evidence of increased dopamine release, inhibition of amine uptake or a direct action on dopamine receptors. Most authors now suggest, although not with much conviction, that increased dopamine release is primarily responsible for the clinical effects.

Amantadine is less effective than levodopa or bromocriptine, and its action declines with time. Its side effects are considerably less severe, although qualitatively similar to those of levodopa.

Acetylcholine antagonists

▼ For more than a century, until levodopa was discovered, atropine and related drugs were the main form of treatment for PD. Muscarinic acetylcholine receptors exert an inhibitory effect on dopaminergic nerve terminals, suppression of which compensates for a lack of dopamine. The side effects of muscarinic antagonists—dry mouth, constipation, impaired vision, urinary retention—are troublesome, and they are now rarely used, except to treat parkinsonian symptoms in patients receiving antipsychotic drugs (which are dopamine antagonists and thus nullify the effect of L-dopa; see Ch. 45).

NEURAL TRANSPLANTATION AND BRAIN STIMULATION

▼ Parkinson's disease is the first neurodegenerative disease for which neural transplantation was attempted in 1982, amid much publicity. Various transplantation approaches have been tried, based on the injection of dissociated fetal cells (neuroblasts) directly into the striatum. Trials in patients with PD (see Björklund & Lindvall, 2000; Barker & Rosser, 2001) have mainly involved injection of midbrain cells from aborted human fetuses. Such transplants have been shown to survive and establish functional dopaminergic connections, and to produce clinical benefit in many cases (see Lindvall & Kokaia, 2009). However, some patients have gone on to develop serious dyskinesias, possibly due to dopamine overproduction. It is not yet known whether the transplanted cells will prove vulnerable to the neurodegenerative process responsible for killing the resident dopaminergic neurons. The use of fetal material is, of course, fraught with difficulties (usually cells from five or more fetuses are needed for one transplant), and hopes for the future rest mainly on the possibility of developing stem cell transplants (see Lindvall & Kokaia, 2009).

Electrical stimulation of the subthalamic nuclei with implanted electrodes (which inhibits ongoing neural activity, equivalent to reversible ablation) is used in severe cases, and can improve motor dysfunction in PD, but does not improve cognitive and other symptoms (see Benabid et al., 2009).

Drugs used in Parkinson's disease

- Drugs act by counteracting deficiency of dopamine in basal ganglia or by blocking muscarinic receptors. None of the available drugs affects the underlying neurodegeneration.
- Drugs include:
  - **levodopa** (dopamine precursor; Ch. 14), given with an inhibitor of peripheral dopa decarboxylase (e.g. **carbidopa**) to minimise side effects; sometimes a catechol-*O*-methyl transferase inhibitor (e.g. **entacapone**) is also given, especially to patients with 'end of dose' motor fluctuations
  - dopamine receptor agonists (**pramipexole**, **ropinirole**, **rotigotine**, **bromocriptine**). Rotigotine is available as a transdermal patch
  - monoamine oxidase B inhibitors (**selegiline**, **rasagiline**)
  - **amantadine** (which may enhance dopamine release)
  - **benztropine** (muscarinic receptor antagonist used for parkinsonism caused by antipsychotic drugs).
- Neurotransplantation, still in an experimental phase, may be effective but results are variable, and slowly developing dyskinesias may occur.

HUNTINGTON'S DISEASE

▼ Huntington's disease (HD) is an inherited (autosomal dominant) disorder resulting in progressive brain degeneration, starting in adulthood and causing rapid deterioration and death. As well as dementia, it causes severe motor symptoms in the form of involuntary writhing movements, which are highly disabling. It is the commonest of a group of so-called *trinucleotide repeat* neurodegenerative diseases, associated with the expansion of the number of repeats of the CAG sequence in specific genes, and hence the number (50 or more) of consecutive glutamine residues at the N-terminal of the expressed protein (see Walker, 2007). The larger the number of repeats, the earlier the appearance of symptoms. The protein coded by the HD gene, *huntingtin*, which normally possesses a chain of fewer than 30 glutamine residues, is a soluble cytosolic protein of unknown function found in all cells. HD develops when the mutant protein contains 40 or more repeats. The long poly-Gln chains reduce the solubility of huntingtin, and favour the formation of aggregates, which are formed from proteolytic N-terminal fragments that include the poly-Gln region. As with AD and PD, aggregation is probably responsible for the neuronal loss, which affects mainly the cortex and the striatum, resulting in progressive dementia and severe involuntary jerky (choreiform) movements. Studies on postmortem brains showed that the dopamine content of the striatum was normal or slightly increased, while there was a 75% reduction in the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis (Ch. 37). It is believed that the loss of GABA-mediated inhibition in the basal ganglia produces a hyperactivity of dopaminergic synapses, so the syndrome is in some senses a mirror image of PD (Fig. 39.4). The effects of drugs that influence dopaminergic transmission are correspondingly the opposite of those that are observed in PD, dopamine antagonists being effective in reducing the involuntary movements, while drugs such as levodopa and bromocriptine make them worse. Drugs used to alleviate the motor symptoms include **tetrabenazine** (an inhibitor of the vesicular monoamine transporter (see Ch. 14) that reduces dopamine storage, dopamine antagonists such as **chlorpromazine** (Ch. 45) and the GABA agonist **baclofen** (Ch. 37). These do not affect dementia or retard the course of the disease, and it is possible that drugs that inhibit excitotoxicity, or possibly neural transplantation procedures when these become available (see above), may prove useful.

NEURODEGENERATIVE PRION DISEASES

▼ A group of human and animal diseases associated with a characteristic type of neurodegeneration, known as *spongiform encephalopathy* because of the vacuolated appearance of the affected brain, has recently been the focus of intense research activity (see Collinge, 2001; Prusiner, 2001). A key feature of these diseases is that they are transmissible through an infective agent, although not, in general, across species. The recent upsurge of interest has been spurred mainly by the discovery that the bovine form of the disease, bovine spongiform encephalopathy (BSE), is transmissible to humans. Different human forms of spongiform encephalopathy include Creutzfeld–Jacob disease (CJD) which is unrelated to BSE, and the new variant form (vCJD), which results from eating, or close contact with, infected beef or human tissue. Another human form is *kuru*, a neurodegenerative

disease affecting cannibalistic tribes in Papua New Guinea. These diseases cause a progressive, and sometimes rapid, dementia and loss of motor coordination, for which no therapies currently exist. *Scrapie*, a common disease of domestic sheep, is another example, and it may have been the practice of feeding sheep offal to domestic cattle that initiated an epidemic of BSE in Britain during the 1980s, leading to the appearance of vCJD in humans in the mid-1990s. Although the BSE epidemic has been controlled, there is concern that more human cases may develop in its wake, because the incubation period—known to be long—is uncertain.

Prion diseases are examples of protein misfolding diseases (see above) in which the prion protein adopts a misfolded conformation that forms insoluble aggregates. The infectious agent responsible for transmissible spongiform encephalopathies such as vCJD is, unusually, a protein, known as a prion. The protein involved (PrP^{C}) is a normal cytosolic constituent of the brain and other tissues, whose functions are not known. As a result of altered glycosylation, the protein can become misfolded, forming the insoluble PrP^{Sc} form, which has the ability to recruit normal PrP^{C} molecules to the misfolded PrP^{Sc}, thus starting a chain reaction. PrP^{Sc}—the infective agent—accumulates and aggregates as insoluble fibrils, and is responsible for the progressive neurodegeneration. In support of this unusual form of infectivity, it has been shown that injection of PrP^{Sc} into normal mice causes spongiform encephalopathy, whereas PrP knockout mice, which are otherwise fairly normal, are resistant because they lack the substrate for the autocatalytic generation of PrP^{Sc}. Fortunately, the infection does not easily cross between species, because there are differences between the *PrP* genes of different species. It is possible that a mutation of the *PrP* gene in either sheep or cattle produced the variant form that became infective in humans.

This chain of events bears some similarity to that of AD, in that the brain accumulates an abnormal form of a normally expressed protein.

There is as yet no known treatment for this type of encephalopathy, but laboratory experiments suggest that two very familiar drugs, namely **quinacrine** (an antimalarial drug) and **chlorpromazine** (a widely used antipsychotic drug; Ch. 45), can inhibit PrP^{Sc} aggregation in mouse models. Both are under investigation for treating human CJD. **Pentosan polyphosphate**, a glycosidic polymer that binds PrP and inhibits disease progression when give by intracerebroventricular injection in animal models, is also being tested in humans. Other possible strategies, none yet tested in patients, are discussed by Mallucci & Collinge (2005).

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40 General anaesthetic agents

OVERVIEW

General anaesthetics are used to render patients unaware of, and unresponsive to, painful stimulation during surgical procedures. They are given systemically and exert their main effects on the central nervous system (CNS), in contrast to local anaesthetics (Ch. 42). Although we now take them for granted, general anaesthetics are the drugs that paved the way for modern surgery. Without them, much of modern medicine would be impossible.

In this chapter, we describe the pharmacology of the main agents in current use, which fall into two main groups: intravenous agents and inhalation agents (gases and volatile liquids). Detailed information on the clinical pharmacology and use of anaesthetic agents can be found in specialised textbooks (e.g. Aitkinhead et al., 2006).

INTRODUCTION

It was only when inhalation anaesthetics were first discovered, in 1846, that most surgical operations became a practical possibility. Until that time, surgeons relied on being able to operate on struggling patients at lightning speed, and most operations were amputations.

▼ The use of **nitrous oxide** to relieve the pain of surgery was suggested by Humphrey Davy in 1800. He was the first person to make nitrous oxide, and he tested its effects on several people, including himself and the Prime Minister, noting that it caused euphoria, analgesia and loss of consciousness. The use of nitrous oxide, billed as 'laughing gas', became a popular fairground entertainment and came to the notice of an American dentist, Horace Wells, who had a tooth extracted under its influence, while he himself squeezed the inhalation bag. Ether also first gained publicity in a disreputable way, through the spread of 'ether frolics', at which it was used to produce euphoria among the guests. William Morton, also a dentist and a student at Harvard Medical School, used it successfully to extract a tooth in 1846 and then suggested to Warren, the illustrious chief surgeon at Massachusetts General Hospital, that he should administer it for one of Warren's operations. Warren grudgingly agreed, and on 16 October 1846 a large audience was gathered in the main operating theatre;[1] after some preliminary fumbling, Morton's demonstration was a spectacular success. 'Gentlemen, this is no humbug', was the most gracious comment that Warren could bring himself to make to the assembled audience.

In the same year, James Simpson, Professor of Midwifery at Edinburgh University, used chloroform to relieve the pain of childbirth, bringing on himself fierce denunciation from the clergy, one of whom wrote:

'Chloroform is a decoy of Satan, apparently offering itself to bless women; but in the end it will harden society and rob God of the deep, earnest cries which arise in time of trouble, for help.'

Opposition was effectively silenced in 1853, when Queen Victoria gave birth to her seventh child under the influence of chloroform, and the procedure became known as *anaesthésie à la reine*.

MECHANISM OF ACTION OF ANAESTHETIC DRUGS

Unlike most drugs, anaesthetics, which include substances as diverse as simple gases (e.g. **nitrous oxide** and **xenon**), halogenated hydrocarbons (e.g. **isoflurane**), barbiturates (e.g. **thiopental**) and steroids (e.g. **alphaxalone**), belong to no recognisable chemical class. At one time it appeared that the shape and electronic configuration of the molecule were relatively unimportant, and the pharmacological action required only that the molecule had certain physicochemical properties. We now know much more about how different anaesthetics interact with neuronal membrane proteins and have come to realise that there are multiple mechanisms by which anaesthesia can be produced and that different anaesthetics work by different mechanisms.

As the concentration of an anaesthetic is increased, the switch from being conscious to unconscious occurs over a very narrow concentration range (approximately 0.2 of a log unit). This is a much steeper concentration–response curve than that seen with drugs that interact as agonists or antagonists at classical receptors (see Ch. 2).

LIPID SOLUBILITY

Overton and Meyer, at the turn of the 20th century, showed a close correlation between anaesthetic potency and lipid solubility in a diverse group of simple and unreactive organic compounds that were tested for their ability to immobilise tadpoles. This led to a bold theory, formulated by Meyer in 1937: 'Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell.'

The relationship between anaesthetic activity and lipid solubility has been repeatedly confirmed. Anaesthetic potency in humans is usually expressed as the minimal alveolar concentration (MAC) required to abolish the response to surgical incision in 50% of subjects. Figure 40.1 shows the correlation between MAC (inversely proportional to potency) and lipid solubility, expressed as oil:water partition coefficient, for a wide range of inhalation anaesthetics. The Overton–Meyer studies did not suggest any particular mechanism, but revealed an impressive correlation, for which any theory of anaesthesia needs to account. Oil:water partition was assumed to predict partition into membrane lipids, consistent with the suggestion that anaesthesia results from an alteration of membrane function.

How the simple introduction of inert foreign molecules into the lipid bilayer could cause a functional disturbance was not explained. Two possible mechanisms, namely

[1]Now preserved as the Ether Dome, a museum piece at Massachusetts General Hospital.

volume expansion and increased membrane fluidity, have been suggested and tested experimentally, but both are now largely discredited (see Halsey, 1989; Little, 1996), and attention has swung from lipids to proteins, the correlation of potency with lipid solubility being explained by molecules of anaesthetic binding to hydrophobic pockets within specific membrane protein targets.

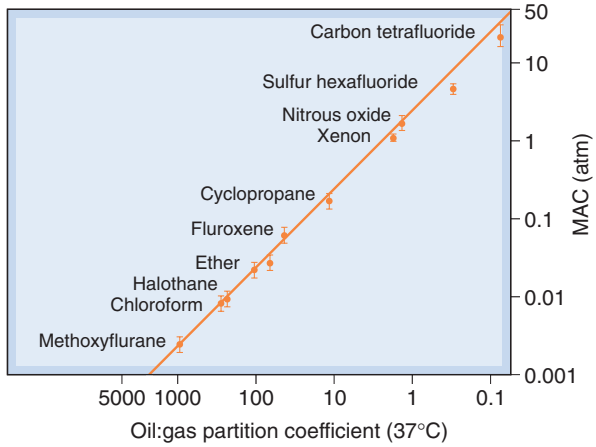

Fig. 40.1 Correlation of anaesthetic potency with oil:gas partition coefficient. Anaesthetic potency in humans is expressed as minimum alveolar partial pressure (MAC) required to produce surgical anaesthesia. There is a close correlation with lipid solubility, expressed as the oil:gas partition coefficient. (From: Halsey, 1989.)

EFFECTS ON ION CHANNELS

Following early studies that showed that anaesthetics can bind to various proteins as well as lipids, it was found that anaesthetics affect several different types of ion channels (see Rudolph & Antkowiak, 2004; Franks, 2008). For most anaesthetics, there are no known competitive antagonists, so this approach to identify sites of action is denied. Therefore the main criterion for identifying putative mechanisms of action of general anaesthetics is that, for an effect to be relevant to the anaesthetic or analgesic actions of these agents, it must occur at therapeutically relevant concentrations.

$GABA_A$ receptors. Almost all anaesthetics (with the exceptions of **cyclopropane**, **ketamine** and **xenon**) potentiate the action of GABA at the $GABA_A$ receptor. As described in detail in Chapter 37, $GABA_A$ receptors are ligand-gated Cl^- channels made up of five subunits (generally comprising two α, two β and one γ or δ subunit). Anaesthetics can bind to hydrophobic pockets within different $GABA_A$ receptor subunits (see Fig. 40.2).

Specific mutations of the amino acid sequence of the α subunit inhibit the actions of volatile anaesthetics but not those of intravenous anaesthetics, whereas mutations of the β subunit inhibit both volatile and intravenous anaesthetics (see Franks, 2008). This suggest that volatile anaesthetics may bind at the interface between α and β subunits (analogous to benzodiazepines that bind at the interface between α and γ/δ subunits, see Ch. 37), whereas the intravenous anaesthetics bind only on the β subunit. A further level of complexity arises because there are different subtypes of each subunit (see Ch. 37). Different subunit compositions give rise to subtly different subtypes of $GABA_A$ receptor. It has recently been shown that the $GABA_A$ receptors clustered at the synapse have different pharmacologi-

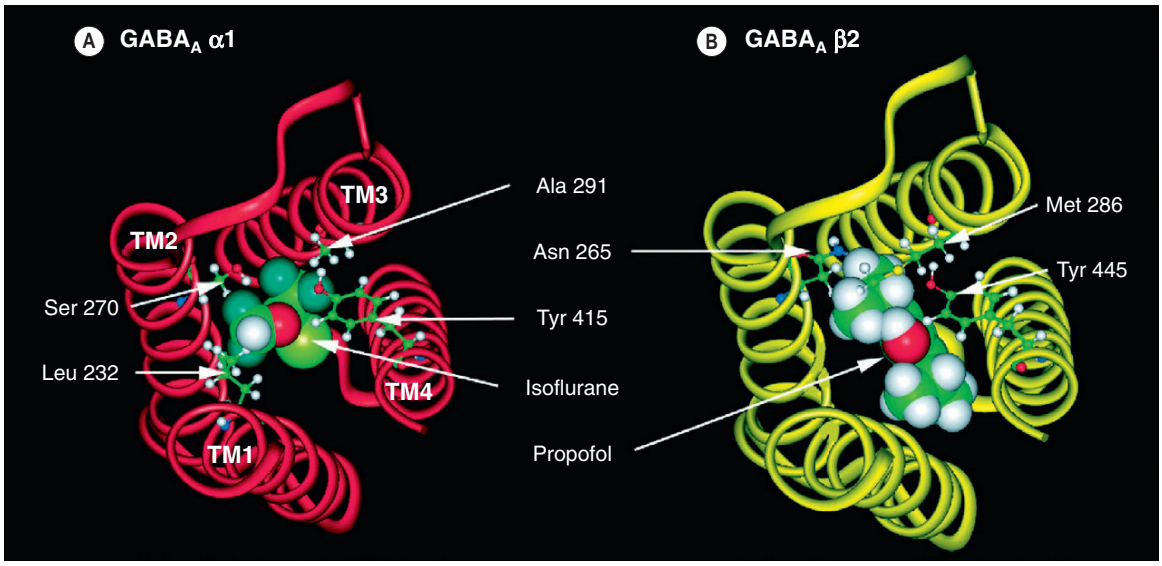

Fig. 40.2 Putative anaesthetic binding sites on $GABA_A$ receptor subunits. [**A**] A model of the α1 subunit of the $GABA_A$ receptor with the amino acids that form the binding site (Leu 232, Ser 270, Ala 291 and Tyr 415) illustrated in ball-and-stick mode. A molecule of isoflurane is shown sitting in the putative binding site. The transmembrane α-helices (TM) are numbered 1–4. [**B**] A model of the β2 subunit of the $GABA_A$ receptor with Asn 265, Met 286 and Tyr 445 illustrated in ball-and-stick mode. A molecule of propofol is shown sitting in the putative binding site. (Reproduced with permission from Hemmings H C et al. 2005 Trends Pharmacol Sci 26: 503–510.)

cal and kinetic properties from those that are distributed elsewhere across the cell (extrasynaptic receptors; see Ch. 4). Anaesthetics appear to have a greater potentiating effect on these extrasynaptic $GABA_A$ receptors.

Two-pore domain K^+ channels. These belong to a family of 'background' K^+ channels that modulate neuronal excitability. They are homomeric or heteromeric assemblies of a family of structurally related subunits (see Ch. 4 and Bayliss & Barrett, 2008). Channels made up of TREK1, TREK2, TASK1, TASK3 or TRESK subunits can be directly activated by low concentrations of volatile and gaseous anaesthetics, thus reducing membrane excitability (see Franks, 2008). This may contribute to the analgesic, hypnotic and immobilising effects of these agents. Two-pore domain K^+ channels do not appear to be affected by intravenous anaesthetics.

NMDA receptors. **Glutamate**, the major excitatory neurotransmitter in the CNS, activates three main classes of ionotropic receptor—AMPA, kainate and NMDA receptors (see Ch. 37). NMDA receptors are an important site of action for anaesthetics such as **nitrous oxide**, **xenon** and **ketamine** which act, in different ways, to reduce NMDA receptor-mediated responses. Xenon appears to inhibit NMDA receptors by competing with glycine for its regulatory site on this receptor whereas ketamine blocks the pore of the channel (see Ch. 37). Other inhalation anaesthetics may also exert effects on the NMDA receptor in addition to their effects on other proteins such as the $GABA_A$ receptor.

Other ion channels. Anaesthetics may also exert actions at other neuronal ligand-gated channels including glycine, nicotinic and 5-hydroxytryptamine receptors as well as at cyclic nucleotide-gated K^+ channels. Some general anaesthetics inhibit certain subtypes of voltage-gated Na^+ channels. Inhibition of presynaptic Na^+ channels may give rise to the inhibition of transmitter release at excitatory synapses. For further reading, see Hemmings et al. (2005) and Franks (2008).

It may be overly simplistic to think of each anaesthetic as having only one mechanism of action: as Little (1996) emphasises, individual anaesthetics differ in their actions and affect cellular function in several different ways, so a single mechanism is unlikely to be sufficient.

Comprehensive reviews of the molecular and cellular actions of general anaesthetics can be found in Schüttler & Schwilden, 2008.

Theories of anaesthesia

- Many simple, unreactive compounds produce general anaesthesia, the extreme example being the inert gas xenon.
- Anaesthetic potency is closely correlated with lipid solubility (Overton–Meyer correlation), not with chemical structure.
- Earlier theories of anaesthesia postulated interaction with the lipid membrane bilayer. Recent work favours interaction with membrane ion channels.
- Most anaesthetics enhance the activity of inhibitory $GABA_A$ receptors. Other important effects are the activation of a subfamily of potassium channels (the two-pore domain K^+ channels) and inhibition of excitatory NMDA receptors.

EFFECTS ON THE NERVOUS SYSTEM

At the cellular level, the effects of anaesthetics are to enhance tonic inhibition (through enhancing the actions of GABA), reduce excitation (opening K^+ channels) and to inhibit excitatory synaptic transmission (by depressing transmitter release and inhibiting ligand-gated ion channels). Effects on axonal conduction are relatively unimportant.

The anaesthetised state comprises several components, including *unconsciousness*, loss of reflexes (*muscle relaxation*) and *analgesia*. Much effort has gone into identifying the brain regions on which anaesthetics act to produce these effects. The most sensitive regions appear to be the midbrain reticular formation, thalamic sensory relay nuclei and, to a lesser extent, parts of the cortex. Inhibition of these regions results in unconsciousness and analgesia. Some anaesthetics—particularly volatile anaesthetics—cause inhibition at the spinal level, producing a loss of reflex responses to painful stimuli, although, in practice, neuromuscular-blocking drugs (Ch. 13) are used as an adjunct to produce muscle relaxation rather than relying on the anaesthetic alone. Anaesthetics, even in low concentrations, cause short-term amnesia. It is likely that interference with hippocampal function produces this effect, because the hippocampus is involved in short-term memory, and certain hippocampal synapses are highly susceptible to inhibition by anaesthetics.

As the anaesthetic concentration is increased, all brain functions are progressively affected, including motor control and reflex activity, respiration and autonomic regulation. Therefore it is not possible to identify a critical 'target site' in the brain responsible for all the phenomena of anaesthesia.

High concentrations of any general anaesthetic affect all parts of the CNS, causing profound inhibition which, in the absence of artificial respiration, leads to death from respiratory failure. The margin between surgical anaesthesia and potentially fatal respiratory and circulatory depression is quite narrow, requiring careful monitoring by the anaesthetist and adjustment of the level of anaesthesia.

EFFECTS ON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

Most anaesthetics decrease cardiac contractility, but their effects on cardiac output and blood pressure vary because of concomitant actions on the sympathetic nervous system and vascular smooth muscle. **Isoflurane** and other halogenated anaesthetics inhibit sympathetic outflow, reduce arterial and venous tone and thus decrease arterial pressure and venous pressure. By contrast, **nitrous oxide** and **ketamine** increase sympathetic discharge and plasma noradrenaline concentration and, if used alone, increase heart rate and maintain blood pressure.

Many anaesthetics, especially **halothane**, cause ventricular extrasystoles. The mechanism involves sensitisation to adrenaline. Electrocardiogram monitoring shows that extrasystolic beats occur commonly in patients under anaesthesia, with no harm coming to the patient. If catecholamine secretion is excessive, however (*par excellence* in phaeochromocytoma; see Ch. 14), there is a risk of precipitating ventricular fibrillation.

With the exception of nitrous oxide, ketamine and xenon, all anaesthetics depress respiration markedly and increase

Pharmacological effects of anaesthetic agents

- Anaesthesia involves three main neurophysiological changes: unconsciousness, loss of response to painful stimulation and loss of reflexes (motor and autonomic).
- At supra-anaesthetic doses, all anaesthetic agents can cause death by loss of cardiovascular reflexes and respiratory paralysis.
- At the cellular level, anaesthetic agents affect synaptic transmission and neuronal excitability rather than axonal conduction. GABA-mediated inhibitory transmission is enhanced by most anaesthetics. The release of excitatory transmitters and the response of the postsynaptic receptors are also inhibited.
- Although all parts of the nervous system are affected by anaesthetic agents, the main targets appear to be the cortex, thalamus, hippocampus, midbrain reticular formation and spinal cord.
- Most anaesthetic agents (with the exception of **ketamine**, **nitrous oxide** and **xenon**) produce similar neurophysiological effects and differ mainly in respect of their pharmacokinetic properties and toxicity.
- Most anaesthetic agents cause cardiovascular depression by effects on the myocardium and blood vessels, as well as on the nervous system. Halogenated anaesthetic agents are likely to cause cardiac dysrhythmias, accentuated by circulating catecholamines.

arterial P_{CO_2}. Nitrous oxide has much less effect, in part because its low potency prevents very deep anaesthesia from being produced with this drug. Some inhalation anaesthetics are pungent, particularly **desflurane** which is liable to cause coughing, laryngospasm and bronchospasm, so desflurane is not used for induction of anaesthesia but only for maintenance.

INTRAVENOUS ANAESTHETIC AGENTS

Even the fastest-acting inhalation anaesthetics, such as nitrous oxide, take a few minutes to act and cause a period of excitement before anaesthesia is induced. Intravenous anaesthetics act more rapidly, producing unconsciousness in about 20 s, as soon as the drug reaches the brain from its site of injection. These drugs (e.g. **propofol**, **thiopental** and **etomidate**) are normally used for induction of anaesthesia. They are preferred by many patients because injection generally lacks the menacing quality associated with a face mask in an apprehensive individual. With propofol, recovery is also fast due to rapid metabolism.

Although many intravenous anaesthetics are not suitable for maintaining anaesthesia because their elimination from the body is relatively slow compared with that of inhalation agents, propofol can be used as a continuous infusion, and the duration of action of ketamine is sufficient that it can be used as a single bolus for short operations without the need for an inhalation agent.

The properties of the main intravenous anaesthetics are summarised in Table 40.1.[2]

PROPOFOL

Propofol, introduced in 1983, has now largely replaced thiopental as an induction agent. It has a rapid onset of action (approximately 30 s) and rapid rate of distribution ($t_{1/2}$ 2–4 min). It has the advantage over thiopental of being very rapidly metabolised to inactive conjugates and quinols; therefore giving rapid recovery with less hangover effect. It has a cardiovascular depressant effect that may lead to hypotension and bradycardia. Respiratory depression and pain with injection may also occur. Propofol has less tendency to cause involuntary movement and adrenocortical suppression seen with etomidate. It is particularly useful for day-case surgery especially as its use is associated with less nausea and vomiting when compared with inhalation anaesthetics.

Propofol can also be given as a continuous infusion to maintain surgical anaesthesia without the need for any inhalation agent. However, there have been reports of a propofol infusion syndrome occurring in approximately 1 in 300 patients when high doses have been given for a prolonged period, particularly to sick patients—especially children—in intensive care units. This is characterised by severe metabolic acidosis, skeletal muscle necrosis (rhabdomyolysis), hyperkalaemia, lipaemia, hepatomegaly, renal failure, arrhythmia and cardiovascular collapse.

THIOPENTAL

Thiopental is the only remaining barbiturate in common use as an anaesthetic. It has very high lipid solubility, and this accounts for the speed of onset and transience of its effect when it is injected intravenously. The free acid is insoluble in water, so thiopental is given as the sodium salt. On intravenous injection, thiopental causes unconsciousness within about 20 s, lasting for 5–10 min. The anaesthetic effect closely parallels the concentration of thiopental in the blood reaching the brain, because its high lipid solubility allows it to cross the blood–brain barrier without noticeable delay.

The blood concentration of thiopental declines rapidly, by about 80% within 1–2 min, following the initial peak after intravenous injection, because the drug is redistributed, first to tissues with a large blood flow (liver, kidneys, brain, etc.) and more slowly to muscle. Uptake into body fat, although favoured by the high lipid solubility of thiopental, occurs only slowly, because of the low blood flow to this tissue. After several hours, however, most of the thiopental present in the body will have accumulated in body fat, the rest having been metabolised. Recovery from the anaesthetic effect of a bolus dose occurs within about 5 min, governed entirely by redistribution of the drug to well-perfused tissues; very little is metabolised in this time. After the initial rapid decline, the blood concentration drops more slowly, over several hours, as the drug is taken up by body fat and metabolised. Consequently, thiopental

[2] **Propanidid** and **alphaxalone** were withdrawn because of allergic reactions including hypotension and bronchoconstriction—probably attributable to the solvent Cremophor—but a new formulation of alphaxalone has been reintroduced to veterinary medicine and is thought to be less allergenic.

Table 40.1 Properties of intravenous anaesthetic agents

Drug	Speed of induction and recovery	Main unwanted effect(s)	Notes
Propofol	Fast onset, very fast recovery	Cardiovascular and respiratory depression	Rapidly metabolised Possible to use as continuous infusion Causes pain at injection site
Thiopental	Fast (accumulation occurs, giving slow recovery) 'Hangover'	Cardiovascular and respiratory depression	Largely replaced by propofol Causes pain at injection site Risk of precipitating porphyria in susceptible patients
Etomidate	Fast onset, fairly fast recovery	Excitatory effects during induction and recovery Adrenocortical suppression	Less cardiovascular and respiratory depression than with thiopental Causes pain at injection site
Ketamine	Slow onset, after effects common during recovery	Psychotomimetic effects following recovery Postoperative nausea, vomiting and salivation Raised intracranial pressure	Produces good analgesia and amnesia
Midazolam	Slower than other agents	—	Little respiratory or cardiovascular depression

produces a long-lasting hangover. Repeated intravenous doses cause progressively longer periods of anaesthesia, because the plateau in blood concentration becomes progressively more elevated as more drug accumulates in the body. For this reason, thiopental is not used to maintain surgical anaesthesia but only as an induction agent.

Thiopental binds to plasma albumin (roughly 85% of the blood content normally being bound). The fraction bound is less in states of malnutrition, liver disease or renal disease, which affect the concentration and drug-binding properties of plasma albumin, and this can appreciably reduce the dose needed for induction of anaesthesia.

Accidental injection of intravenous thiopental—a strongly alkaline solution—around rather than into the vein, or into an artery, can cause pain, local tissue necrosis and ulceration or severe arterial spasm that can result in gangrene. If the injection is into an artery then immediate injection of **procaine**, through the same needle, is the recommended procedure to encourage vasodilatation.

The actions of thiopental on the nervous system are very similar to those of inhalation anaesthetics, although it has little analgesic effect and can cause profound respiratory depression even in amounts that fail to abolish reflex responses to painful stimuli. Its long after-effect, associated with a slowly declining plasma concentration, means that drowsiness and some degree of respiratory depression persist for some hours.

ETOMIDATE

Etomidate has gained favour over thiopental on account of the larger margin between the anaesthetic dose and the dose needed to produce cardiovascular depression. It is more rapidly metabolised than thiopental, and thus less likely to cause a prolonged hangover. It causes less hypotension than propofol or thiopental. In other respects, etomidate is very similar to thiopental, although it appears more likely to cause involuntary movements during induction, postoperative nausea and vomiting, and pain at the injection site. Etomidate, particularly with prolonged infusion, suppresses the production of adrenal steroids, an effect that has been associated with an increase in mortality in severely ill patients. It should be avoided in patients at risk of having adrenal insufficiency, e.g. in sepsis. It is preferable to thiopental in patients at risk of circulatory failure.

OTHER INTRAVENOUS AGENTS

KETAMINE

▼ **Ketamine** closely resembles, both chemically and pharmacologically, **phencyclidine**, which is a 'street drug' with a pronounced effect on sensory perception (see Ch. 47). Both drugs produce a similar anaesthesia-like state and profound analgesia, but ketamine produces less euphoria and sensory distortion than phencyclidine and is thus more useful in anaesthesia. Both drugs are believed to act by blocking activation of the NMDA receptor (see Ch. 37).

Given intravenously, ketamine takes effect more slowly (1–2 min) than thiopental, and produces a different effect, known as 'dissociative anaesthesia', in which there is a marked sensory loss and analgesia, as well as amnesia, without complete loss of consciousness. During induction and recovery, involuntary movements and peculiar sensory experiences often occur. Ketamine does not act simply as a CNS depressant, and it produces cardiovascular and respiratory effects quite different from those of most anaesthetics. Blood pressure and heart rate are usually increased, and respiration is unaffected by effective anaesthetic doses. This makes it relatively safe to use in low-technology healthcare situations or in emergencies in the field. However, ketamine, unlike other intravenous anaesthetic drugs, can increase intracranial pressure, so it should not be given to patients with raised intracranial pressure or at risk of cerebral ischaemia. The other main drawback of ketamine is that hallucinations, and sometimes delirium and irrational behaviour, are common during recovery. These after-effects limit the usefulness of ketamine but are said to be less marked in children,[3] and ketamine, often in conjunction with a benzodiazepine, is sometimes still used for minor procedures in paediatrics.

[3]A cautionary note: many adverse effects are claimed to be less marked in children, perhaps because they cannot verbalise their experiences. At one time, muscle relaxants alone were used without anaesthesia during cardiac surgery in neonates. The babies did not complain of pain, but their circulating catecholamines reached extreme levels.

MIDAZOLAM

Midazolam, a benzodiazepine (Ch. 43), is slower in onset and offset than the drugs discussed above but, like ketamine, causes less respiratory or cardiovascular depression. Midazolam (or **diazepam**) is often used as a preoperative sedative and during procedures such as endoscopy, where full anaesthesia is not required. It can be administered in combination with an analgesic such as **alfentanyl**. In the event of overdose it can be reversed by **flumazenil** (see Ch. 43).

Neuroleptanalgesia

The combined use of a sedative (e.g. the dopamine antagonist **droperidol**) related to antipsychotic drugs (Ch. 45) and an opiate analgesic such as **fentanyl** (Ch. 41) can produce a state of deep sedation and analgesia (known as neuroleptanalgesia) in which the patient remains responsive to simple commands and questions, but does not respond to painful stimuli or retain any memory of the procedure. This can be used for minor procedures such as endoscopy but is less used since the advent of midazolam which has a shorter duration of action. Use of neuroleptanalgesics is more common in veterinary medicine; they are the pharmacological component in chemical darts used to immobilise wild animals.

INHALATION ANAESTHETICS

Many inhalation anaesthetics that were once widely used, such as ether, chloroform, trichloroethylene, cyclopropane, methoxyflurane and enflurane, have now been replaced in clinical practice, particularly by **isoflurane**, **sevoflurane** and **desflurane** which have improved pharmacokinetic properties, fewer side effects and are non-flammable. Of the older agents, nitrous oxide is still used widely (especially in obstetric practice), and halothane now only occasionally. Inhalation anaesthetics are most commonly used for the maintenance of anaesthesia.

PHARMACOKINETIC ASPECTS

An important characteristic of an inhalation anaesthetic is the speed at which the arterial blood concentration, which governs the pharmacological effect in the brain, follows changes in the partial pressure of the drug in the inspired air. Ideally, the blood concentration should follow as quickly as possible, so that the depth of anaesthesia can be controlled rapidly. In particular, the blood concentration should fall to a subanaesthetic level rapidly when administration is stopped, so that the patient recovers consciousness with minimal delay. A prolonged semicomatose state, in which respiratory reflexes are weak or absent, is particularly hazardous.

The lungs are the only quantitatively important route by which inhalation anaesthetics enter and leave the body. For modern inhalation anaesthetics, metabolic degradation is generally insignificant in determining their duration of action. Inhalation anaesthetics are all small, lipid-soluble molecules that readily cross alveolar membranes. It is therefore the rates of delivery of drug to and from the lungs, via (respectively) the inspired air and bloodstream, that determine the overall kinetic behaviour of an anaesthetic. The reason that anaesthetics vary in their kinetic behaviour is that their relative solubilities in blood, and in body fat, vary between one drug and another.

Intravenous anaesthetic agents

- Most commonly used for induction of anaesthesia, followed by inhalation agent. Propofol can also be used to maintain anaesthesia during surgery.
- Propofol, thiopental and etomidate are most commonly used; all act within 20–30 s if given intravenously.
- **Propofol**:
  - potent
  - rapid onset and distribution
  - rapidly metabolised
  - very rapid recovery; limited cumulative effect
  - useful for day-case surgery
  - low incidence of nausea and vomiting
  - risk of bradycardia
  - may induce an adverse 'propofol infusion syndrome' when administered at high doses for prolonged periods of time.
- **Thiopental**:
  - barbiturate with very high lipid solubility
  - rapid action due to rapid transfer across blood–brain barrier; short duration (about 5 min) due to redistribution, mainly to muscle
  - reduces intracranial pressure
  - slowly metabolised and liable to accumulate in body fat, therefore may cause prolonged effect if given repeatedly
  - narrow margin between anaesthetic dose and dose causing cardiovascular depression
  - risk of tissue damage if accidentally injected extravascularly or into an artery
  - can precipitate an attack of porphyria in susceptible individuals (see Ch. 57).
- **Etomidate**:
  - similar to thiopental but more quickly metabolised
  - less risk of cardiovascular depression
  - may cause involuntary movements during induction and high incidence of nausea
  - possible risk of adrenocortical suppression.
- **Ketamine**:
  - analogue of phencyclidine, with similar properties
  - action differs from other agents, probably related to inhibition of NMDA-type glutamate receptors
  - onset of effect is relatively slow (1–2 min)
  - powerful analgesic
  - produces 'dissociative' anaesthesia, in which the patient may remain conscious although amnesic and insensitive to pain
  - high incidence of dysphoria, hallucinations, etc. during recovery; used mainly for minor procedures in children
  - can raise intracranial pressure.

The main factors that determine the speed of induction and recovery can be summarised as follows:

- Properties of the anaesthetic:
  - blood:gas partition coefficient (i.e. solubility in blood)
  - oil:gas partition coefficient (i.e. solubility in fat).

- Physiological factors:
  - alveolar ventilation rate
  - cardiac output.

SOLUBILITY OF INHALATION ANAESTHETICS

Inhalation anaesthetics can be regarded physicochemically as ideal gases: their solubility in different media is expressed as *partition coefficients*, defined as the ratio of the concentration of the agent in two phases at equilibrium.

The *blood:gas partition coefficient* is the main factor that determines the rate of induction and recovery of an inhalation anaesthetic, and the lower the blood:gas partition coefficient, the faster is induction and recovery (Table 40.2). This is because it is the partial pressure of the gas in the alveolar space that governs the concentration in the blood. The lower the blood:gas partition coefficient, the more rapidly the partial pressure of the gas in the alveolar space will equal that being administered in the inspired air (see below).

The *oil:gas partition coefficient*, a measure of fat solubility, determines the potency of an anaesthetic (as already discussed) and also influences the kinetics of its distribution in the body, the main effect being that high lipid solubility delays recovery from anaesthesia. Values of blood:gas and oil:gas partition coefficients for some anaesthetics are given in Table 40.2.

INDUCTION AND RECOVERY

Cerebral blood flow is a substantial fraction of cardiac output (~15%), and the blood–brain barrier is freely permeable to anaesthetics, so the concentration of anaesthetic in the brain closely tracks that in the arterial blood. The kinetics of transfer of anaesthetic between the inspired air and the arterial blood therefore determine the kinetics of the pharmacological effect.

When a volatile anaesthetic is first administered, the initial breaths are diluted into the residual gas volume in the lungs resulting in a reduction in the alveolar partial pressure of the anaesthetic as compared with the inspired gas mixture. With subsequent breaths, the alveolar partial pressure rises towards equilibrium. For an anaesthetic with a low blood:gas partition coefficient, the absorption into the blood will be slower, so with repeated breaths the partial pressure in the alveolar space will rise faster than with an agent of high blood:gas partition coefficient. Thus a smaller number of breaths (i.e. a shorter time) will be needed to reach equilibrium. Therefore, contrary to what one might intuitively suppose, the *lower* the solubility in blood, the *faster* is the process of equilibration. Figure 40.3 shows the much faster equilibration for **nitrous oxide**, a low-solubility agent, than for **ether**, a high-solubility agent.

The transfer of anaesthetic between blood and tissues also affects the kinetics of equilibration. Figure 40.4 shows

Table 40.2 Characteristics of inhalation anaesthetics

Drug	Partition coefficient		Minimum alveolar concentration (% v/v)	Induction/ recovery	Main adverse effect(s) and disadvantage(s)	Notes
	Blood:gas	Oil:gas				
Nitrous oxide	0.5	1.4	100[a]	Fast	Few adverse effects Risk of anaemia (with prolonged or repeated use) Accumulation in gaseous cavities	Good analgesic effect Low potency precludes use as sole anaesthetic agent—normally combined with other inhalation agents
Isoflurane	1.4	91	1.2	Medium	Few adverse effects Possible risk of coronary ischemia in susceptible patients	Widely used Has replaced halothane
Desflurane	0.4	23	6.1	Fast	Respiratory tract irritation, cough, bronchospasm	Used for day-case surgery because of fast onset and recovery (comparable with nitrous oxide)
Sevoflurane	0.6	53	2.1	Fast	Few reported Theoretical risk of renal toxicity owing to fluoride	Similar to desflurane
Halothane	2.4	220	0.8	Medium	Hypotension Cardiac arrhythmias Hepatotoxicity (with repeated use) Malignant hyperthermia (rare)	Little used nowadays Significant metabolism to trifluoracetate
Enflurane	1.9	98	0.7	Medium	Risk of convulsions (slight) Malignant hyperthermia (rare)	Has declined in use May induce seizures
Ether	12.0	65	1.9	Slow	Respiratory irritation Nausea and vomiting Explosion risk	Now obsolete, except where modern facilities are lacking

[a] Theoretical value based on experiments under hyperbaric conditions.

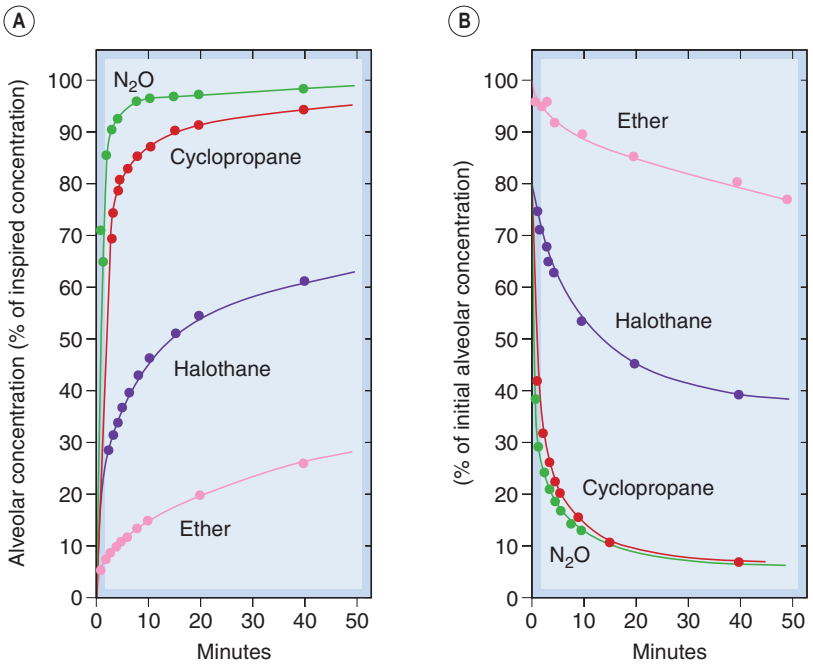

Fig. 40.3 Rate of equilibration of inhalation anaesthetics in humans. The curves show alveolar concentration (which closely reflects arterial blood concentration) as a function of time during induction and recovery. The initial rate of equilibration reflects solubility in blood. There is also a slow phase of equilibration, most marked with highly lipid-soluble drugs (ether and halothane), owing to the slow transfer between blood and fat (Fig. 40.4). [**A**] Induction. [**B**] Recovery. (From Papper E M, Kitz R (eds) 1963 Uptake and distribution of anaesthetic agents. McGraw-Hill, New York.)

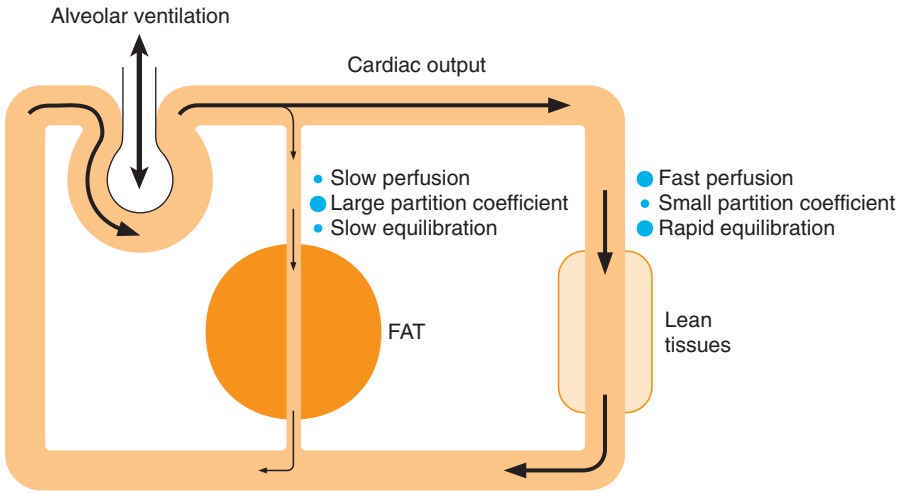

Fig. 40.4 Factors affecting the rate of equilibration of inhalation anaesthetics in the body. The body is represented as two compartments. Lean tissues, including the brain, have a large blood flow and low partition coefficient for anaesthetics, and therefore equilibrate rapidly with the blood. Fat tissues have a small blood flow and large partition coefficient, and therefore equilibrate slowly, acting as a reservoir of drug during the recovery phase.

a very simple model of the circulation, in which two tissue compartments are included. Body fat has a low blood flow but has a high capacity to take up anaesthetics, and constitutes about 20% of the volume of a representative man. Therefore, for a drug such as **halothane**, which is about 100 times more soluble in fat than in water, the amount present in fat after complete equilibration would be roughly 95% of the total amount in the body. Because of the low blood flow to adipose tissue, it takes many hours for the drug to enter and leave the fat, which results in a pronounced slow phase of equilibration following the rapid phase associated with the blood–gas exchanges (Fig. 40.3). The more fat-soluble the anaesthetic and the fatter the patient, the more pronounced this slow phase becomes and recovery will also be delayed.

Of the physiological factors affecting the rate of equilibration of inhalation anaesthetics, alveolar ventilation is the most important. The greater the minute volume (respiration rate × tidal volume), the faster is equilibration, particularly for drugs that have high blood:gas partition coefficients. Respiratory depressant drugs such as **morphine** (see Ch. 41) can thus retard recovery from anaesthesia.

Recovery from anaesthesia involves the same processes as induction but in reverse (Fig. 40.3), the rapid phase of recovery being followed by a slow 'hangover'. Because of these kinetic factors, the search for improved inhalation anaesthetics has focused on agents with low blood and tissue solubility. Newer drugs, which show kinetic properties similar to those of nitrous oxide but have higher potency, include **sevoflurane** and **desflurane** (Table 40.2).

METABOLISM AND TOXICITY

Metabolism, although not quantitatively important as a route of elimination of inhalation anaesthetics, can generate toxic metabolites.[4] **Chloroform** (now obsolete) causes hepatotoxicity associated with free radical formation in liver cells. **Methoxyflurane**, a halogenated ether, is no longer used because about 50% is metabolised to fluoride and oxalate, which cause renal toxicity. **Halothane** is less used nowadays because it undergoes substantial metabolism, about 30% being converted to bromide, trifluoroacetic acid and other metabolites that are implicated in rare instances of liver toxicity (see below). **Enflurane** and **sevoflurane** also generate fluoride, but at much lower (non-toxic) concentrations (Table 40.2).

Malignant hyperthermia is caused by heat production in skeletal muscle, due to excessive release of Ca^{2+} from the sarcoplasmic reticulum. The result is muscle contracture, acidosis, increased metabolism and an associated dramatic rise in body temperature that can be fatal unless treated promptly. Triggers include halogenated anaesthetics and depolarising neuromuscular-blocking drugs (see Ch. 13). Susceptibility has a genetic basis, being associated with mutations in the gene encoding the ryanodine receptor, which controls Ca^{2+} release from the sarcoplasmic reticulum (Ch. 4). Malignant hyperthermia is treated with **dantrolene**, a muscle relaxant drug that blocks these calcium release channels.

Pharmacokinetic properties of inhalation anaesthetics

- Rapid induction and recovery are important properties of an anaesthetic agent, allowing flexible control over the depth of anaesthesia.
- Speed of induction and recovery are determined by two properties of the anaesthetic: solubility in blood (blood:gas partition coefficient) and solubility in fat (lipid solubility).
- Agents with low blood:gas partition coefficients produce rapid induction and recovery (e.g. **nitrous oxide**, **desflurane**); agents with high blood:gas partition coefficients show slow induction and recovery (e.g. **halothane**).
- Agents with high lipid solubility (e.g. halothane) accumulate gradually in body fat and may produce a prolonged 'hangover' if used for a long operation.
- Some halogenated anaesthetics (especially halothane and **methoxyflurane**) are metabolised. This is not very important in determining their duration of action, but contributes to toxicity (e.g. renal toxicity associated with fluoride production with methoxyflurane—no longer used).

INDIVIDUAL INHALATION ANAESTHETICS

The main inhalation anaesthetics currently used in developed countries are i**soflurane**, **desflurane** and **sevoflurane** sometimes used in combination with **nitrous oxide**. Due to its relatively rapid onset of action sevoflurane can, under some circumstances, be used on its own to induce anaesthesia. **Xenon**, an inert gas shown many years ago to have anaesthetic properties, is making something of a comeback in the clinic because—not surprisingly for an inert gas—it lacks toxicity, but its relatively low potency and high cost are disadvantages.

Halothane is still used in veterinary medicine in species that do not metabolise it to toxic products, and is occasionally used in human medicine when a slow recovery from anaesthesia is desirable. **Enflurane** has decreased in use because of its propensity to induce seizures.

ISOFLURANE, DESFLURANE, SEVOFLURANE, ENFLURANE AND HALOTHANE

Isoflurane is now the most widely used volatile anaesthetic. It is not appreciably metabolised and lacks the proconvulsive property of enflurane. It can cause hypotension and is a powerful coronary vasodilator. This can exacerbate cardiac ischaemia in patients with coronary disease, because of the 'steal' phenomenon (see Ch. 21).

Desflurane is chemically similar to isoflurane, but its lower solubility in blood and fat means that titration of anaesthetic depth and recovery are faster, so it is increasingly used as an anaesthetic for day-case surgery. It is not appreciably metabolised. It is less potent than the drugs described above. At the concentrations used for induction of anaesthesia (about 10%), desflurane causes some respiratory tract irritation, which can lead to coughing and bronchospasm. Rapid increases in the depth of desflurane anaesthesia can be associated with a striking increase in sympathetic activity which is undesirable in patients with ischaemic heart disease.

Sevoflurane resembles desflurane but is more potent and does not cause the same degree of respiratory irritation. It is partially (about 3%) metabolised, and detectable levels of fluoride are produced, although this does not appear to be sufficient to cause toxicity.

Enflurane has a moderate speed of induction but is little used nowadays. It was originally introduced as an alternative to methoxyflurane. It can cause seizures, either during induction or following recovery from anaesthesia, especially in patients suffering from epilepsy. In this connection, it is interesting that a related substance, the fluorine-substituted diethyl-ether hexafluoroether, is a powerful convulsant agent, although the mechanism is not understood.

Halothane was an important drug in the development of volatile inhalation anaesthetics, but its use has declined in favour of isoflurane due to the potential for accumulation of toxic metabolites. Halothane has a marked relaxant effect on the uterus which can cause postpartum bleeding and limits its usefulness for obstetric purposes.

NITROUS OXIDE

Nitrous oxide (N_2O, not to be confused with nitric oxide, NO) is an odourless gas with many advantageous features for anaesthesia. It is rapid in onset of action because of its

[4]The problem of toxicity of low concentrations of anaesthetics inhaled over long periods by operating theatre staff has been a cause for concern. Strict measures are now used to minimise the escape of anaesthetics into the air of operating theatres.

Individual inhalation anaesthetics

- The main agents in current use in developed countries are isoflurane, desflurane and sevoflurane sometimes supplemented with nitrous oxide.
- As a rare but serious hazard, inhalation anaesthetics can cause malignant hyperthermia.
- **Nitrous oxide**:
  - low potency, therefore must be combined with other agents
  - rapid induction and recovery
  - good analgesic properties
  - risk of bone marrow depression with prolonged administration
  - accumulates in gaseous cavities.
- **Isoflurane**:
  - similar to enflurane but lacks epileptogenic property
  - may precipitate myocardial ischaemia in patients with coronary disease
  - irritant to respiratory tract.
- **Desflurane**:
  - similar to isoflurane but with faster onset and recovery
  - respiratory irritant, so liable to cause coughing and laryngospasm
  - useful for day-case surgery.
- **Sevoflurane**:
  - similar to desflurane, with lack of respiratory irritation.
- **Halothane**:
  - no longer widely used
  - potent, non-irritant
  - may cause hypotension and dysrhythmias; about 30% metabolised
  - can be useful when slow recovery is desirable but otherwise the 'hangover' due to high lipid solubility is unwanted
  - risk of liver damage if used repeatedly in susceptible individuals.
- **Enflurane**:
  - halogenated anaesthetic similar to halothane
  - less metabolism than halothane, therefore less risk of toxicity
  - faster induction and recovery than halothane (less accumulation in fat)
  - risk of epilepsy-like seizures.
- **Ether**:
  - obsolete except where modern facilities are not available
  - easy to administer and control
  - slow onset and recovery, with postoperative nausea and vomiting
  - analgesic and muscle relaxant properties
  - highly explosive
  - irritant to respiratory tract.

low blood:gas partition coefficient (Table 40.2), and is an effective analgesic in concentrations too low to cause unconsciousness. Its potency is low. It is used as a 50:50 mixture with O_2 to reduce pain during childbirth. It must never be given as 100% of the inspired gas as patients do need to breathe oxygen! Even at 80% in the inspired gas mixture, nitrous oxide does not produce surgical anaesthesia. It is not therefore used on its own as an anaesthetic, but is used (as 70% nitrous oxide in oxygen) as an adjunct to volatile anaesthetics, allowing them to be used at lower concentrations. During recovery from nitrous oxide anaesthesia, the transfer of the gas from the blood into the alveoli can be sufficient to reduce, by dilution, the alveolar partial pressure of oxygen, producing transient hypoxia (known as *diffusional hypoxia*). This is important for patients with respiratory disease.

Nitrous oxide tends to enter gaseous cavities in the body causing them to expand. This can be dangerous if a pneumothorax or vascular air embolus is present, or if the intestine is obstructed.

Given for brief periods, nitrous oxide is devoid of any serious toxic effects, but prolonged exposure (> 6 h) causes inactivation of methionine synthase, an enzyme required for DNA and protein synthesis, resulting in bone marrow depression that may cause anaemia and leucopenia, so its use should be avoided in patients with anaemia related to vitamin B_{12} deficiency. Bone marrow depression does not occur with brief exposure to nitrous oxide, but prolonged or repeated use (for example, in intermittently painful conditions such as sickle cell anaemia) should be avoided. Nitrous oxide 'sniffers' are subject to this danger.

Clinical uses of general anaesthetics

- *Intravenous anaesthetics* are used for:
  - induction of anaesthesia (e.g. **propofol** or **thiopental**)
  - maintenance of anaesthesia throughout surgery ('total intravenous anaesthesia', e.g. **propofol** sometimes in combination with muscle relaxants and analgesics).
- *Inhalational anaesthetics* (gases or volatile liquids) are used for maintenance of anaesthesia. Points to note are that:
  - volatile anaesthetics (e.g. **isoflurane**, **sevoflurane**) are delivered in air, oxygen or oxygen–nitrous oxide mixtures as the carrier gas
  - nitrous oxide must always be given with oxygen
  - because of its potential for inducing hepatotoxicity, **halothane** has largely been replaced by newer volatile anaesthetics such as **isoflurane**
  - all inhalational anaesthetics can trigger *malignant hyperthermia* in susceptible individuals (Ch.13).

USE OF ANAESTHETICS IN COMBINATION WITH OTHER DRUGS

Only in simple, short surgical procedures would a single anaesthetic be used on its own. In complex surgery, an array of drugs will be given at various times throughout the procedure. These may include a sedative or anxiolytic premedication (e.g. a benzodiazepine, see Ch. 43), an intravenous anaesthetic for rapid induction (e.g. **propofol**), a perioperative opioid analgesic (e.g. **remifentanyl**, see Ch. 41), an inhalation anaesthetic to maintain anaesthesia during surgery (e.g. **nitrous oxide** and **isoflurane**), a neuromuscular blocking agent to produce adequate muscle relaxation (e.g. **vecuronium**, see Ch. 13), an antiemetic agent (e.g. **ondansetron**, see Ch. 29) and a muscarinic antagonist to prevent or treat bradycardia or to reduce bronchial and salivary secretions (e.g. **atropine** or **glycopyrrolate**, see Ch. 13) and, towards the end of the procedure, an anticholinesterase agent (e.g. **neostigmine**, see Ch. 13) to reverse the neuromuscular blockade and an analgesic for postoperative pain relief (e.g. an opioid such as **morphine** and/or a non-steroidal anti-inflammatory drug such as **diclofenac**, see Ch. 41). Such combinations of drugs result in much faster induction and recovery, avoiding long (and potentially hazardous) periods of semiconsciousness, and it enables surgery to be carried out with less undesirable cardiorespiratory depression.

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