Bone metabolism

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OVERVIEW

In this chapter we consider first the cellular and biochemical processes involved in bone remodelling, and the various mediators that regulate these processes. We then describe the drugs used to treat disorders of bone, including new agents.

INTRODUCTION

The human skeleton undergoes a continuous process of remodelling throughout life – some bone being resorbed and new bone being laid down continuously – resulting in the complete skeleton being replaced every 10 years. Structural deterioration and decreased bone mass (osteoporosis) occur with advancing age and constitute a worldwide health problem. Other conditions that lead to treatable pathological changes in bone include nutritional deficiencies and malignancy. There have recently been significant advances in the understanding of bone biology, which have led in turn to several valuable new drugs.

BONE STRUCTURE AND COMPOSITION

The human skeleton consists of 80% cortical bone and 20% trabecular bone. Cortical bone is the dense, compact outer part and trabecular bone, the inner meshwork. The former predominates in the shafts of long bones, the latter in the vertebrae, the epiphyses of long bones and the iliac crest. Trabecular bone, having a large surface area, is metabolically more active and more affected by factors that lead to bone loss (see opposite).

The main minerals in bone are calcium and phosphates. More than 99% of the calcium in the body is in the skeleton, mostly as crystalline hydroxyapatite but some as non-crystalline phosphates and carbonates; together, these make up half the bone mass.

The main bone cells are *osteoblasts*, *osteoclasts* and *osteocytes*.

- Osteoblasts are bone-forming cells derived from precursor cells in the bone marrow and the periosteum: they secrete important components (particularly collagen) of the extracellular matrix of bone – which is known as *osteoid*. They also have a role in the activation of osteoclasts (see Figs 36.1 and 36.2).
- Osteoclasts are multinucleated bone-resorbing cells derived from precursor cells of the macrophage/monocyte lineage.
- Osteocytes are derived from osteoblasts which, during the formation of new bone, become embedded in the bony matrix and differentiate into osteocytes. These cells form a connected cellular network that, along with nerve fibres located in bone, influences the response to mechanical loading. Osteocytes sense mechanical strain, and respond by triggering bone remodelling and secreting *sclerostin*, a mediator that reduces bone formation (Khosla et al., 2008).
- Other important cells in bone include monocytes/macrophages, lymphocytes and vascular endothelial cells; these secrete cytokines and other mediators implicated in bone remodelling.

Osteoid is the organic matrix of bone and its principal component is collagen. Other components such as *proteoglycans*, *osteocalcin* and various phosphoproteins are also important; one of these, *osteonectin*, binds to both calcium and collagen and thus links these two major constituents of bone matrix.

Calcium phosphate crystals are deposited as hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ in the osteoid, converting it into hard bone matrix.

In addition to its structural function, bone plays a major role in calcium homeostasis.

BONE REMODELLING

There has been substantial progress in our understanding of bone remodelling (see reviews by Boyce & Xing, 2008; Gallagher, 2008; Deal, 2009; Wright et al., 2009.)

The process of remodelling involves:

- activity of osteoblasts and osteoclasts (Fig. 36.1)
- actions of various cytokines (Figs 36.1 and 36.2)
- turnover of bone minerals – particularly calcium and phosphate
- actions of several hormones: parathyroid hormone (PTH), the vitamin D family, oestrogens, growth hormone, steroids, calcitonin and various cytokines.

Diet, drugs and physical factors (exercise, loading) also affect remodelling. Bone loss – of 0.5–1% per year – starts aged 35–40 in both sexes, and accelerates by as much as 10-fold during the menopause in women or with castration in men, and then gradually settles at 1–3% per year. The loss during the menopause is due to increased osteoclast activity and affects mainly trabecular bone; the later loss in both sexes with increasing age is due to decreased osteoblast numbers and affects mainly cortical bone.

THE ACTION OF CELLS AND CYTOKINES

A cycle of remodelling starts with recruitment of osteoclast precursors followed by cytokine-induced differentiation of these to mature multinucleated osteoclasts (Fig. 36.1). The osteoclasts adhere to an area of trabecular bone, developing a ruffled border at the attachment site. They move along the bone, digging a pit by secreting hydrogen ions and proteolytic enzymes, mainly *cathepsin*
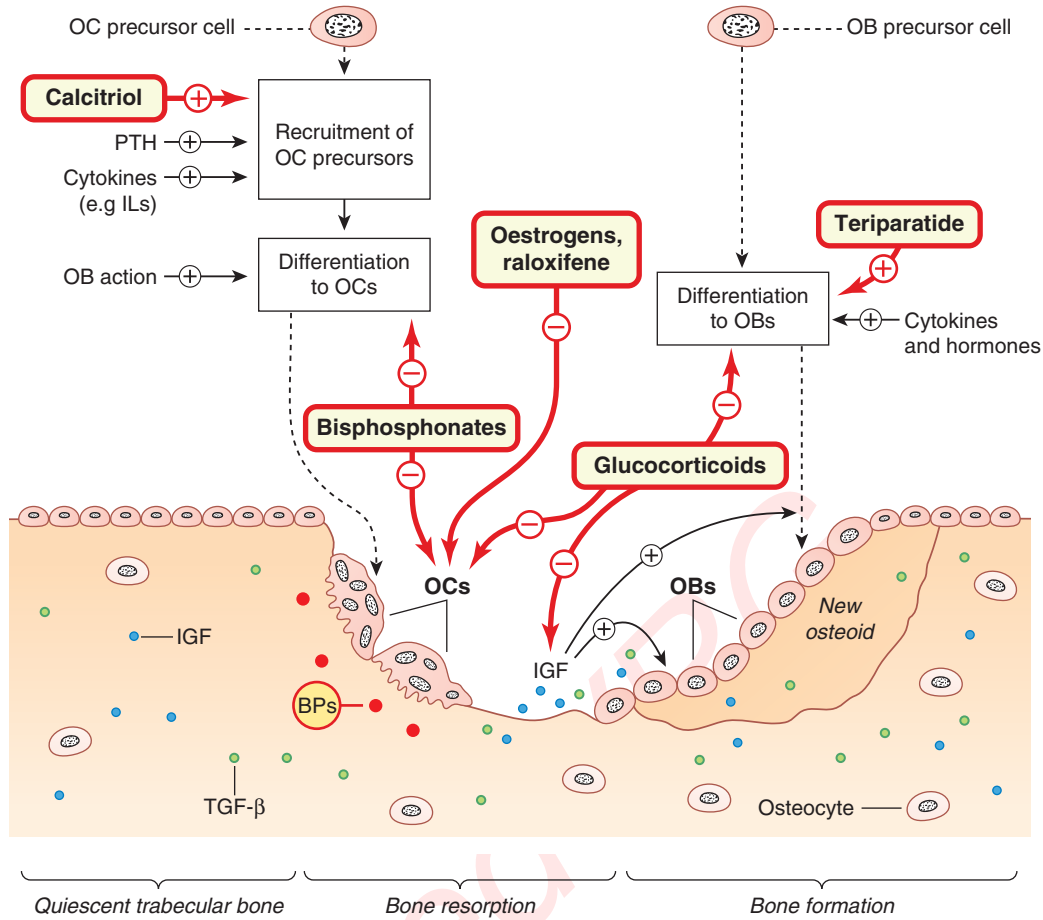
Fig. 36.1 **The bone-remodelling cycle and the action of hormones, cytokines and drugs.** *Quiescent trabecular bone:* Cytokines such as insulin-like growth factor (IGF) and transforming growth factor (TGF)-β, shown as dots, are embedded in the bone matrix. *Bone resorption* and *bone formation* are illustrated. Embedded bisphosphonates (BPs), are ingested by osteoclasts (OCs) when bone is resorbed (not shown); IL, interleukin; PTH, parathyroid hormone.
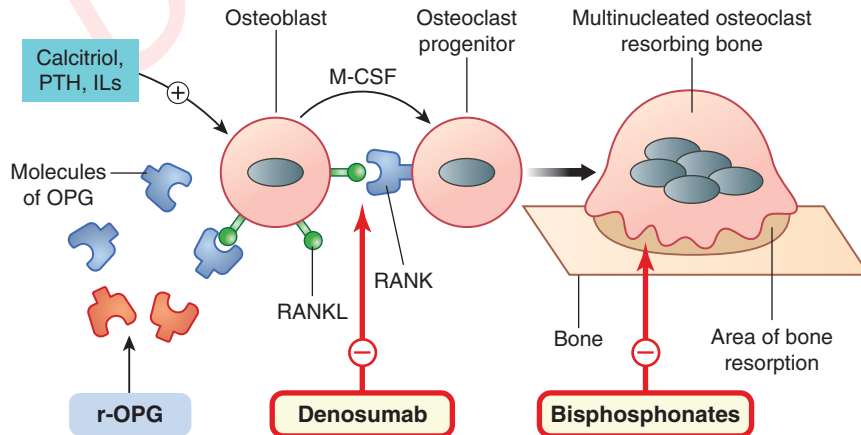

Fig. 36.2 **Schematic diagram of the role of the osteoblast and cytokines in the differentiation and activation of the osteoclast and the action of drugs thereon.** The osteoblast is stimulated to express a surface ligand, the RANK ligand (RANKL). RANKL interacts with a receptor on the osteoclast – an osteoclast differentiation and activation receptor termed RANK (receptor activator of nuclear factor kappa B), which causes differentiation and activation of the osteoclast progenitors to form mature osteoclasts. Bisphosphonates inhibit bone resorption by osteoclasts. Anti-RANKL antibodies (e.g. denosumab) bind RANKL and prevent the RANK–RANKL interaction. Drugs used clinically are in red-bordered boxes.

K. This process gradually liberates cytokines such as insulin-like growth factor (IGF)-1 and transforming growth factor (TGF)-β, which have been embedded in the osteoid (Fig. 36.1); these in turn recruit and activate successive teams of osteoblasts that have been stimulated to develop from precursor cells and are awaiting the call to duty (see Fig. 36.1). The osteoblasts invade the site, synthesising and secreting osteoid and secreting IGF-1 and TGF-β (which become embedded in the osteoid; see above). Some osteoblasts become embedded in the osteoid, forming osteocytes; others interact with and activate osteoclast precursors – and we are back to the beginning of the cycle.

Cytokines other than IGF-1 and TGF-β involved in bone remodelling include other members of the TGF-β family, including *bone morphogenic proteins* (BMPs), several interleukins, various hormones and members of the tumour necrosis factor (TNF) family. A member of this last family – a ligand for a receptor on the osteoclast precursor cell – is of particular importance. The receptor is termed (wait for it – biological terminology has fallen over its own feet here) *RANK*, which stands for *receptor activator of nuclear factor kappa B* (NFκB), NFκB being the principal transcription factor involved in osteoclast differentiation and activation. And the ligand is termed, unsurprisingly, RANK ligand (RANKL).

▼ Osteoblasts synthesise and release *osteoprotegerin* (OPG) which is identical with RANK and functions as a decoy receptor. In a sibling-undermining process by osteoblast and osteoclast precursor cells, OPG can bind to RANKL[1] (generated by the very same cells as OPG) and inhibit RANKL's binding to the functional receptor, RANK, on the osteoclast precursor cell (Fig. 36.2). The ratio of RANKL to OPG is critical in the formation and activity of osteoclasts and the RANK, RANKL, OPG system is fundamental to bone remodelling (reviewed by Boyce & Xing, 2008; Wright et al., 2009).

THE TURNOVER OF BONE MINERALS

The main bone minerals are calcium and phosphates.

CALCIUM METABOLISM

The daily turnover of bone minerals during remodelling involves about 700 mg of calcium. Calcium has numerous roles in physiological functioning. Intracellular Ca^{2+} is part of the signal transduction mechanism of many cells (see Ch. 4), so the concentration of Ca^{2+} in the extracellular fluid and the plasma, normally about 2.5 mmol/l, needs to be controlled with great precision. The plasma Ca^{2+} concentration is regulated by interactions between PTH and various forms of vitamin D (Figs 36.3 and 36.4); calcitonin also plays a part.

Calcium absorption in the intestine involves a Ca^{2+}-binding protein whose synthesis is regulated by calcitriol (see Fig. 36.3). It is probable that the overall calcium content of the body is regulated largely by this absorption mechanism, because urinary Ca^{2+} excretion normally remains more or less constant. However, with high blood Ca^{2+} concentrations urinary excretion increases, and with low blood concentrations urinary excretion can be reduced by PTH and calcitriol, both of which enhance Ca^{2+} reabsorption in the renal tubules (Fig. 36.3).

PHOSPHATE METABOLISM

Phosphates are important constituents of bone, and are also critically important in the structure and function of all the cells of the body. They are constituents of nucleic acids, provide energy in the form of ATP, and control – through phosphorylation – the activity of many functional proteins. They also have roles as intracellular buffers and in the excretion of hydrogen ions in the kidney.

Phosphate absorption is an energy-requiring process regulated by *calcitriol*. Phosphate deposition in bone, as hydroxyapatite, depends on the plasma concentration of PTH, which, with calcitriol, mobilises both Ca^{2+} and phosphate from the bone matrix. Phosphate is excreted by the kidney; here PTH inhibits reabsorption and thus increases excretion.

> ### Bone remodelling
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> - Bone is continuously remodelled throughout life. The events of the remodelling cycle are as follows:
>   - osteoclasts, having been activated by osteoblasts, resorb bone by digging pits in trabecular bone. Into these pits the bone-forming osteoblasts secrete osteoid (bone matrix), which consists mainly of collagen but also contains osteocalcin, osteonectin, phosphoproteins and the cytokines insulin growth factor (IGF) and transforming growth factor (TGF)-β
>   - the osteoid is then mineralised, i.e. complex calcium phosphate crystals (hydroxyapatites) are deposited.
> - Bone metabolism and mineralisation involve the action of parathyroid hormone, the vitamin D family, and various cytokines (e.g. IGF, the TGF-β family and interleukins). Declining physiological levels of oestrogens and therapeutic levels of glucocorticoids can result in bone resorption not balanced by bone formation – leading to osteoporosis.

HORMONES INVOLVED IN BONE METABOLISM AND REMODELLING

The main hormones involved in bone metabolism and remodelling are parathyroid hormone (PTH), members of the vitamin D family, oestrogens and calcitonin. Glucocorticoids and thyroid hormone also affect bone.

PARATHYROID HORMONE

Parathyroid hormone, which consists of a single-chain polypeptide of 84 amino acids, is an important physiological regulator of Ca^{2+} metabolism. It acts on PTH receptors in various tissues (bone, kidney, gastrointestinal tract) to maintain the plasma Ca^{2+} concentration. It mobilises Ca^{2+} from bone, promotes its reabsorption by the kidney and stimulates the synthesis of calcitriol, which in turn increases Ca^{2+} absorption from the intestine and synergises with PTH in mobilising bone Ca^{2+} (Figs 36.3 and 36.4). PTH promotes phosphate excretion, and thus its net effect is to increase the concentration of Ca^{2+} in the plasma and lower that of phosphate.

The mobilisation of Ca^{2+} from bone by PTH is mediated, at least in part, by stimulation of the recruitment and activation of osteoclasts. Pathological oversecretion of
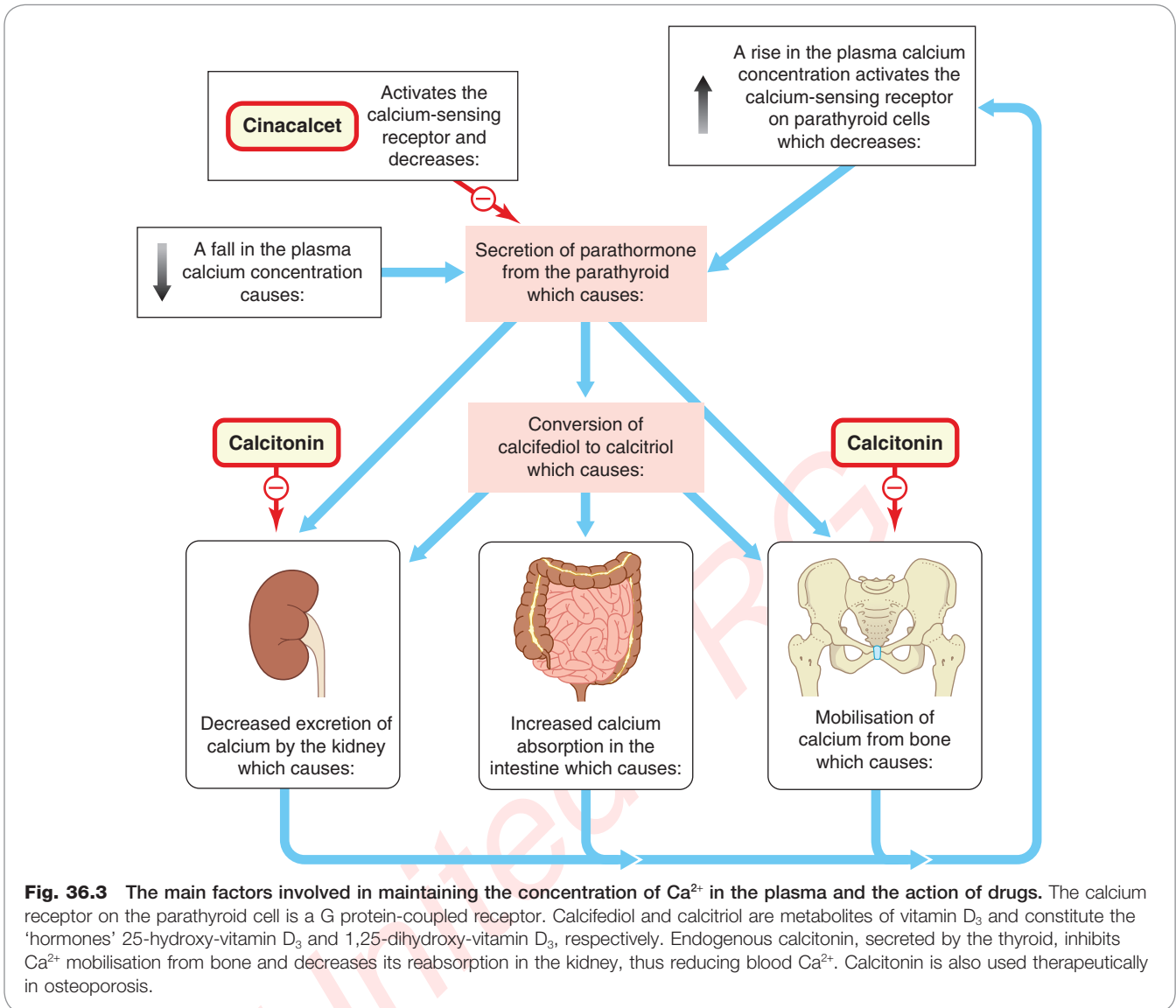
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[1]RANKL is also sometimes confusingly termed OPG ligand.

Fig. 36.3 **The main factors involved in maintaining the concentration of Ca^{2+} in the plasma and the action of drugs.** The calcium receptor on the parathyroid cell is a G protein-coupled receptor. Calcifediol and calcitriol are metabolites of vitamin D$_3$ and constitute the 'hormones' 25-hydroxy-vitamin D$_3$ and 1,25-dihydroxy-vitamin D$_3$, respectively. Endogenous calcitonin, secreted by the thyroid, inhibits Ca^{2+} mobilisation from bone and decreases its reabsorption in the kidney, thus reducing blood Ca^{2+}. Calcitonin is also used therapeutically in osteoporosis.

PTH (hyperparathyroidism) inhibits osteoblast activity (not shown in Fig. 36.1). But given therapeutically in a low intermittent dose, PTH and fragments of PTH paradoxically stimulate osteoblast activity and enhance bone formation.

Parathyroid hormone is synthesised in the cells of the parathyroid glands and stored in vesicles. The principal factor controlling secretion is the concentration of ionised calcium in the plasma, low plasma Ca^{2+} stimulating secretion, high plasma Ca^{2+} decreasing it by binding to and activating a Ca^{2+}-sensing G protein-coupled surface receptor (see Ch. 3 and Fig. 36.3). (For reviews, see Stewart, 2004; Deal, 2009.)

VITAMIN D

Vitamin D (calciferol) consists of a group of lipophilic precursors that are converted in the body into biologically active metabolites that function as true hormones, circulating in the blood and regulating the activities of various cell types (see Reichel et al., 1989). Their main action, mediated by nuclear receptors of the steroid receptor superfamily (see Ch. 3), is the maintenance of plasma Ca^{2+} by increasing Ca^{2+} absorption in the intestine, mobilising Ca^{2+} from bone and decreasing its renal excretion (see Fig. 36.3). In humans, there are two important forms of vitamin D, termed D$_2$ and D$_3$:

1. Dietary *ergocalciferol* (D$_2$), derived from ergosterol in plants.
2. *Cholecalciferol* (D$_3$), generated in the skin from 7-dehydrocholesterol by the action of ultraviolet irradiation during sun exposure, or formed from cholesterol in the wall of the intestine.

Cholecalciferol is converted to *calcifediol* (25-hydroxy-vitamin D$_3$) in the liver, and this is converted to a series of other metabolites of varying activity in the kidney, the most potent of which is *calcitriol* (1,25-dihydroxy-vitamin D$_3$); see Figure 36.4.

The synthesis of calcitriol from calcifediol is regulated by PTH, and is also influenced by the phosphate concentration in the plasma and by the calcitriol concentration itself through a negative feedback mechanism (Fig. 36.4).
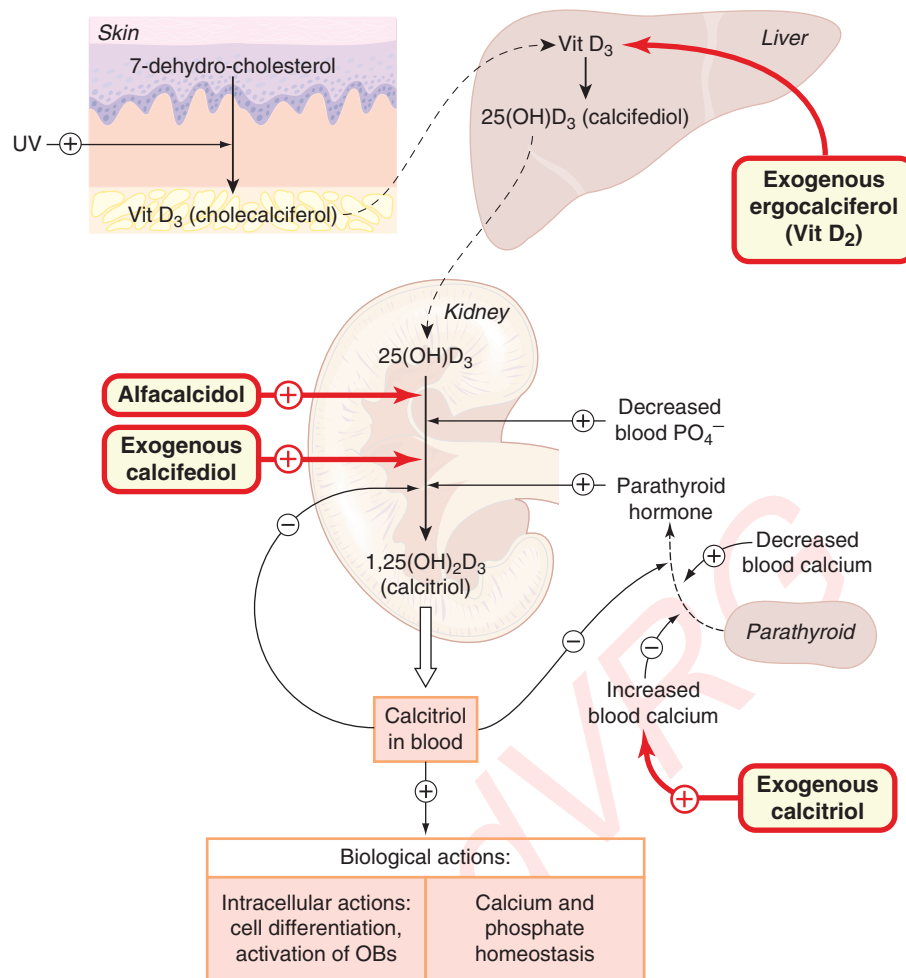
Fig. 36.4 **Summary of the actions of the vitamin D endocrine system and the action of drugs.** Exogenous ergocalciferol, vitamin (Vit) D₂ (formed in plants by ultraviolet [UV] light), is converted to the corresponding D₂ metabolites in liver and kidney, as is the D₂ analogue dihydrotachysterol (not shown). Alfacalcidol (1α-hydroxycholecalciferol) is 25-hydroxylated to calcitriol in the liver. OB, osteoblast.

Receptors for calcitriol are ubiquitous, and calcitriol is important in the functioning of many cell types.

The main actions of calcitriol are the stimulation of absorption of Ca^{2+} and phosphate in the intestine, and the mobilisation of Ca^{2+} from bone, but it also increases Ca^{2+} reabsorption in the kidney tubules (Fig. 36.3). Its effect on bone involves promotion of maturation of osteoclasts and indirect stimulation of their activity (Figs 36.1 and 36.3). It decreases collagen synthesis by osteoblasts. However, the effect on bone is complex and not confined to mobilising Ca^{2+}, because in clinical vitamin D deficiency (see p. 444), in which the mineralisation of bone is impaired, administration of vitamin D restores bone formation. One explanation may lie in the fact that calcitriol stimulates synthesis of *osteocalcin*, the Ca^{2+}-binding protein of bone matrix.

OESTROGENS

Oestrogens have an important role in maintaining bone integrity in adult women, acting on osteoblasts and osteoclasts. Oestrogen inhibits the cytokines that recruit osteoclasts and opposes the bone-resorbing, Ca^{2+}-mobilising action of PTH. It increases osteoblast proliferation, augments production of TGF-β and bone morphogenic

proteins, and inhibits apoptosis (see Ch. 5). Withdrawal of oestrogen, as happens physiologically at the menopause, frequently leads to osteoporosis.

CALCITONIN

Calcitonin is a peptide hormone secreted by 'C' cells found in the thyroid follicles (see Ch. 34).

The main action of calcitonin is on bone; it inhibits bone resorption by binding to an inhibitory receptor on osteoclasts. In the kidney, it decreases the reabsorption of Ca^{2+} and phosphate in the proximal tubules. Its overall effect is to decrease the plasma Ca^{2+} concentration (Fig. 36.3).

Secretion is determined mainly by the plasma Ca^{2+} concentration.

OTHER HORMONES

Physiological concentrations of glucocorticoids are required for osteoblast differentiation. Higher concentrations inhibit bone formation by inhibiting osteoblast differentiation and activity, and may stimulate osteoclast action – leading to osteoporosis, which is a feature of Cushing's syndrome (Fig. 33.7) and an important adverse effect of glucocorticoid administration (Ch. 33).

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Thyroxine stimulates osteoclast action, reducing bone density and liberating Ca^{2+}. Osteoporosis occurs in association with thyrotoxicosis, and it is important not to use excessive thyroxine for treating hypothyroidism (see Ch. 34).

> ### Parathyroid hormone, vitamin D and bone mineral homeostasis
>
> - The vitamin D family give rise to true hormones; precursors are converted to calcifediol in the liver, then to the main hormone, calcitriol, in the kidney.
> - Calcitriol increases plasma Ca^{2+} by mobilising it from bone, increasing its absorption in the intestine and decreasing its excretion by the kidney.
> - Parathyroid hormone (PTH) increases blood Ca^{2+} by increasing calcitriol synthesis, mobilising Ca^{2+} from bone and reducing renal Ca^{2+} excretion. Paradoxically, small doses of PTH given intermittently *increase* bone formation through an anabolic effect.
> - Calcitonin (secreted from the thyroid) reduces Ca^{2+} resorption from bone by inhibiting osteoclast activity.

DISORDERS OF BONE

The reduction of bone mass with distortion of the microarchitecture is termed *osteoporosis*; a reduction in the mineral content is termed *osteopenia*. Dual-energy X-ray absorptiometry (DXA) and quantitative computed tomography are the standard methods for assessing osteoporosis severity and monitoring the effect of treatment (Riggs et al., 2012). Osteoporotic bone fractures easily after minimal trauma. The commonest causes of osteoporosis are postmenopausal deficiency of oestrogen and age-related deterioration in bone homeostasis. It is estimated that 50% of women and 20% of men over the age of 50 will have a fracture due to osteoporosis. With increasing life expectancy, osteoporosis has increased to epidemic proportions and is an important public health problem, affecting about 75 million people in the USA, Japan and Europe. Other predisposing factors include catabolic hormones that favour protein breakdown such as excessive thyroxine or glucocorticoid administration. Other preventable or treatable diseases of bone include *osteomalacia* and *rickets* (the juvenile form of osteomalacia), in which there are defects in bone mineralisation due to vitamin D deficiency, either due to dietary deficiency of vitamin D and lack of sunlight, or to renal disease resulting in reduced synthesis of the active calcitriol hormone (Ch. 29) and *Paget's disease*, in which there is distortion of the processes of bone resorption and remodelling as a consequence of mutation in the gene that codes for a ubiquitin-binding protein[2] called sequestosome 1 (Rea et al., 2013) which is

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[2]Ubiquitin (Ch. 5) is a small regulatory protein present in almost all cells of the body ('ubiquitous'). It directs proteins to compartments in the cell, including the proteasome which destroys and recycles proteins. Ubiquitin-binding proteins interact with ubiquitinated targets and regulate diverse biological processes, including endocytosis, signal transduction, transcription and DNA repair.

a scaffold protein in the RANK/NFκB signalling pathway (see p. 441).

DRUGS USED IN BONE DISORDERS

Two types of agent are currently used for treatment of osteoporosis:

1. *Antiresorptive drugs* that decrease bone loss, e.g. bisphosphonates, calcitonin, selective [o]estrogen receptor modulators (SERMs), **denusomab**, calcium.
2. *Anabolic agents* that increase bone formation, e.g. PTH, **teriparatide**.

Strontium has both actions.

Rickets and osteomalacia are treated with vitamin D preparations.

Paget's disease is common but only a small percentage of patients are symptomatic; if medical treatment is needed, bisphosphonates such as **pamidronate** or **zoledronate** (see below) are very effective and much more convenient than frequent injections of **salmon calcitonin**, previously the only effective medical treatment. A single intravenous dose of zoledronate (5 mg) can suppress the elevated plasma alkaline phosphatase that signals disease activity in Paget's disease for more than 2 years.

BISPHOSPHONATES

Bisphosphonates (Fig. 36.5) are enzyme-resistant analogues of pyrophosphate, a normal constituent of tissue fluids that accumulates in bone, and has a role in regulating bone resorption. Bisphosphonates inhibit bone resorption by an action mainly on the osteoclasts. They form tight complexes with calcium in the bone matrix, and are

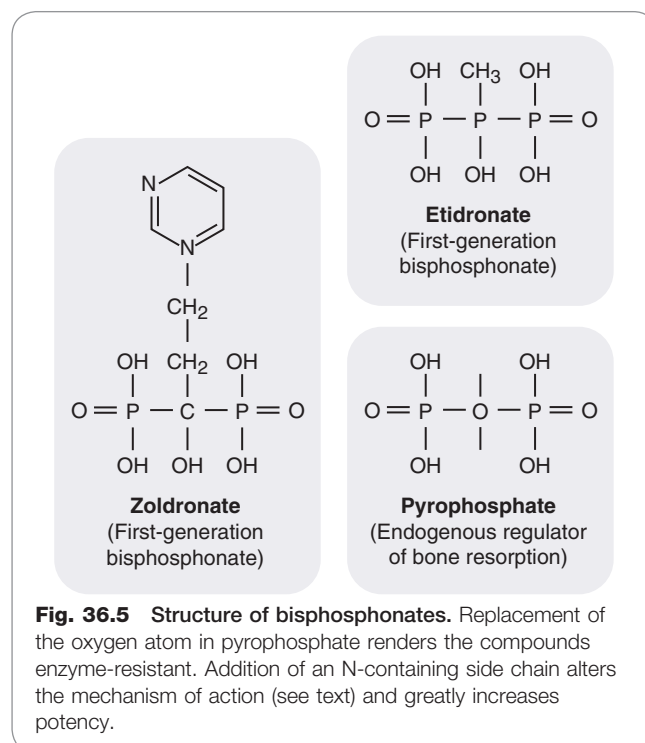**Fig. 36.5** **Structure of bisphosphonates.** Replacement of the oxygen atom in pyrophosphate renders the compounds enzyme-resistant. Addition of an N-containing side chain alters the mechanism of action (see text) and greatly increases potency.

released slowly as bone is resorbed by the osteoclasts, which are thus exposed to high local bisphosphonate concentrations.

Mechanism of action

Bisphosphonates reduce the rate of bone turnover. They can be grouped into two classes:

1. Simple compounds that are very similar to pyrophosphate (e.g. **etidronate**). These are incorporated into ATP analogues that accumulate within the osteoclasts and promote their apoptosis.
2. Potent, amino-bisphosphonates (e.g. **pamidronate**, **alendronate**, **risedronate**, **ibandronate**, **zoledronate**). These prevent bone resorption by interfering with the anchoring of cell surface proteins to the osteoclast membrane by prenylation, thereby preventing osteoclast attachment to bone (see Strewler, 2005).

Pharmacokinetic aspects

Bisphosphonates are given orally on an empty stomach with plenty of water in a sitting or standing position at least 30 minutes before breakfast because of their propensity to cause severe oesophageal problems or, in the case of pamidronate, ibandronate and of zoledronate, intravenously. They are poorly absorbed from the gut. About 50% of absorbed drug accumulates at sites of bone mineralisation, where it remains adsorbed onto hydroxyapatite crystals, potentially for months or years, until the bone is resorbed. The free drug is excreted unchanged by the kidney.

Absorption is impaired by food, particularly milk, so the drugs must be taken on an empty stomach.

Unwanted effects include gastrointestinal disturbances including peptic ulcers and oesophagitis (sometimes with erosions or stricture formation). Bone pain occurs occasionally. Atypical femoral fractures are described during long-term treatment, especially of osteoporosis, and the need for continued use should be re-evaluated periodically (e.g. after 5 years). Given intravenously, some bisphosphonates (in particular zoledronate) can lead to osteonecrosis (literally 'death of bone') of the jaw, especially in patients with malignant disease; a dental check is needed before treatment (followed by any indicated remedial work). After zoledronate infusion supplemental calcium and vitamin D are administered for at least ten days.

Clinical use

Alendronate and risedronate are given orally for prophylaxis and treatment of osteoporosis. Etidronate is an alternative. Clodronate is used in patients with malignant disease involving bone and pamidronate is given by intravenous infusion to treat hypercalcaemia of malignancy or to treat Paget's disease. Ibandronate is given intravenously every 3–4 weeks in patients with breast cancer metastatic to bone, or every 3 months to treat postmenopausal osteoporosis. Zoledronate, which is given as an intravenous infusion, is used for advanced malignancy involving bone, for Paget's disease and for selected cases of osteoporosis (postmenopausal or in men) when it is administered once a year or even less frequently (see clinical box below).

Bisphosphonates

- Orally active, stable analogues of pyrophosphate, which are incorporated into remodelling bone and remain there for months to years.
- Released when osteoclast-mediated bone resorption occurs, exposing osteoclasts to their effects.
- First-generation compounds (e.g. **etidronate**) act by promoting apoptosis of osteoclasts.
- Second-generation compounds (e.g. **risedronate**) with N-containing side chains are much more potent, and prevent osteoclast action by inhibiting prenylation reactions required for membrane anchoring of functional proteins.
- Used long term for prevention and treatment of osteoporosis, and for symptomatic Paget's disease.
- Main unwanted effect is gastrointestinal (especially oesophageal) disturbance; a rare but serious adverse effect of the most potent drugs (notably **zoledronate**) is osteonecrosis of the jaw.

Clinical uses of bisphosphonates

- *Osteoporosis*:
  - 'primary' prevention of fractures in high-risk individuals (e.g. with established osteoporosis, several risk factors for osteoporosis, chronic treatment with systemic glucocorticoids)
  - 'secondary' prevention after an osteoporotic fracture
  - **alendronate** by mouth, given daily or once weekly in addition to calcium with vitamin D_3. **Risedronate** or **etidronate** are alternatives; **zoledronate** is given annually or even less often by intravenous infusion; it is the most potent bisphosphonate and more likely to cause osteonecrosis of the jaw – dental check and remedial dental work are prerequisites of treatment.
- *Malignant disease* involving bone (e.g. metastatic breast cancer, multiple myeloma):
  - to reduce bone damage, pain and hypercalcaemia (e.g. **clodronate**, **ibandronate**, **zoledronate**).
- *Paget's disease* of bone (e.g. **etidronate**, **pamidronate**) administered intermittently and with monitoring of serum phosphate, alkaline phosphatase and urinary hydroxyproline (a marker of collagen turnover).

OESTROGENS AND RELATED COMPOUNDS

The decline in endogenous oestrogen is a major factor in postmenopausal osteoporosis, and there is evidence that giving oestrogen as hormone replacement therapy (HRT; see Ch. 35) can ameliorate this. But HRT has actions on many systems, and newer agents (e.g. **raloxifene**, see Ch. 35) have been developed that exhibit agonist actions

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on some tissues and antagonist actions on others. These are termed *selective oestrogen receptor modulators* (SERMs).

RALOXIFENE

Raloxifene is a SERM that stimulates osteoblasts and inhibits osteoclasts. It also has agonist actions on the cardiovascular system, and antagonist activity on mammary tissue and the uterus.

It is well absorbed in the gastrointestinal tract, and undergoes extensive first-pass metabolism in the liver to give the glucuronide, which undergoes enterohepatic recycling. Overall bioavailability is only about 2%. Despite the low plasma concentration, raloxifene is concentrated in tissues, and is converted to an active metabolite in liver, lungs, bone, spleen, uterus and kidney. Its half-life averages 32 h. It is excreted mainly in the faeces.

Unwanted effects include hot flushes, leg cramps, flu-like symptoms and peripheral oedema. Less common are thrombophlebitis and thromboembolism. Other rarer adverse effects are thrombocytopenia, gastrointestinal disturbances, rashes, raised blood pressure and arterial thromboembolism. Raloxifene is not recommended for primary prevention of osteoporotic fractures, but is one alternative to a bisphosphonate for secondary prevention in postmenopausal women who cannot tolerate a bisphosphonate.

PARATHYROID HORMONE AND TERIPARATIDE

PTH and fragments of PTH given in small doses paradoxically *stimulate* osteoblast activity and *enhance* bone formation, and are used by specialists to treat selected male or female patients with osteoporosis, especially those with severe disease. The main compound currently used is **teriparatide** – the peptide fragment (1–34) of recombinant PTH. Another peptide analogue (**ostabolin,** cyclic PTH1–35, which it is hoped will increase bone mass with less effect on bone resorption and hence on plasma calcium concentration than PTH or teriparatide) is in development.

Teriparatide reverses osteoporosis by stimulating new bone formation (Yasothan & Santwana, 2008). It increases bone mass, structural integrity and bone strength by increasing the number of osteoblasts and by activating those osteoblasts already in bone. It also reduces osteoblast apoptosis.

It acts on PTH_1 and PTH_2, G protein-coupled receptors in the cell membranes of target cells, and its effects are mediated through activation of adenylyl cyclase and phospholipases A, C and D, and consequent increases in cyclic AMP and intracellular Ca^{2+} (see Deal, 2009).

Teriparatide is given subcutaneously once daily. It is well tolerated, and serious adverse effects are few. Nausea, dizziness, headache and arthralgias can occur. Mild hypercalcaemia, transient orthostatic hypotension and leg cramps have been reported.

STRONTIUM

Strontium (a Scottish element discovered in the tin mines around Strontian and given as the ranelate salt) inhibits bone resorption and also stimulates bone formation. It prevents vertebral and non-vertebral fractures in older women (see Fogelman & Blake, 2005). However, like barium it blocks potassium channels responsible for basal vasodilator tone, and is associated with an increased risk of cardiovascular disease, including myocardial infarction. It can also cause severe allergic reactions, and its use is restricted to specialists treating severe forms of osteoporosis.

The precise mechanism is not clear. Like calcium, strontium is absorbed from the intestine, incorporated into bone and excreted via the kidney. Strontium ions stimulate the calcium-sensing receptor causing pre-osteoblasts to differentiate into osteoblasts, which increase bone formation and secrete osteoprotegerin. Strontium inhibits osteoclasts so decreasing bone resorption. Strontium atoms are adsorbed onto the hydroxyapatite crystals, but eventually exchange for calcium in the bone minerals and remain in bone for many years.

The drug is well tolerated; a low incidence of nausea and diarrhoea is reported.

VITAMIN D PREPARATIONS

Vitamin D preparations are used in the treatment of vitamin D deficiencies, bone problems associated with renal failure ('renal osteodystrophy') and hypoparathyroidism – acute hypoparathyroidism is treated with intravenous calcium and injectable vitamin D preparations.

The main vitamin D preparation used clinically is **ergocalciferol**. Other preparations are **alfacalcidol** and **calcitriol**. All can be given orally and are well absorbed unless there is obstructive liver disease (vitamin D is fat soluble, and bile salts are necessary for absorption). **Paricalcitol,** a synthetic vitamin D analogue with less potential to cause hypercalcaemia, is used to treat and prevent the secondary hyperparathyroidism that occurs in patients with chronic renal failure because of associated hyperphosphataemia (Salusky, 2005).

Given orally, vitamin D is bound to a specific α-globulin in the blood and exogenous vitamin D can be found in fat for many months after dosing. The main route of elimination is in the faeces.

The clinical uses of vitamin D preparations are given in the box.

Excessive intake of vitamin D causes hypercalcaemia. If hypercalcaemia persists, especially in the presence of elevated phosphate concentrations, calcium salts are deposited in the kidney and urine, causing renal failure and kidney stones.

Clinical uses of vitamin D

- Deficiency states: prevention and treatment of *rickets*, *osteomalacia* and vitamin D deficiency owing to *malabsorption* and *liver disease* (**ergocalciferol**).
- Hypocalcaemia caused by *hypoparathyroidism* (**ergocalciferol**).
- *Osteodystrophy* of *chronic renal failure*, which is the consequence of decreased calcitriol generation (**calcitriol** or **alphacalcidol**).
  Plasma Ca^{2+} levels should be monitored during therapy with vitamin D.

BIOLOGICALS

Denosumab is a recombinant human monoclonal antibody that inhibits RANKL, the primary signal for bone resorption (see p. 441). It was approved by the US Food and Drug Administration in 2010 for use in postmenopausal women at risk of osteoporosis, and to prevent skeleton-related events in patients with bone metastases from solid tumours. Trials in other indications are ongoing. It is especially useful when bisphosphonates are not appropriate. Calcium and vitamin D deficiencies need to be corrected and necessary dental work needs to be undertaken before treatment with denosumab to reduce the risk of osteonecrosis of the jaw (as with potent bisphosphonates, see clinical box, p. 445). It is administered as subcutaneous injections (60 mg) every 6 months for women with postmenopausal osteoporosis or men with prostate cancer at increased risk of osteoporosis because of hormone ablation, or more frequently (monthly) in patients with bone metastases. Adverse effects include altered bowel habit (diarrhoea or constipation), dyspnoea, hypocalcaemia, hypophosphataemia, infection (including respiratory, ear, cellulitis) or rash as well as (rarely) osteonecrosis of the jaw.

CALCITONIN

The main preparation available for clinical use (see the clinical box) is **salcatonin** (synthetic salmon calcitonin). Synthetic human calcitonin is also available. Calcitonin is given by subcutaneous or intramuscular injection, and there may be a local inflammatory action at the injection site. It can also be given intranasally, which is more convenient but less effective. Its plasma half-life is 4–12 min, but its action lasts for several hours.

Unwanted effects include nausea and vomiting. Facial flushing may occur, as may a tingling sensation in the hands and an unpleasant taste in the mouth.

Clinical uses of calcitonin/salcatonin

These agents are now less used.
- *Hypercalcaemia* (e.g. associated with neoplasia).
- *Paget's disease* of bone (to relieve pain and reduce neurological complications) – but it is much less convenient than an injected high potency bisphosphonate.
- Postmenopausal and corticosteroid-induced *osteoporosis* (with other agents).

CALCIUM SALTS

Calcium salts used therapeutically include **calcium gluconate** and **calcium lactate**, given orally. Calcium gluconate is also used for intravenous injection in emergency treatment of hyperkalaemia (Ch. 29); intramuscular injection is not used because it causes local necrosis.

Calcium carbonate, an antacid and phosphate binder (Ch. 29), is usually very little absorbed from the gut (an advantage since an effect within the stomach or intestine is the desired outcome for a drug intended to buffer gastric acid and to reduce ileal phosphate absorption), but there is concern that low level systemic absorption has the potential to cause arterial calcification in patients with renal failure, especially if complicated by hyperphosphataemia (the product of calcium and phosphate ion concentrations is sometimes used clinically to estimate the risk of tissue deposition of insoluble calcium phosphate).

Unwanted effects: oral calcium salts can cause gastrointestinal disturbance. Intravenous administration in emergency treatment of hyperkalaemia requires care, especially in patients receiving cardiac glycosides, the toxicity of which is influenced by extracellular calcium ion concentration (see Ch. 21).

The clinical uses of calcium salts are given in the clinical box.

Clinical uses of calcium salts

- Dietary deficiency.
- Hypocalcaemia caused by *hypoparathyroidism* or *malabsorption* (intravenous for acute tetany).
- Calcium carbonate is an antacid; it is poorly absorbed and binds phosphate in the gut. It is used to treat *hyperphosphataemia* (Ch. 29).
- Prevention and treatment of *osteoporosis* (often with oestrogen or SERM in women, bisphosphonate, vitamin D).
- Cardiac dysrhythmias caused by severe *hyperkalaemia* (intravenous; see Ch. 21).

CALCIMIMETIC COMPOUNDS

Calcimimetics enhance the sensitivity of the parathyroid Ca^{2+}-sensing receptor to the concentration of blood Ca^{2+}, with a consequent decrease in secretion of PTH and reduction in serum Ca^{2+} concentration. There are two types of calcimimetics:

1. Type I are agonists, and include various inorganic and organic cations; Sr^{2+} is an example (see p. 446).
2. Type II are allosteric activators (see Ch. 3) that activate the receptor indirectly. **Cinacalcet**, which is used for the treatment of hyperparathyroidism (Fig. 36.3; Peacock et al., 2005) is an example.

POTENTIAL NEW THERAPIES

Improved understanding of bone remodelling (Yasothan & Kar, 2008; Deal, 2009) has opened several therapeutic approaches that will hopefully yield useful new drugs in the foreseeable future. These include cathepsin K inhibitors (e.g. **odanacatib** – which may be submitted for regulatory review in 2014). Other promising targets are discussed by Deal (2009).

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Chemical transmission and drug action in the central nervous system

37

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. 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. 40) 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 40, 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 and neuropharmacology textbooks such as Kandel et al. (2013), 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 37.1. 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 long-lasting effects; that they can act rather diffusely, at a considerable distance from their site of release (e.g. GABA acting at extrasynaptic $GABA_A$ receptors, see Ch. 38); and that they can also produce other diverse effects, for example on transmitter synthesis, on the

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[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).
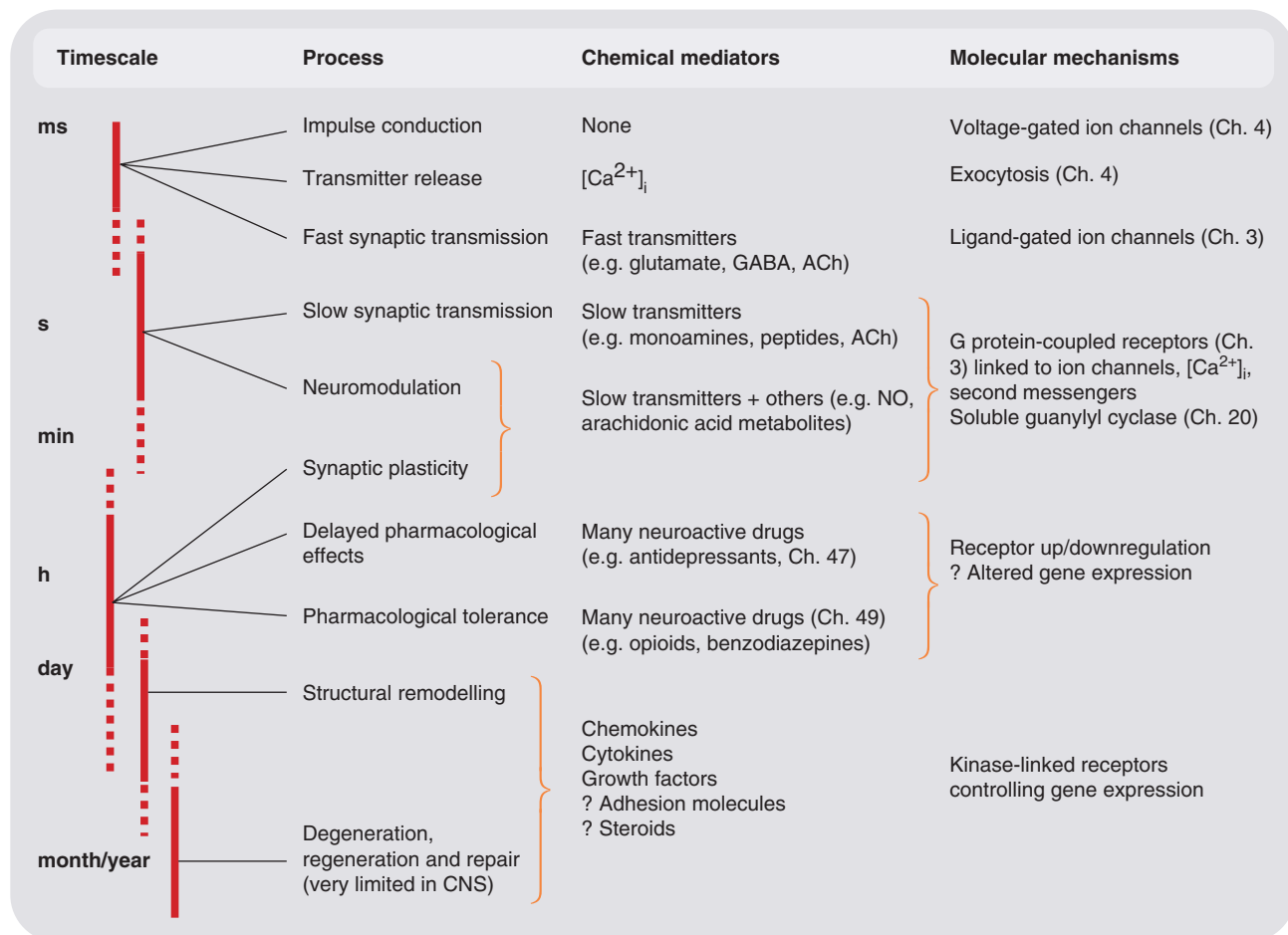
Fig. 37.1   **Chemical signalling in the nervous system.** Knowledge of the mediators and mechanisms becomes sparser as we move from the rapid events of synaptic transmission to the slower ones involving remodelling and alterations of gene expression. ACh, acetylcholine; CNS, central nervous system; NO, nitric oxide.

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, Ch. 20) and arachidonic acid metabolites (Ch. 17), 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 37.1 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 Matsas & Tsacopolous, 2013),

albeit on a slower timescale than that of neuronal communication. These cells express a range of receptors and transporters, and also release a wide variety of mediators, including glutamate, D-serine, ATP, 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.

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

Table 37.1 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
'Gaseous' mediators	Nitric oxide Carbon monoxide	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 are now being targeted 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.

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receptors, kinase-linked receptors and nuclear receptors – current neuroactive drugs target mainly the first two.

In the last 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.
- Considerable molecular diversity of known receptor molecules and ion channels (see Ch. 3) has been revealed.
- The receptors and channels are each expressed in several subtypes, with characteristic distributions in different brain areas. In most cases, we are only beginning to discover what this diversity means at a functional level, mainly through the study of transgenic animals. 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. 44). The potential of these new approaches in terms of improved drugs for neurological and psychiatric diseases is large but as yet unrealised.

- The pathophysiology of neurodegeneration is beginning to be understood (see Ch. 40), and progress is being made in understanding the mechanisms underlying drug dependence (see Ch. 49), suggesting new strategies for treating these disabling conditions. The neurobiology of epilepsy, schizophrenia and depressive illnesses is also advancing.
- Cognitive dysfunction in CNS disorders such as schizophrenia, depressive illness and drug addiction is a potential target for drug therapy.

DRUG ACTION IN THE CENTRAL NERVOUS SYSTEM

As already emphasised, the molecular and cellular mechanisms underlying drug action in the CNS and in the periphery have much in common. Understanding how drugs affect brain function is, however, problematic. One difficulty is the complexity of neuronal interconnections in

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the brain – the wiring diagram. Figure 37.2 illustrates in a schematic way the kind of interconnections that typically exist for, say, a noradrenergic neuron in the *locus coeruleus* (see Ch. 39), 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 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 adaptive responses and slowly developing changes in perception rather than the immediate pharmacodynamic effects of the drug. This is well documented for antipsychotic and antidepressant drugs (Chs 46 and 47). The development of dependence on opioids, benzodiazepines and psychostimulants is similarly gradual in onset (Ch. 49). 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 key factor in CNS pharmacology is the blood–brain barrier (see Ch. 8), penetration of which requires molecules to traverse the vascular endothelial cells rather than going between them. Inflammation can disrupt the integrity of the blood–brain barrier, allowing previously impermeable drugs such as **penicillin** to cross. 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 **levodopa** (Ch. 40), **valproate** (Ch. 45) 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). Many antibacterial and anticancer drugs are excluded from the brain while some CNS-acting drugs – including certain opioid, antidepressant, antipsychotic and antiepileptic drugs – are actively extruded from the brain see (Linnet & Ejsing, 2008). Variation in the activity of efflux transporters between individuals is an important consideration (Chs 8 and 11).

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.

Some drugs defy classification in this scheme, for example **lithium** (see Ch. 47), which is used in the treatment of manic depressive psychosis, and **ketamine** (see Ch. 41), 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 bipolar depression (Ch. 47). Antidepressant drugs are often used to treat anxiety (Ch. 44) and neuropathic pain (Ch. 42), and certain psychostimulants are of proven efficacy for treating hyperactive children (Ch. 48). Here we will adhere to the conventional pharmacological categories, but it needs to be emphasised that in clinical use these distinctions are often disregarded.
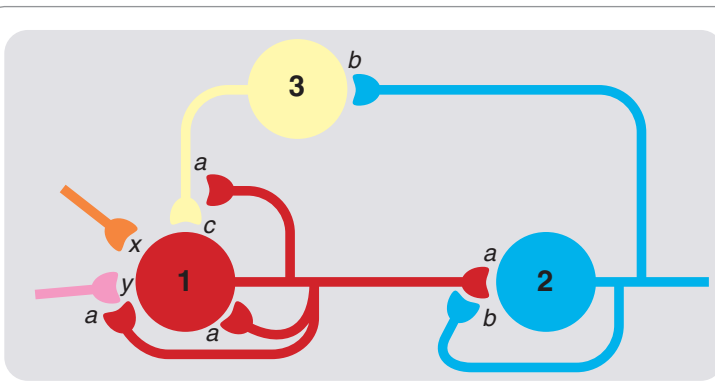
Fig. 37.2 **Simplified scheme of neuronal interconnections in the central nervous system.** Neurons 1, 2 and 3 are shown releasing transmitters *a*, *b* and *c*, respectively, which may be excitatory or inhibitory. Boutons of neuron 1 terminate on neuron 2, but also on neuron 1 itself, and on presynaptic 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.

Table 37.2   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, desflurane, propofol, etomidate	41
Analgesic drugs	Drugs used clinically for controlling pain	Opiates Neuropathic pain – carbamazepine, gabapentin, amitriptyline, duloxetine	42
Anxiolytics and sedatives	Drugs that reduce anxiety and cause sleep	Benzodiazepines (e.g. diazepam, chlordiazepoxide, flurazepam, clonazepam)	44
Antiepileptic drugs Synonym: anticonvulsants	Drugs used to reduce seizures	Carbamazepine, valproate, lamotrigine	45
Antipsychotic drugs Synonym: antischizophrenic drugs	Drugs used to relieve the symptoms of schizophrenic illness	Clozapine, haloperidol, risperidone	46
Antidepressant drugs	Drugs that alleviate the symptoms of depressive illness	Selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors	47
Psychomotor stimulants Synonym: psychostimulants	Drugs that cause wakefulness and euphoria	Amphetamine, cocaine, methylphenidate, caffeine	48
Psychotomimetic drugs Synonym: hallucinogens	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, MDMA (ecstasy)	48
Cognition enhancers Synonym: nootropic drugs	Drugs that improve memory and cognitive performance	Acetylcholinesterase inhibitors: donepezil, galantamine, rivastigmine NMDA receptor antagonists: memantine Others: piracetam, modafinil	40 37

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).

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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 central nervous system. **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 p. 462) 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 25 years is beyond the range of this book; for more detail see Traynelis et al. (2010) and Nicoletti et al. (2011). Here we concentrate on pharmacological aspects. With regard to novel drug development, many promising new compounds interacting with EAAs commenced development for the treatment of a wide range of neurological and psychiatric disorders but have failed because of lack of efficacy or adverse effects and only a few drugs[1] have made it into clinical use. The field has yet to make a major impact on therapeutics. 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.

METABOLISM AND RELEASE OF EXCITATORY 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. 38.1). 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 38.1, 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 nerve terminals and neighbouring astrocytes (Fig. 38.2) by $Na^+/H^+/K^+$ dependent transporters (cf. monoamine transporters – Chs 12 and 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 Beart & O'Shea, 2007). Glutamate transport can, under some circumstances (e.g. depolarisation by increased extracellular $[K^+]$), operate in reverse and constitute a source of glutamate release, a process that may occur under pathological conditions such as brain ischaemia (see Ch. 40). Glutamate taken up by astrocytes is converted to glutamine and recycled, via transporters, back to the neurons, which convert the glutamine back to glutamate (Fig. 38.2). Glutamine, which lacks the pharmacological activity of glutamate, thus

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[1]**Perampanel**, a non-competitive AMPA receptor antagonist, has recently been approved for the treatment of epilepsy (Ch. 45).
Memantine, an N-methyl-D-aspartate (NMDA) antagonist, licensed for the treatment of moderate to severe Alzheimer's disease (Ch. 40), has been used for some time, as has the dissociative anaesthetic **ketamine**, an NMDA channel blocker (Ch. 41).
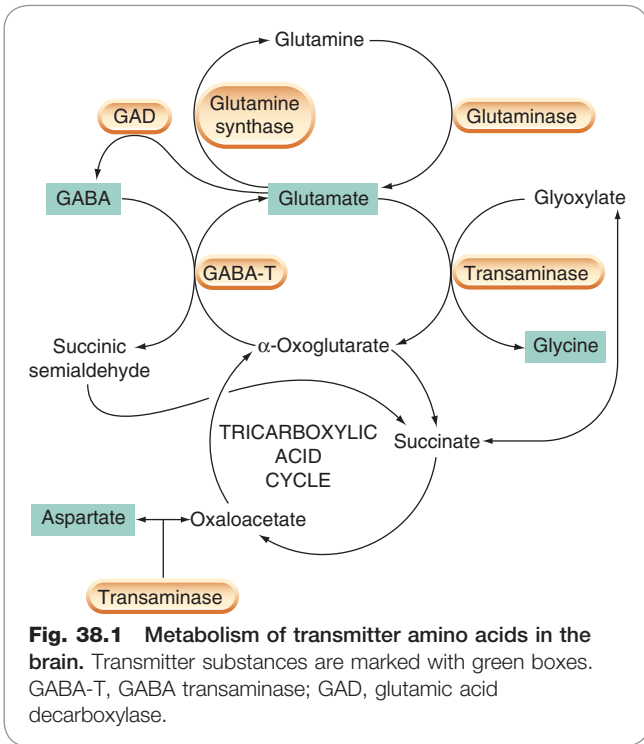
Fig. 38.1 **Metabolism of transmitter amino acids in the brain.** Transmitter substances are marked with green boxes. GABA-T, GABA transaminase; GAD, glutamic acid decarboxylase.
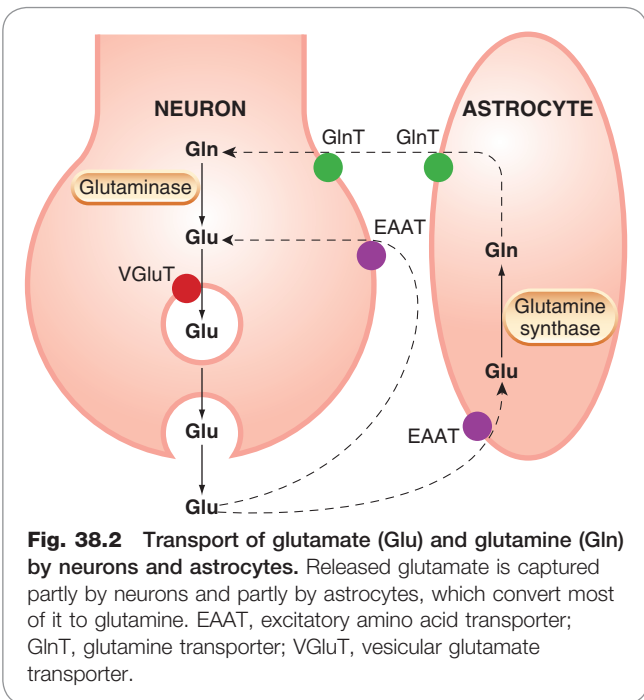

Fig. 38.2 **Transport of glutamate (Glu) and glutamine (Gln) by neurons and astrocytes.** Released glutamate is captured partly by neurons and partly by astrocytes, which convert most of it to glutamine. EAAT, excitatory amino acid transporter; GlnT, glutamine transporter; VGluT, vesicular glutamate transporter.

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.

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. 40), schizophrenia (see Ch. 46) and depression (see Ch. 47). In contrast to the situation with monoamine synthesis and transport (Chs 14 and 39), few drugs (none

in clinical use) are known that interfere specifically with glutamate metabolism.

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. 38.3), 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 38.1). These ligand-gated channels can be homomeric or heteromeric assemblies of four subunits, each with the 'pore loop' structure shown in Figure 3.18 (Ch. 3). 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, serve to mediate fast excitatory synaptic transmission in the CNS – absolutely essential for our brains to function. NMDA receptors (which often coexist with AMPA receptors) contribute a slow component to the excitatory synaptic potential (Fig. 38.4B), the magnitude of which varies in different pathways. 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.

[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 and www.guidetopharmacology.org). 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. 39) control neurotransmitter release. An explanation of how this control can be either facilitatory or inhibitory is given in Khakh & Henderson (2000).
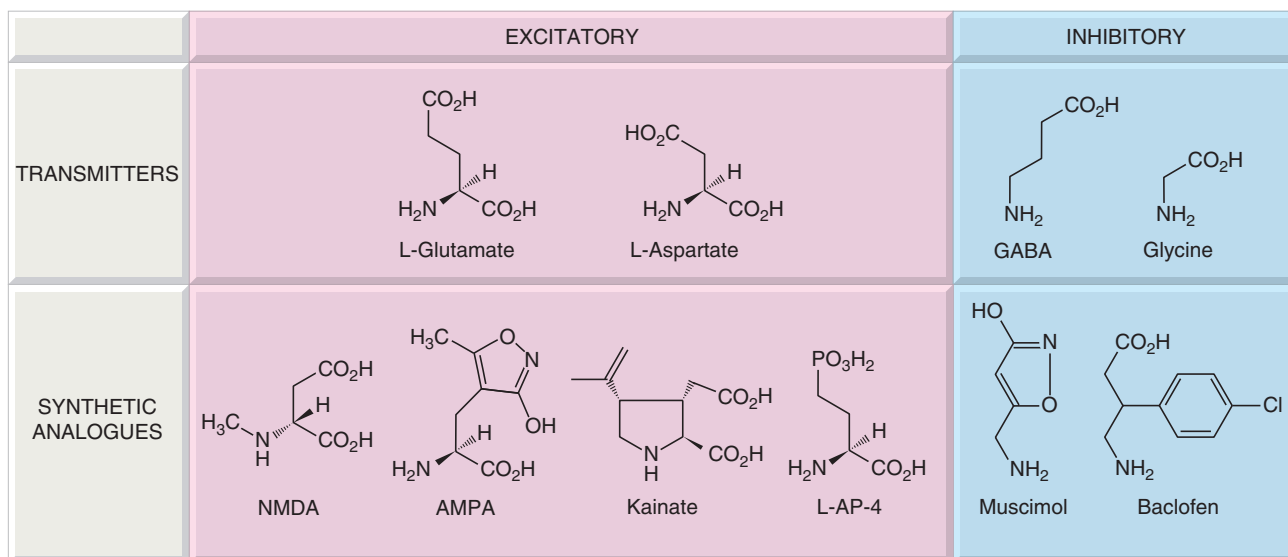
Fig. 38.3 **Structures of agonists acting on glutamate, GABA and glycine receptors.** The receptor specificity of these compounds is shown in Tables 38.1 and 38.2. AMPA, (S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; L-AP4, L-2-amino-4-phosphonopentanoic acid; NMDA, N-methyl-D-aspartic acid.

Table 38.1 **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]	AP5, CPP	7-Chloro-kynurenic acid, HA-966	NBQX	NBQX ACET
Other modulators	Polyamines (e.g. spermine, spermidine) Mg^{2+}, Zn^{2+}		Cyclothiazide Perampanel 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 GluA2 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).

[b]A neurotoxin from mussels (see Ch. 40).

ACET, -(S)-1-(2-amino-2-carboxyethyl)-3-(2-carboxy-5-phenylthiophene-3-yl-methyl)-5-methylpyrimidine-2,4-dione; AP5, 2-amino-5-phosphonopentanoic acid; CPP, 3-(2-carboxypiperazin-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 38.3.)
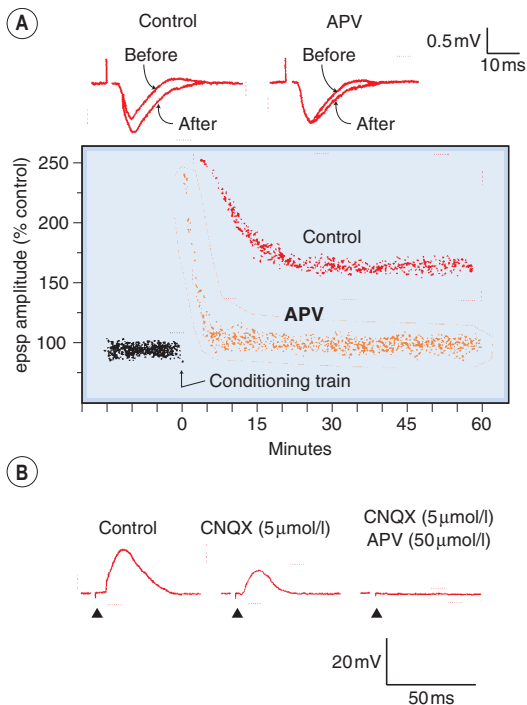
Fig. 38.4 Effects of excitatory amino acid receptor antagonists on synaptic transmission. [**A**] AP5 (NMDA antagonist) prevents long-term potentiation (LTP) in the rat hippocampus without affecting the fast excitatory postsynaptic potential (epsp). Top records show the extracellularly recorded fast 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 AP5 (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 AP5, 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 AP5 (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 AP5 (50 μmol/l). *(Panel [A] from Malinow R, Madison D, Tsien R W 1988 Nature 335, 821; panel [B] from Andreasen M, Lambert J D, Jensen M S 1989 J Physiol 414, 317–336.)*

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.

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. 38.5, that are postulated to play a role in pathophysiological mechanisms.
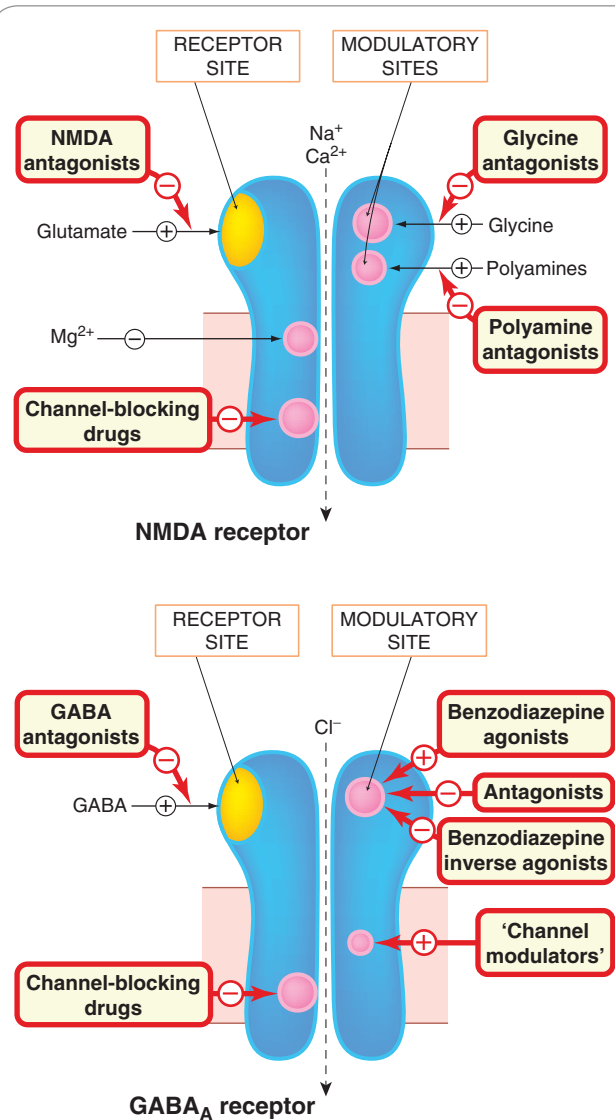
Fig. 38.5 Main sites of drug action on NMDA and GABA_A receptors. Both receptors are multimeric ligand-gated ion channels. Drugs can act as agonists or antagonists at the neurotransmitter receptor site or at modulatory sites associated with the receptor. They can also act to block the ion channel at one or more distinct sites. In the case of the GABA_A receptor, the mechanism by which 'channel modulators' (e.g. ethanol, anaesthetic agents, neurosteroids) 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 38.1 and 38.3.

- 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. 38.6). The binding site for
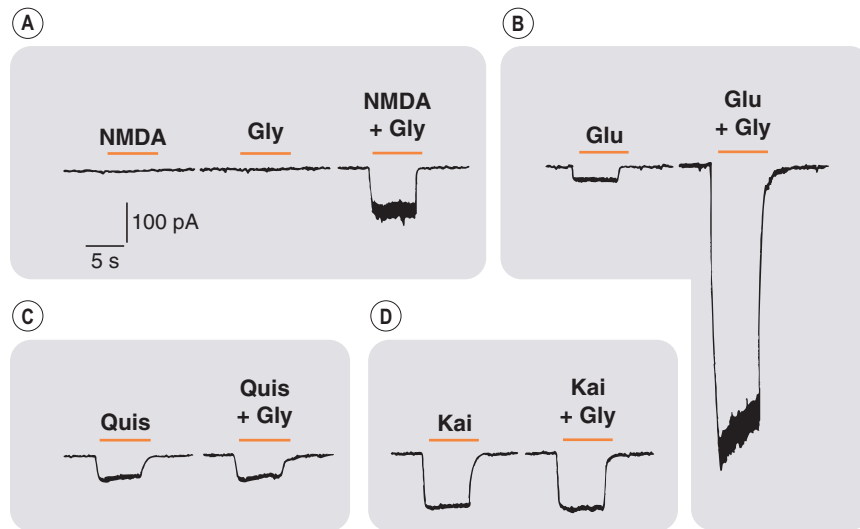
Fig. 38.6   Facilitation of NMDA by glycine. Recordings from mouse brain neurons in culture (whole-cell patch clamp technique). Downward deflections represent inward current through excitatory amino acid-activated ion channels. [**A**] NMDA (10 μmol/l) or glycine (1 μmol/l) applied separately had little 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 JW, Ascher P 1987 Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325, 529–531.)*

glycine is distinct from the glutamate binding site, i.e. glycine is an allosteric modulator (see Ch. 2), 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 p. 465), 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 38.1) 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.

• Some endogenous polyamines (e.g. **spermine**, **spermidine**) act at an allosteric site distinct from that of glycine to facilitate channel opening. The experimental drugs **ifenprodil** and **eliprodil** block their action.

• Recently other allosteric sites have been identified on the NMDA receptor and positive and negative allosteric modulators with novel patterns of GluN2 subunit selectivity have been discovered (Monaghan et al., 2012).

• Some well-known anaesthetic and psychotomimetic agents, such as **ketamine** (Ch. 41) and **phencyclidine** (Ch. 48), are selective blocking agents for NMDA-operated channels. The experimental compound **dizocilpine** shares this property.

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 homo- and heterodimers[7] (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 38.2). 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. 38.7). 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.

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

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[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.

[7]It has been suggested that mGlu receptors may form heterodimers with non mGlu receptors such as the $5\text{-}HT_{2A}$ receptor (Gonzalez-Maeso et al., 2008).

Table 38.2 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-carboxyphenylglycine.

brain, and are of particular interest as potential drug targets. AMPA receptors, on the other hand, are mainly responsible for fast excitatory transmission. 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. 40).

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 Bear et al., 2006; Bliss & Cooke, 2011) 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; Bliss & Cooke, 2011). 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. 38.4). 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 Kullman, 2012). The response of postsynaptic AMPA 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 enhance AMPA receptor internalisation.

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. Endogenous cannabinoids released by the postsynaptic cell, may also act as retrograde messengers to enhance glutamate release (see Chs 19 and 39).

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 the postsynaptic cell activates protein kinases, phospholipases and nitric oxide synthase, which act jointly with other cellular processes (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 38.1. 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 the glycine site is an alternative way to produce antagonism. **Kynurenic acid** and the more potent analogue **7-chlorokynurenic acid** act in this way. Another site of block is the channel itself, where substances such as ketamine, phencyclidine and **memantine** act. 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. 40), as well as in the treatment of epilepsy (Ch. 45) and Alzheimer's disease (Ch. 40). They have also been considered for indications such as drug dependence (Ch. 49),
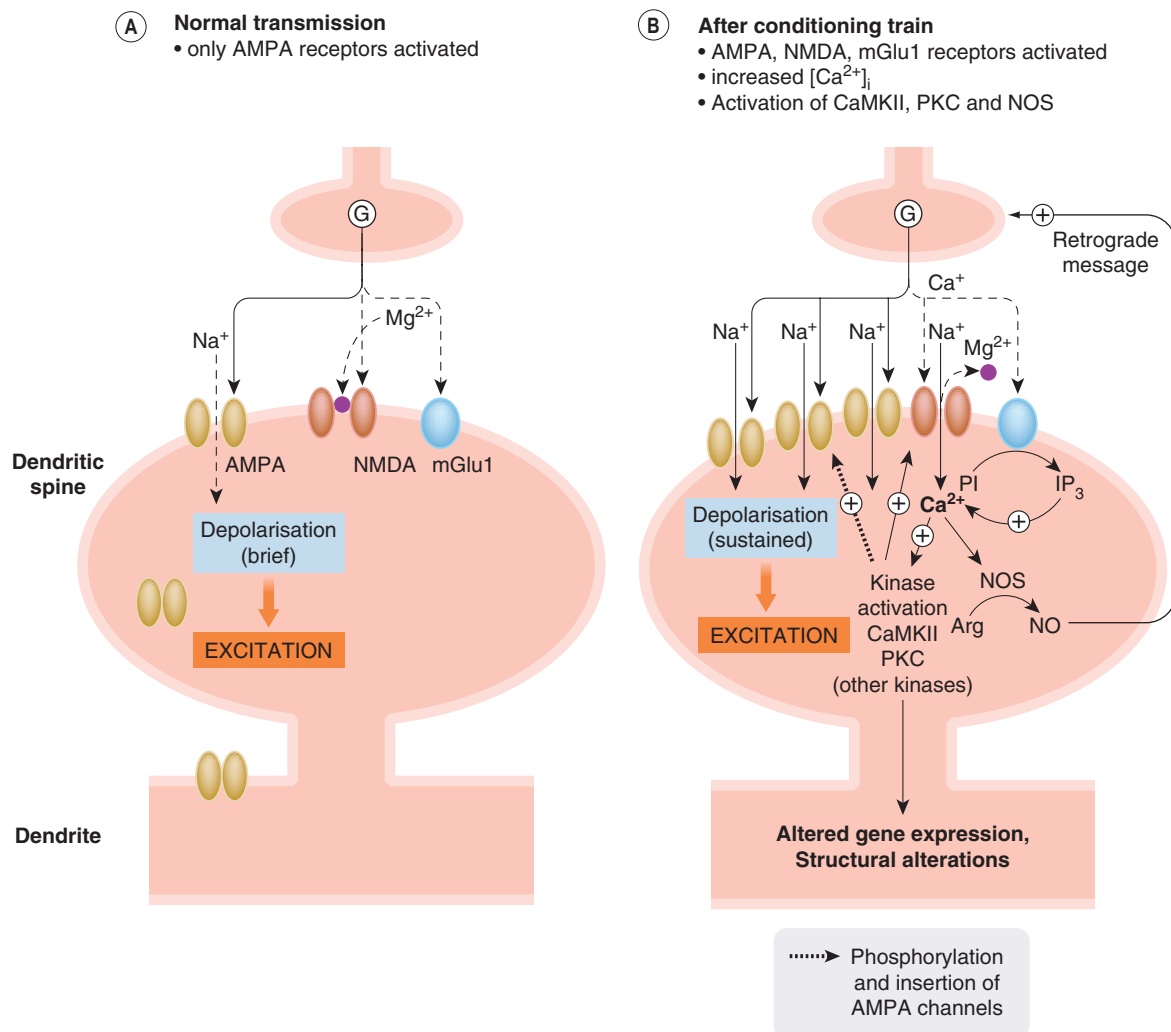
Fig. 38.7 Mechanisms of long-term potentiation. [**A**] With infrequent synaptic activity, glutamate (G) activates mainly AMPA receptors. There is insufficient glutamate to activate metabotropic receptors, and NMDA receptor channels are blocked by Mg^{2+}. [**B**] After a conditioning train of stimuli, 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) phosphorylate 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 endocannabinoids (Ch. 19) that act as retrograde messengers to enhance glutamate release.
- The neurotrophic factor BDNF released from nerve terminals and postsynaptic structures (not shown) plays a multimodal role in the early and later stages of LTP.

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

schizophrenia (Ch. 46) and depression (Ch. 47). 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. 48). Only two NMDA receptor antagonists, **ketamine** (anaesthesia, analgesia and depression; see Chs 41, 42 and 47) and **memantine** (Alzheimer's disease; Ch. 40), 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. The noncompetitive AMPA receptor antagonist **perampanel** has been introduced as an antiepileptic drug. The prospects for kainate receptor antagonists appear 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 been less successful than was hoped. 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. The new hope is that subunit selective negative allosteric modulators may have fewer side effects than previous generations of orthosteric antagonists.

Metabotropic glutamate receptor antagonists

While antagonists that discriminate between the different groups of mGlu receptors are available (see Table 38.2), 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, which can be either inhibitory or facilitatory (see Ch. 3). Antagonists or negative allosteric modulators acting at group 1 mGlu receptors have potential for the treatment of fragile X syndrome,[8] various pain states, Parkinson's disease (including the control of **levodopa**-induced dyskinesias, see Ch. 40), neuroprotection, epilepsy and drug abuse; whereas antagonists or negative allosteric modulators of group 2 mGlu receptors have potential as cognition enhancers (see Nicoletti et al., 2011).

AGONISTS AND POSITIVE MODULATORS

Ionotropic glutamate receptors

Various agonists at ionotropic glutamate receptors that are used experimentally are shown in Table 38.1. From the clinical perspective, interest centres on the theory that positive AMPA receptor modulators may improve memory and cognitive performance. Early examples include **cyclothiazide**, **piracetam** and CX-516 (**Ampalex**). These positive allosteric modulators, known as *ampakines*, 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). Originally ampakines were thought to have therapeutic potential as cognition enhancers and for the treatment of schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and Parkinson's disease (see Lynch, 2006) but so far clinical trials have been disappointing. A more recently developed ampakine, CX1739, is in Phase II clinical trial for the treatment of drug-induced respiratory depression. Inhibition of the glycine transporter GlyT1 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 various neurological disorders (see Harvey & Yee, 2013).

Metabotropic glutamate receptors

Developing selective agonists of mGlu receptors has proven to be quite difficult; recently, selective positive allosteric modulators have been developed (see Nicoletti et al., 2011). Group 2 and 3 mGlu receptors are located presynaptically on nerve terminals and agonists at these receptors decrease glutamate release. Group 2 mGlu agonists and positive allosteric modulators were therefore thought to have therapeutic potential to decrease neuronal cell death in stroke and in the treatment of epilepsy, but to date clinical trials have been disappointing. Agonists and positive allosteric modulators may be useful in treating anxiety as well as in controlling the positive symptoms of schizophrenia. Group 3 mGlu receptor positive allosteric modulators may be useful in treating anxiety and Parkinson's disease.

Excitatory amino acids

- Excitatory amino acids (EAAs), namely glutamate and aspartate, 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 **AP5** (2-amino-5-phosphonopentanoic acid) and **CPP** (3-(2-carboxypirazin-4-yl)-propyl-1-phosphonic acid); the NMDA-operated ion channel is blocked by **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. 40).
- 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.
- Some specific metabotropic glutamate receptor agonists and antagonists are available, as are positive and negative allosteric modulators.

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[8]Fragile X syndrome is caused by mutation of a single gene on the X chromosome. It affects about 1:4000 children of either sex, causing mental retardation, autism and motor disturbances.

γ-AMINOBUTYRIC ACID (GABA)

GABA is the main inhibitory transmitter in the brain. In the spinal cord and brain stem, glycine is also important (see p. 465).

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. 38.1) 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. GAT1 is the predominant GABA transporter in the brain and is located primarily on GABAergic nerve terminals where it recycles GABA. GAT3 is located predominantly on astrocytes around the GABAergic synapse. GABA transport is inhibited by **guvacine**, **nipecotic acid** and **tiagabine**. Tiagabine is used to treat epilepsy (Ch. 45). In astrocytes 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. 45).

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. 40 and Fig. 40.4). 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** 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 $GABA_B$ receptors are G protein-coupled.

$GABA_A$ RECEPTORS

$GABA_A$ receptors[9] are members of the *cys-loop* family of receptors that also includes the glycine, nicotinic and $5\text{-}HT_3$ receptors (see Ch. 3, Fig. 3.18). The $GABA_A$ receptors are pentamers made up of different subunits. The reader should not despair when informed that 19 $GABA_A$ 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. The most common are α1β2γ2 (by far the most abundant), α2β3γ2 and α3β3γ2 subunits. To make up the pentamer, each receptor contains two α, two β and one 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 each of the interfaces between the α and β subunits whereas benzodiazepines (see Ch. 44) bind at the α/γ interface. A novel benzodiazepine binding site at the α/β interface has recently been described but its function is unclear at present. 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 both fast and tonic postsynaptic inhibition. The $GABA_A$ channel is selectively permeable to Cl^- and 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.[10] In the postsynaptic cell $GABA_A$ receptors are located both at areas of synaptic contact and extrasynaptically (see Fig 38.8 and 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 and α6 subunits as well as the δ subunit, and are highly sensitive to general anaesthetic agents (see Ch. 41) and ethanol (see Ch. 49), 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. 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' 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. 38.9).

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[9]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, benzodiazepines and baclofen, should be subtypes of the $GABA_A$ receptor family as they are pentameric Cl^--permeable ligand-gated channels comprising homo- or heteromeric assemblies of ρ subunits. Their functional significance is slowly being worked out (see Chebib, 2004).

[10]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.
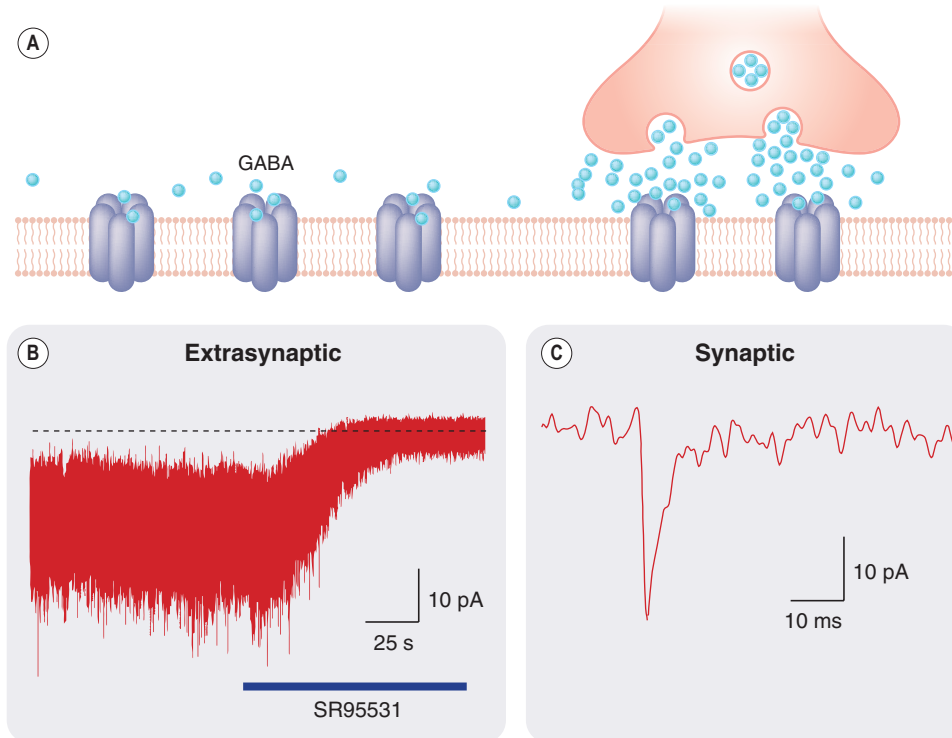
Fig. 38.8 **Synaptic and extrasynaptic GABA$_A$ receptors.** [**A**] Diagram depicting GABA$_A$ receptors at synaptic and extrasynaptic sites in the plasma membrane. The blue dots represent GABA molecules. [**B**] Tonic activation of extrasynaptic GABA$_A$ receptors gives rise to a steady-state inward current (distance from the baseline indicated by the dashed line) and increased 'noise' on the trace. The current is blocked on application of the GABA$_A$ receptor antagonist SR95531. [**C**] Phasic release of GABA from the presynaptic terminal evokes a fast synaptic current (rapid downward deflection). Note the different timescales in [**B**] and [**C**]. *(Figure courtesy of M Usowicz.)*
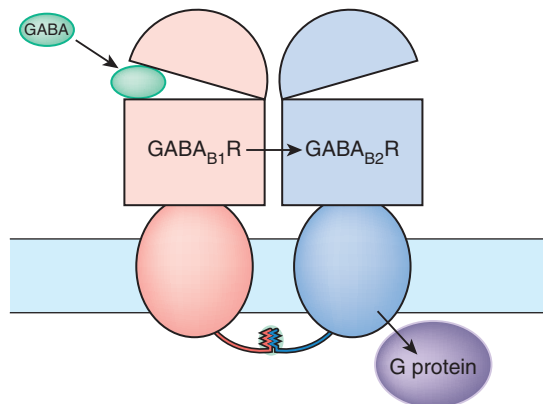
Fig 38.9 **Dimeric structure of the GABA$_B$ receptor.** The receptor is made up of two seven-transmembrane domain subunits held together by a coil/coil interaction between their C-terminal tails. Activation of the receptor occurs when GABA binds to the extracellular domain of the B1 subunit (known as the venus fly trap, because it snaps shut when GABA binds). This produces an allosteric change in the B2 subunit which is coupled to the G protein. *(Adapted from Kubo Y, Tateyama M 2005 Towards a view of functioning dimeric metabotropic receptors. Curr Opin Neurobiol 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. 38.5). 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 (see Ch. 44), alcohol (see Ch. 49), barbiturates, neurosteroids (see p. 464, Table 38.3) and many general anaesthetics (see Ch. 41). The main agonists, antagonists and modulatory substances that act on GABA receptors are shown in Table 38.3.

Muscimol, derived from a hallucinogenic mushroom, resembles GABA chemically (see Fig. 38.3) and is a powerful GABA$_A$ receptor agonist. A synthetic analogue, **gaboxadol** is a partial agonist that was developed as a hypnotic drug (Ch. 44) but has now been withdrawn. **Bicuculline**, a naturally occurring convulsant compound, is a specific antagonist 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. 44), selectively potentiate the effects of GABA on some GABA$_A$ receptors

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Table 38.3 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] a partial agonist)	Anxiolytic benzodiazepines (e.g. diazepam)	Barbiturates Steroid anaesthetics (e.g. alphaxalone)	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 Ca^{2+} channels, activation of K$^+$ channels, inhibition of adenylyl cyclase	Ligand-gated chloride channel
Location	Widespread; primarily postsynaptic			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 glycine receptors.
ipsp, inhibitory postsynaptic potential.

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. 38.5), include other CNS depressants such as barbiturates (Ch. 44), anaesthetic agents (Ch. 41) and neurosteroids. Neurosteroids (see Lambert et al., 2009) are compounds that are related to steroid hormones but that act to enhance activation of GABA$_A$ receptors – those containing δ subunits appear most sensitive. 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 **alphaxalone**, developed as an anaesthetic agent (Ch. 41).

Picrotoxin, a plant product, is a convulsant that acts by blocking the GABA$_A$ receptor chloride channel, thus blocking the postsynaptic inhibitory effect of GABA. It also blocks glycine receptors. 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. 38.3), 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. Baclofen is used to treat spasticity and related motor disorders (Ch. 45) and may also be useful in the treatment of drug dependence (see Ch. 49).

Competitive antagonists for the GABA$_B$ receptor include a number of experimental compounds (e.g. **2-hydroxy-saclofen** and more potent compounds with improved brain penetration, such as CGP 35348). Tests in animals showed 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. 45), together with enhanced cognitive performance. However, as in many areas of pharmacology, such preclinical promise has not resulted in the development of a new therapeutic drug.

γ-HYDROXYBUTYRATE

γ-Hydroxybutyrate (**sodium oxybate** or GHB; see Wong et al., 2004) occurs naturally in the brain as a side product of GABA synthesis. As a synthetic drug it can be used to treat narcolepsy and alcoholism. In addition 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. It is also used as an intoxicant and date rape drug. In common with many abused drugs (see Ch. 49), it activates 'reward pathways' in the brain, and its use is now illegal in most countries. GHB is an agonist at GABA$_A$ receptors containing α4 and δ subunits, a weak partial agonist at GABA$_B$ receptors and an agonist at an 'orphan' G protein-coupled receptor, GPR172A.

GLYCINE

Glycine is an important inhibitory neurotransmitter in the spinal cord and brain stem. It 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. 457-458).

▼ The glycine receptor (see Dutertre et al., 2012) 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 forms of glycine receptor are homomers of α subunits or a heteromeric complex of α and β subunits, probably with a stoichiometry of 2α and 3β. Receptors made up only of α subunits are sensitive to glycine and strychnine, indicating that the binding site for these drugs is on the α subunit. The situation for glycine is therefore much simpler than for GABA (see p. 462). Glycine receptors are involved in the regulation of respiratory rhythms, motor control and muscle tone as well as in the processing of pain signals. Mutations of the receptor have been identified in some inherited neurological disorders associated with muscle spasm and reflex hyperexcitability. There are as yet no therapeutic drugs that act specifically by modifying 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 GlyT1 and GlyT2 (Eulenburg et al., 2005). GlyT1 is located primarily on astrocytes and expressed throughout most regions of the CNS. GlyT2 on the other hand is expressed on glycinergic neurons in the spinal cord, brain stem and cerebellum. GlyT2 inhibitors may have potential 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
>   – CNS depressants, such as barbiturates and many 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 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 25 years, 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 have been undertaken, there have been few 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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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 38 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. 19) 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 18, and information on specific neuropeptides (e.g. endorphins, neurokinins and orexins) 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 Nestler et al. (2008) and Iversen et al. (2009).

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[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. 39.1). 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. 42).

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
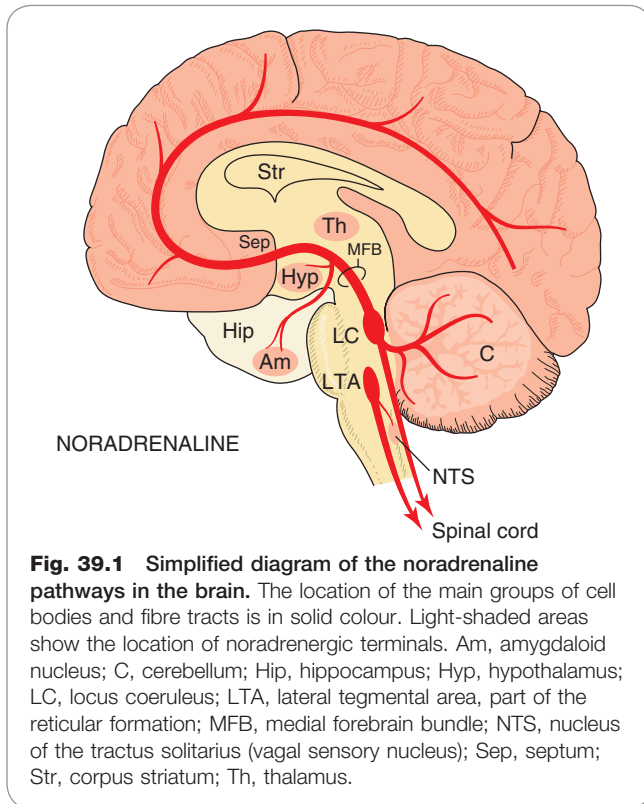
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Fig. 39.1 Simplified diagram of the noradrenaline pathways in the brain. The location of the main groups of cell bodies and fibre tracts is in solid colour. Light-shaded areas show the location of noradrenergic terminals. Am, amygdaloid nucleus; C, 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.

distributed, located both on postsynaptic neurons and on glial cells, and may be involved in motor control, cognition and fear. 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. 47).

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 (see Ch. 22); 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. 48).

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. 47) 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.

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. 40), 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. 33).

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,

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.

Fig. 39.2   The main pathways for dopamine metabolism in the brain. COMT, catechol-*O*-methyl transferase; MAO, monoamine oxidase.

one of the large family of monoamine transporters (see Ch. 14). It is metabolised by monoamine oxidase and catechol-*O*-methyl transferase (Fig. 39.2), the main products being *dihydroxyphenylacetic acid* (DOPAC) and *homovanillic acid* (HVA), the methoxy derivative of DOPAC. The brain content of HVA is often used in animal experiments 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.

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. 39.3):

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 39.4, which was obtained by injecting a dopa derivative containing radioactive fluorine, and scanning for

Fig. 39.3   Simplified diagram of the dopamine pathways in the brain, drawn as in Figure 39.1. 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 39.1.

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.

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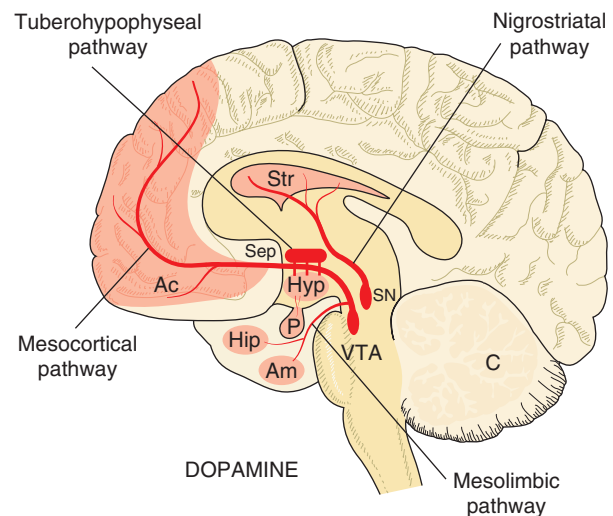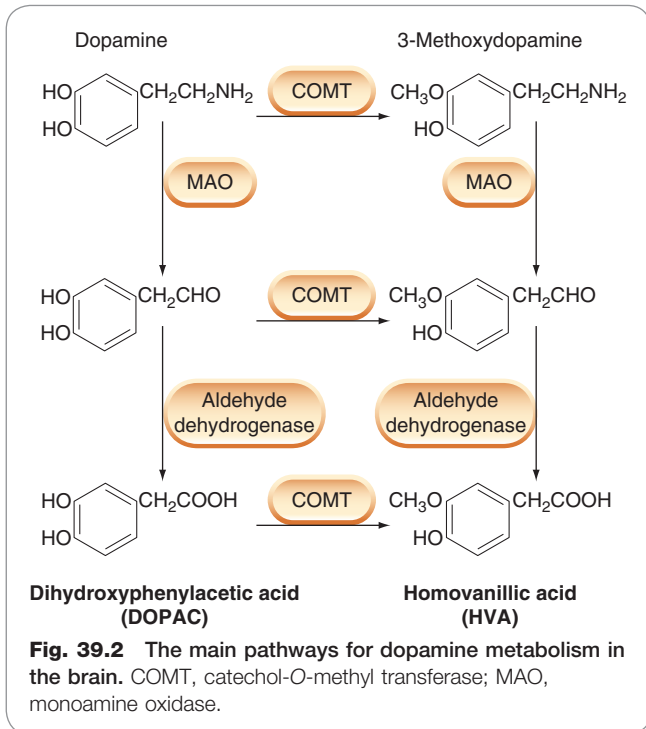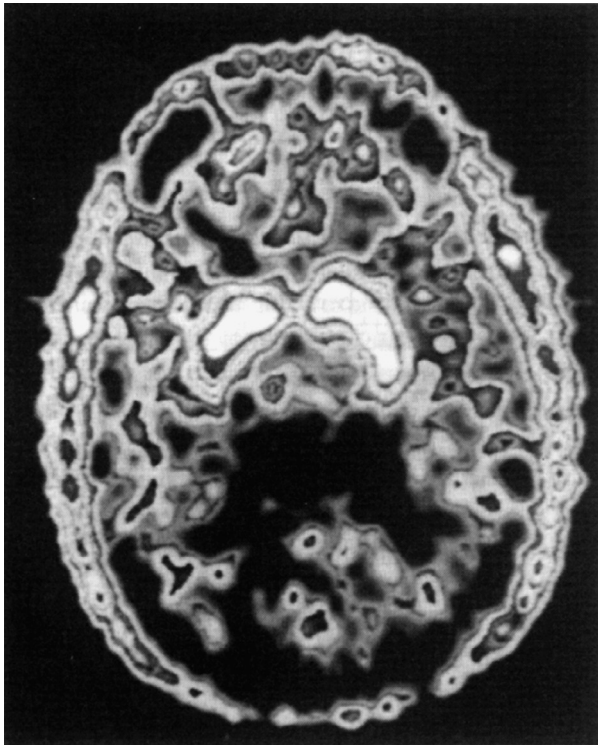
Fig. 39.4 **Dopamine in the basal ganglia of a human subject.** The subject was injected with 5-fluoro-dopa labelled with the positron-emitting isotope ^{18}F, which was localised 3 h later by the technique of positron emission tomography. The isotope is accumulated (white areas) by the dopa uptake system 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 ES, Firnau G, Nahmias C 1983 Nature 305, 137–138.)*

Fig. 39.5 **The role of the neuron-specific phosphoprotein DARPP-32 in signalling by dopamine receptors (see text).** PKA, protein kinase A.

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.

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. The original D_1 family now includes D_1 and D_5, while the D_2 family consists of D_2, D_3 and D_4 (see Table 39.1). Splice variants, leading to long and short forms of D_2, and genetic polymorphisms, particularly of D_4, have subsequently been identified.

▼ All belong to the family of G protein-coupled transmembrane receptors described in Chapter 3. D_1 and D_5 receptors link through G_s to stimulate adenylyl cyclase and activation of protein kinase A (PKA). PKA mediates many of the effects of D_1 and D_5 receptors by phosphorylating a wide array of proteins, including voltage-activated sodium, potassium and calcium channels as well as ionotropic glutamate and GABA receptors. D_2, D_3, and D_4 receptors 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). An interesting component in the dopamine signal transduction pathway is the protein DARPP-32 (*32-kDa dopamine- and cAMP-regulated phosphoprotein* also known as *protein phosphatase 1 regulatory subunit 1B*; see Girault & Greengard, 2004), which is highly expressed in dopamine-sensitive neurons. When intracellular cAMP is increased through activation of D_1 receptors, activating protein kinase A, DARPP-32 is phosphorylated (Fig. 39.5). Phosphorylated DARPP-32 acts as an inhibitor of protein phosphatase-1, thus acting in concert with protein kinases and favouring protein phosphorylation – effectively an amplifying mechanism. In general, activation of D_2 receptors opposes the effects 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. 39.3), 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.

Dopamine, like many other transmitters and modulators, acts presynaptically as well as postsynaptically. Presynaptic D_2 receptors act as autoreceptors 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. Inhibitory D_2 receptors are also located on glutamatergic, GABAergic and cholinergic nerve terminals.

Table 39.1 Dopamine receptors

	Functional role	D1 type		D2 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 in the IUPHAR/BPS Guide to Pharmacology database (www.guidetopharmacology.org)

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. 22).

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. 40) 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 a given receptor type throughout the brain. For example, many antipsychotic drugs (see Ch. 46) 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.

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. 49) 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.

Neuroendocrine function

The tuberohypophyseal dopaminergic pathway (see Fig. 39.3) is involved in the control of prolactin secretion. The hypothalamus secretes various mediators (mostly small peptides; see Ch. 33), 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. 46), 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 (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. 33). 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. 40) cause nausea and vomiting as side effects, while many dopamine antagonists (e.g. phenothiazines, **metoclopramide**; Ch. 30) have antiemetic activity. D_2 receptors occur in the area of the medulla (chemoreceptor trigger zone) associated with the initiation of vomiting (Ch. 30), 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. 48), 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 5-HT 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, mania, anxiety, obsessive–compulsive disorders, schizophrenia, autism and drug abuse.

In its formation, storage and release, 5-HT resembles noradrenaline. Its precursor is tryptophan, an amino acid

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> ### 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.
> - 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.

derived from dietary protein, the plasma content of which varies considerably according to food intake and time of day. 5-HT does not cross the blood–brain barrier and is synthesised in the CNS. 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 form 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 Chs 44 and Ch. 47) constitute an important group of antidepressant and antianxiety 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.

Fig. 39.6 Simplified diagram of the 5-hydroxytryptamine pathways in the brain, drawn as in Figure 39.1. Abbreviations as in Figure 39.1.
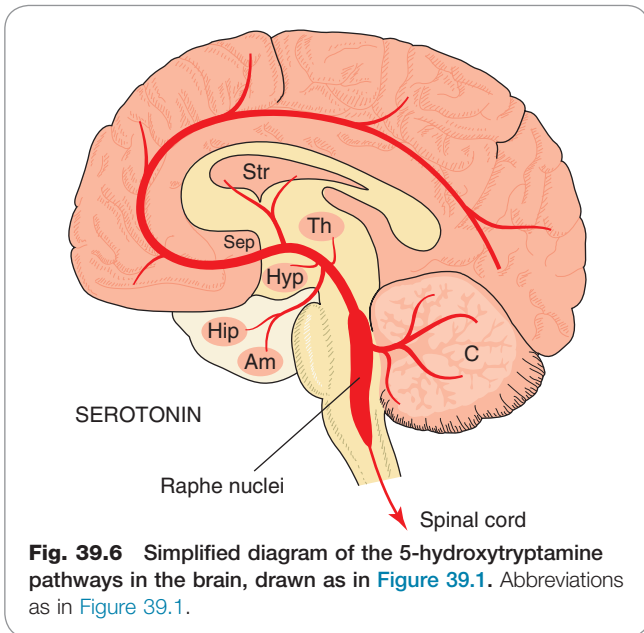
5-HT PATHWAYS IN THE CNS

The distribution of 5-HT-containing neurons (Fig. 39.6) 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 Filip & Bader (2009).

Certain generalisations can be made:

- 5-HT$_1$ receptors (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$)[2] 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 44 and 47). 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 5-HT$_{1B}$

and 5-HT$_{1D}$ receptors such as **sumatriptan** are used to treat migraine (see Ch. 15).
- 5-HT$_2$ receptors (5-HT$_{2A}$, 5-HT$_{2B}$ 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. 47) and antipsychotic drugs (see Ch. 46) as well as various hallucinogenic drugs (see Ch. 48). **Lorcaserin**, a 5-HT$_{2C}$ agonist is an anti obesity drug (see Ch 32). 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-HT3A and 5-HT3B 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. 30) 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. **granisetron** and **ondansetron**; see Chs 15 and 30) are used to treat nausea and vomiting.
- 5-HT$_4$ receptors are important in the gastrointestinal tract (see Chs 15 and 30), 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. 40). Activation of medullary 5-HT$_4$ receptors opposes the respiratory depressant actions of opioids (see Ch. 42).
- There are two 5-HT$_5$ receptors, 5-HT$_{5A}$ and 5-HT$_{5B}$. In the human only 5-HT$_{5A}$ is functional. Antagonists may have anxiolytic, antidepressant and antipsychotic activity.
- 5-HT$_6$ receptors occur primarily in the CNS, particularly in the hippocampus, cortex and limbic system. Blockade of 5-HT$_6$ receptors increases glutamate and Ach release and 5HT$_6$-antagonists are considered potential drugs to improve cognition or relieve symptoms of schizophrenia.
- 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

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[2]There is no 5-HT$_{1C}$ receptor. The original 5-HT$_{1C}$ receptor has been reclassified as 5-HT$_{2C}$.

produced 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 namely:

- hallucinations and behavioural changes
- sleep, wakefulness and mood
- feeding behaviour
- control of sensory transmission (especially pain pathways; see Ch. 42).

Hallucinatory effects

Many hallucinogenic drugs (e.g. LSD; Ch. 48) 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. 46) are antagonists at 5-HT$_{2A}$ receptors in addition to blocking dopamine D$_2$ receptors. The psychostimulant properties of MDMA ('ecstasy'; see Ch. 48) 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. 47), 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. 47) cause loss of appetite, as does the 5-HT$_{2C}$ receptor agonist **lorcaserin**.

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. 42). 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 roles

Other 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 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. 47) and anxiolytic agents (Ch. 44)
- 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. 44)
- 5-HT$_3$ receptor antagonists, such as ondansetron, used as antiemetic agents (see Ch. 30)
- antipsychotic drugs (e.g. clozapine, Ch. 46), 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. 39.7). 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. 40). 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 striatum, these being

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. 44)
  - '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. 47)
  - **ondansetron**, a 5-HT$_3$ antagonist, used to treat chemotherapy-induced emesis (see Chs 15 and 30)
  - **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. 48).

Fig. 39.7 Simplified diagram of the acetylcholine pathways in the brain, drawn as in Figure 39.1. PPT/LD, pedunculopontine and laterodorsal tegmental nuclei; other abbreviations as in Figure 39.1.

important in relation to Parkinson's disease and Huntington's chorea (Ch. 40).

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 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 39.2). The heteromeric α4β2 and the homomeric α7 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. 49) exerts its central effects by agonist action on nAChRs.

In large part, nAChRs are located presynaptically and act usually to facilitate the release of other transmitters such as glutamate, dopamine and GABA.[3] In some 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 occur in disorders such as schizophrenia,

Table 39.2 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.

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

FUNCTIONAL ASPECTS

The main functions ascribed to cholinergic pathways are related to arousal, reward, 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. 40).

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 the dopaminergic VTA to accumbens 'reward' pathway, nicotine affects neuronal firing at the level of the cell soma in the VTA and modulates dopamine release from terminals in the

nucleus accumbens to modify dopamine release in this reward pathway (see Ch. 49).

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.

The importance of cholinergic neurons in neurodegenerative conditions such as dementia and Parkinson's disease is discussed in Chapter 40. The role of nAChRs in addiction to nicotine is described in Chapter 49 and their role 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; Khakh & North, 2012) as they do in the periphery (Ch. 16). Mapping the pathways is difficult, because purinergic neurons are not easily identifiable histochemically. It is likely that adenosine and ATP serve as neuromodulators.

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. 40) 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

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

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. 40), are associated with abnormalities in cholinergic pathways.
- Both nicotinic and muscarinic (predominantly M₁) 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.

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). 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. 48), which are antagonists at A_2 receptors, produce arousal and alertness.

For ATP there are two forms of receptor – P2X and P2Y receptors (see Ch. 16 also). P2X receptor subunits (P2X1-7) are trimeric ligand-gated cation channels that can be homomeric or heteromeric in composition. The evidence in favour of ATP acting on postsynaptic P2X receptors mediating fast synaptic transmission in the brain remains weak. P2X receptors are located on the postsynaptic cell membrane away from sites of synaptic contact, on nerve terminals and on astrocytes. Like acetylcholine at nicotinic receptors (see p. 475), ATP acting on P2X receptors appears to play a neuromodulatory role. There are eight P2Y receptors,[4] all are G protein coupled (see Table 16.1).

While there is little doubt that purinergic signalling plays a significant 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; Chen et al., 2013).

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 four types of receptor (H_{1-4}; Ch. 17) in the brain H_1–H_3 occur in most brain regions, H_4 has a more restricted distribution. All are G protein coupled – H_1 receptors to G_q, H_2 to G_s and H_3 and H_4 to G_i/G_o. H_3 receptors are inhibitory receptors on histamine-releasing neurons as well as on terminals releasing other neurotransmitters.

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). Antihistamines are widely used to control nausea and vomiting, for example in motion sickness and middle ear disorders, as well as to induce sleep. Recent pharmaceutical industry activity has centred on the development of selective H_3 receptor antagonists as they may have potential for the treatment of cognitive impairment associated with Alzheimer's disease (see Ch. 40), schizophrenia (see Ch. 46), attention deficit hyperactivity disorder (see Ch. 48) and Parkinson's disease (see Ch. 40) as well as for the treatment of narcolepsy, obesity and pain states (Leurs et al., 2011).

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

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[4]Unfortunately the nomenclature for P2Y receptors has developed in a rather haphazard manner. There is compelling evidence for the existence of P2Y$_{1,2,4,6,11,12,13}$ and $_{14}$ receptors but not for others.

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. Melatonin has antioxidant properties and may be neuroprotective in Alzheimer's disease and Parkinson's disease (see Ch. 40).

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 but detailed analysis does not support this view (Buscemi et al., 2006). It may be useful for the treatment of insomnia in the elderly and in autistic children with disturbed sleep. **Ramelteon**, an agonist at MT_1 and MT_2 receptors, is used to treat insomnia (see Ch. 44) and **agomelatine**, which has agonist actions at MT_1 and MT_2 receptors as well as antagonist actions at $5\text{-}HT_{2C}$ receptors, is a novel antidepressant drug (see Ch. 47).

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. 40).

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 itself or through activation of protein kinase G can affect membrane ion channels (Steinert et al., 2010). 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. 38), 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. 40). 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. 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. 19), also take place in the CNS (for review see 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 39.8 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 eicosanoids play important roles in neural function including pain,
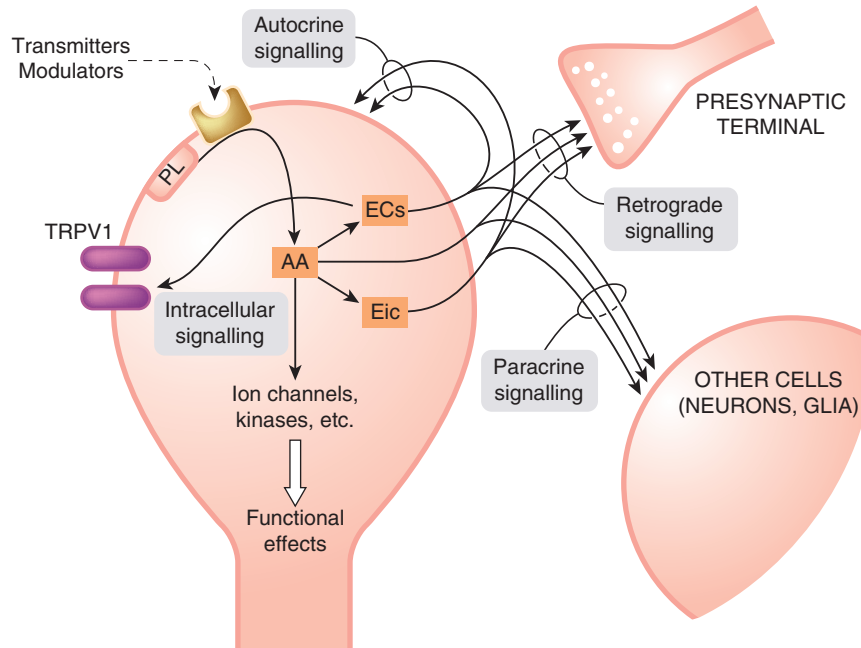
Fig. 39.8 **Postulated modes of signalling by lipid mediators.** Arachidonic acid (AA) is formed by receptor-mediated cleavage of membrane phospholipid. It can act directly as an intracellular messenger on ion channels or components of different kinase cascades, producing various long- and short-term effects. It can also be converted to eicosanoids (prostaglandins, leukotrienes or hydroxyeicosatetraenoic acids [HETEs]) or to the endocannabinoids (ECs), anandamide and 2-arachidonoylglycerol. Endocannabinoids can also act as intracellular messengers to activate TRPV1 channels. HETEs can also act directly as intracellular messengers. All these mediators also 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.

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 and the production of both long-term and short-term depression (see Castillo et al., 2012). 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). Endocannabinoids such as anandamide are metabolised by fatty acid amyl hydrolase (FAAH; see Ch. 19). Inhibitors of FAAH potentiate the effects of endocannabinoids and are effective analgesics in animal models of pain (Roques et al., 2012). 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. 19). One surprise in this field has been the discovery that endocannabinoids, besides being agonists at cannabinoid receptors, also activate TRPV1 channels (see Fig. 39.8 and Ch. 42), 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 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.

Other transmitters and modulators

Purines
- ATP functions as a neurotransmitter, being stored in vesicles and released by exocytosis. It acts via ionotropic P2X receptors and metabotropic P2Y receptors.
- 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.
- 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. 19) and also of the TRPV1 receptor (Ch. 42).

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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 p. 491). 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 new pathophysiological understanding 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.

Looking into the future, the hope is that stem cell therapies will be developed for these disorders. The main topics discussed in this chapter 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).**

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[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 Peden & Ironside, 2012). Misfolding means the adoption of abnormal conformations, by certain normally expressed proteins, such that they tend to form large insoluble aggregates (Fig. 40.1). 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 lack 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. 40.1), 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.

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 40.1.

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
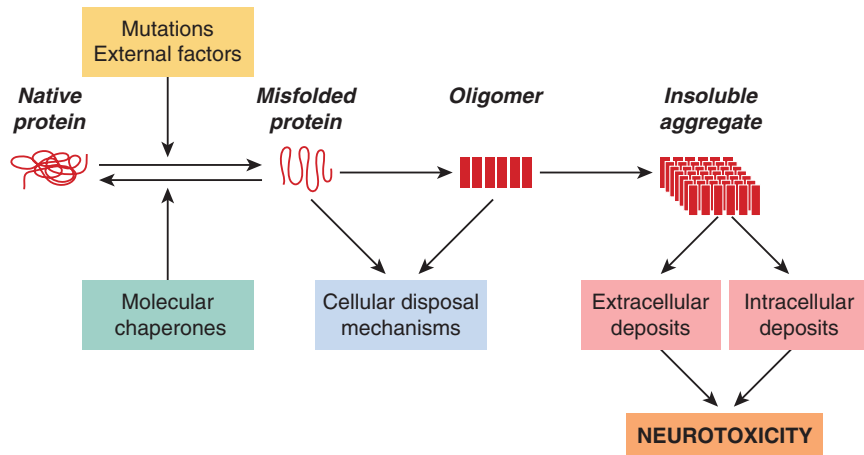
Fig. 40.1 Protein misfolding: a process involved in many chronic neurodegenerative diseases.

Table 40.1 Examples of neurodegenerative diseases associated with protein misfolding and aggregation[a]

Disease	Protein	Characteristic pathology	Notes
Alzheimer's disease	β-Amyloid (Aβ)	Amyloid plaques	Aβ mutations occur in rare familial forms of Alzheimer's disease
	Tau	Neurofibrillary tangles	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.

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.

membrane damage (see p. 484). Necrotic cells typically spill their contents into the surrounding tissue, evoking an inflammatory response. Chronic inflammation is a feature 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

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(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. 38). 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 receptors, and also metabotropic receptors (Ch. 38), 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. 40.2):

- Glutamate activates NMDA, AMPA and metabotropic receptors (sites 1, 2 and 3). Activation of AMPA receptors depolarises the cell, which removes the Mg^{2+} block of NMDA channels (see Ch. 38), permitting Ca^{2+} entry. Depolarisation also opens voltage-dependent calcium channels (site 4). 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 cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, 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 $[Ca^{2+}]_i$ 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 from nerve terminals
  - 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 and inflammatory mediator 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 p. 486), 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.
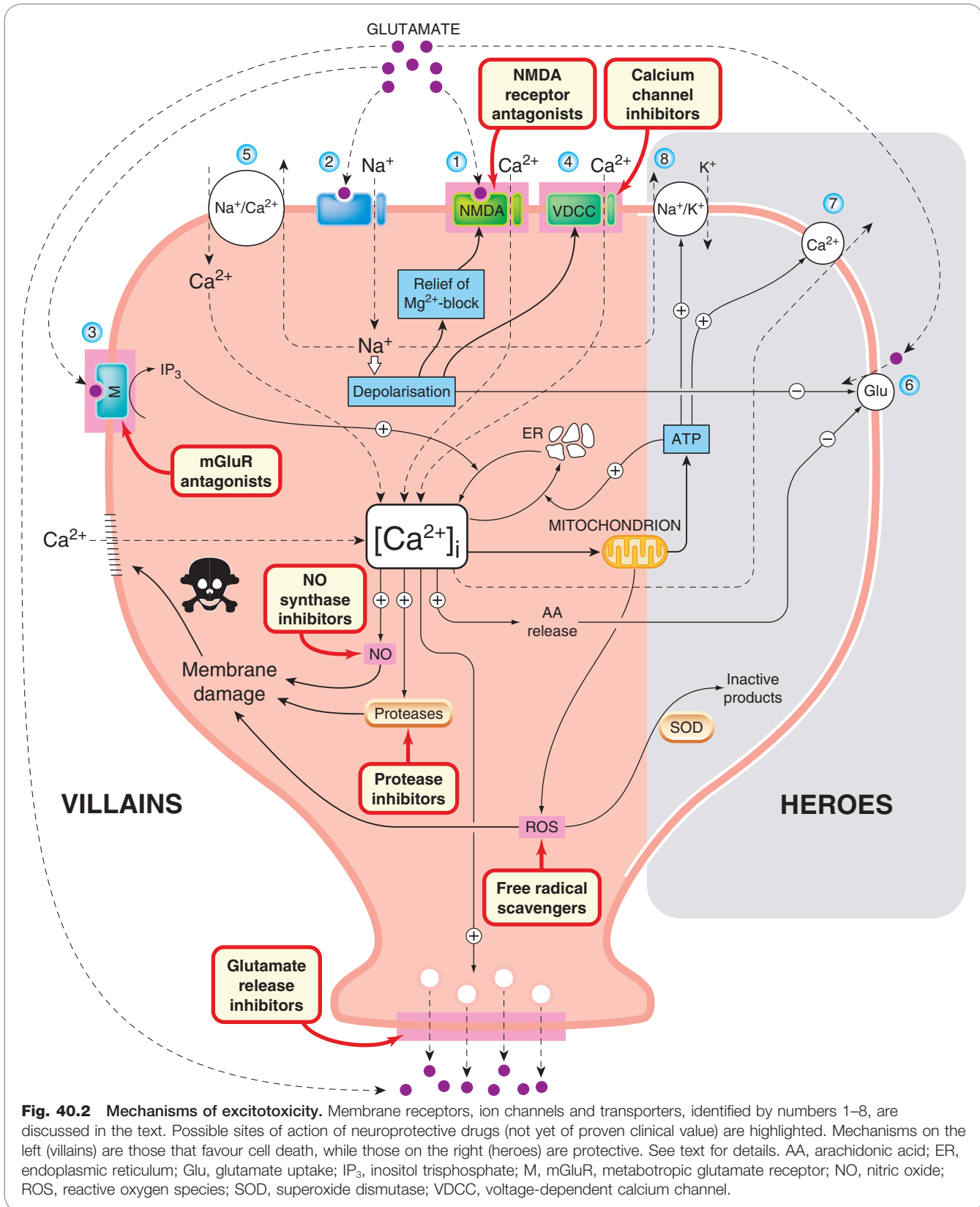
The role of excitotoxicity in ischaemic brain damage is well established (see p. 486), and it is also believed to be a factor in other neurodegenerative diseases, such as those discussed below.

▼ 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** retards to some degree the deterioration of patients with amyotrophic lateral sclerosis. Its precise mechanism of action is unclear. **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.

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

Fig. 40.2 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.

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.

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$_2$O. Under certain conditions, highly reactive oxygen species (ROS), for example oxygen and hydroxyl free radicals and H$_2$O$_2$, 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 p. 484), 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. During periods of tissue reperfusion following ischaemia (e.g. in stroke), delinquent leukocytes may exacerbate this problem by releasing their own cytotoxic oxygen products. 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.18), 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. 40.2) 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 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 Itoh et al., 2013). 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 40.2.

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

> ### Excitotoxicity and oxidative stress
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> - 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.

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 40.2, which lead in turn to later consequences, including cerebral oedema and inflammation, which can also contribute to brain damage. 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, surrounded by a penumbra of compromised tissue in which inflammation

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[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.

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, as both Ca^{2+} entry and cell death following cerebral ischaemia are inhibited by drugs that block NMDA receptors or channels (see Ch. 38). 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 a 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. The use of early surgical procedures to remove clots, in combination with alteplase, is increasing in specialised acute stroke treatment centres.

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 40.2 (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 in these animal models. However, of the many drugs that have been tested in over 100 clinical trials, none was effective. 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**). There is still hope that mGlu1-receptor antagonists or negative allosteric modulators might be effective in the treatment of ischemic brain damage.

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.

One area of promise is the use of subanaesthetic doses of **xenon**, which has NMDA receptor antagonist properties (Ch. 41), in combination with hypothermia to treat hypoxia-induced brain damage in neonates (Esencan et al., 2013).

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.[4]

> ### 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 the 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; haemorrhagic stroke must be excluded by imaging before its administration.

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[5] 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 (see Querfurth & LaFerla, 2010). These advances have raised hopes of more effective treatments, 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

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[4]Eating dark chocolate is believed to reduce the risk of stroke. Flavonoids in the chocolate may be protective due to antioxidant, anti-clotting and anti-inflammatory properties. However, this is not a reason to over indulge!

[5]The term *dementia* is used to describe progressive loss of cognitive function rather than being 'demented', i.e. behaving irrationally due to anger.

deficit and loss of short-term memory that occur in AD. Two microscopic features are characteristic of the disease, namely 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) 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 (e.g. for presenilins and sortilin-related receptor 1) that control amyloid processing, have been discovered. The APP gene resides on chromosome 21, of which an extra copy is the cause of Down's syndrome, in which early AD-like dementia occurs in association with overexpression of APP.

▼ Amyloid deposits consist of aggregates of Aβ (Fig. 40.3), 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. 40.3). 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
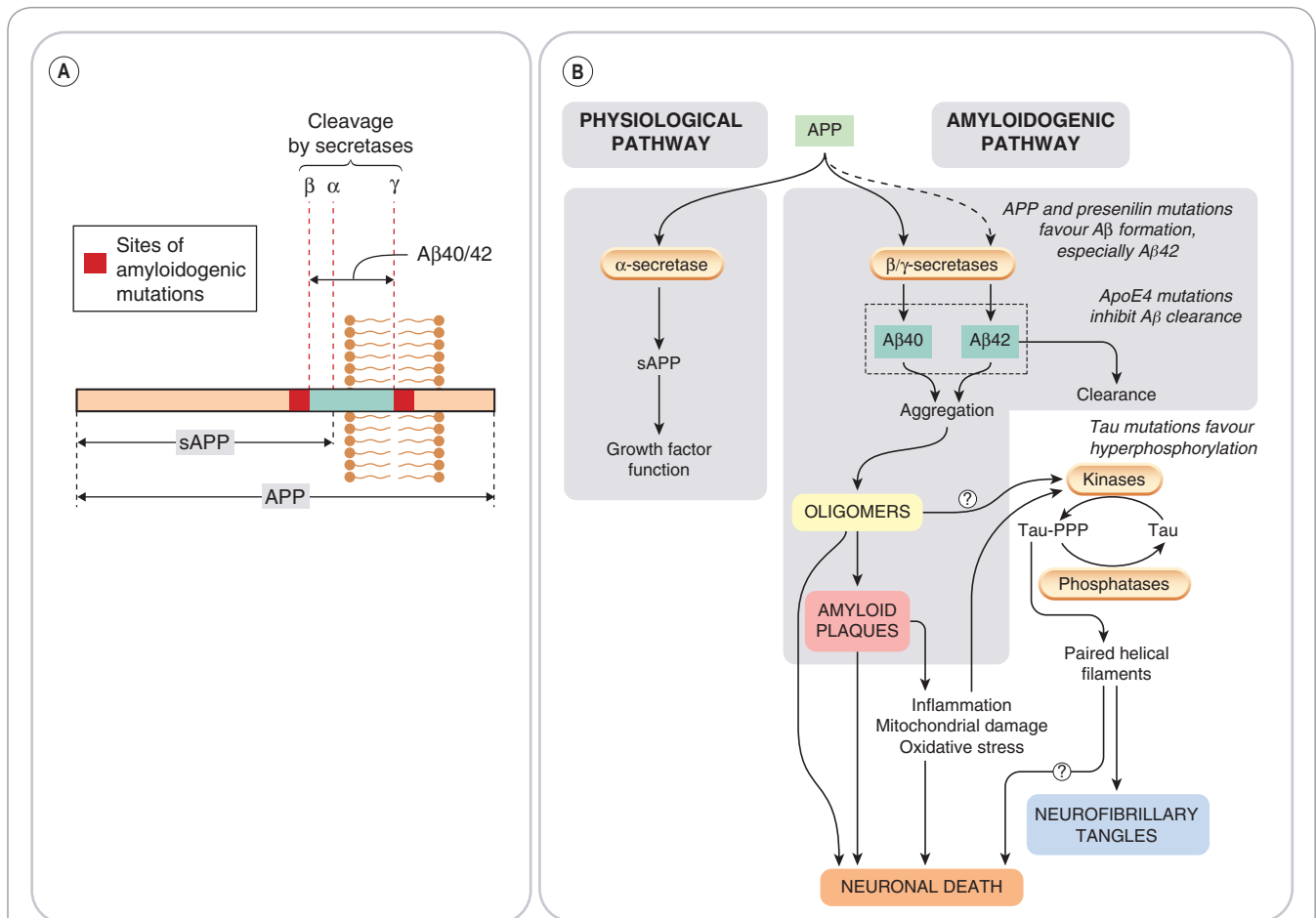

Fig. 40.3 Pathogenesis of Alzheimer's disease. [A] Structure of amyloid precursor protein (APP), showing origin of secreted APP (sAPP) and Aβ amyloid protein. The regions involved in amyloidogenic mutations discovered in some cases of familial Alzheimer's disease are shown flanking the Aβ sequence. APP 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.

transmembrane domain, 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 potentially of great value in testing 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. 40.3). Its 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, including glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5 (CDK5), 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. 39) 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** are the only drugs approved for treating AD. Many

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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.

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other approaches have been explored, based on the amyloid hypothesis as well as other ideas for neuroprotection (see Spencer et al., 2007), so far without success in clinical trials.[6]

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 40.2). 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

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[6]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 40.2   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.

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 inhibitors and a variety of muscarinic and nicotinic receptor agonists. To date the lack of selectivity of muscarinic orthosteric agonists has hindered their use to treat CNS disorders due to the incidence of side effects, but the hope is that positive allosteric modulators (see Ch. 3) that are selective (e.g. for the M_1 receptor) will be developed.

MEMANTINE

The other drug currently approved for the treatment of AD is **memantine**, an orally active weak antagonist at NMDA 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 may work by selectively inhibiting excessive, pathological NMDA receptor activation while preserving more physiological activation. It has a long plasma half-life, and its adverse effects include headache, dizziness, drowsiness, constipation, shortness of breath and hypertension as well as a raft of less common problems. The potential for other drugs acting as agonists or allosteric modulators at NMDA receptors to enhance cognition is discussed by Collingridge et al. (2013).

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. Although several well-characterised targets were identified, such as Aβ formation by the β- and γ-secretases, and

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. 38).
- Used in moderate to severe Alzheimer's disease.

Aβ neurotoxicity, together with a range of transgenic animal models of AD on which compounds can be tested, subsequent clinical trials of drugs targeting these processes have been disappointing (Corbett et al., 2012). Inhibitors of β- and γ-secretase were identified. Though they are effective in reducing Aβ formation, they appear to make cognition impairment worse. Several proved toxic to the immune system and gastrointestinal tract, and development has been halted. Kinase inhibitors aimed at preventing Tau phosphorylation were also investigated (see Brunden et al., 2009). The large number of

phosphorylation sites and different kinases make this a difficult approach.

An ingenious 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. More recent clinical trials with monoclonal Aβ antibodies have been disappointing but it is possible that the antibody treatments were given too late in the progression of the disease and that earlier intervention might reveal therapeutic benefit.

Epidemiological studies suggested that some non-steroidal anti-inflammatory drugs (NSAIDs; see Ch. 26) used routinely to treat arthritis reduced the likelihood of developing AD. This idea has been supported by numerous animal studies in which genetic mouse models lacking specific prostaglandin receptor subtypes have proved resistant to experimental models of neurodegenerative disease. Unfortunately, clinical trials with various NSAIDs have so far failed to show evidence of consistent benefit (Breitner et al., 2011). Indeed, NSAIDs can have adverse effects in later stages of AD, but in asymptomatic individuals **naproxen** can reduce the long-term incidence of AD.

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.

Other approaches. These include the development of new drugs as well as the use of established drugs already in use to treat other unrelated conditions (see Corbett et al., 2012).

New potent and selective histamine H_3 antagonists may improve cognition in AD (see Brioni et al., 2011). They also increase wakefulness and may be used to treat narcolepsy (see Ch. 48).

Levetiracetam, an anticonvulsant with a novel mechanism of action, may slow the development of AD.

Longitudinal cohort studies suggest that antihypertensive therapy may be correlated with a reduction in the incidence of AD (see Corbett et al., 2012). The reasons for this are unclear but could relate to a reduction in inflammatory processes in the brain.

Caprylidene (caprylic triglyceride) is formulated from coconut oil.[7] It is broken down in the body to release ketones which provide an alternative energy source to glucose. There is some evidence that in AD glucose utilisation is impaired. It may be useful in mild to moderate AD to improve memory and cognitive function but it does not reverse neuronal degeneration.

Latrepirdine is in clinical trials for the treatment of AD. It has a complex pharmacology and which of its actions are responsible for any therapeutic benefit still needs to be determined.

Observational studies suggested that statins might prevent dementia, but this has not been prospectively confirmed in clinical trials.

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 (*bradykinesia*), 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, because the degenerative process is not confined to the basal ganglia but also affects other parts of the brain. Non-motor symptoms may appear before motor symptoms and often predominate in the later stages of the disease.
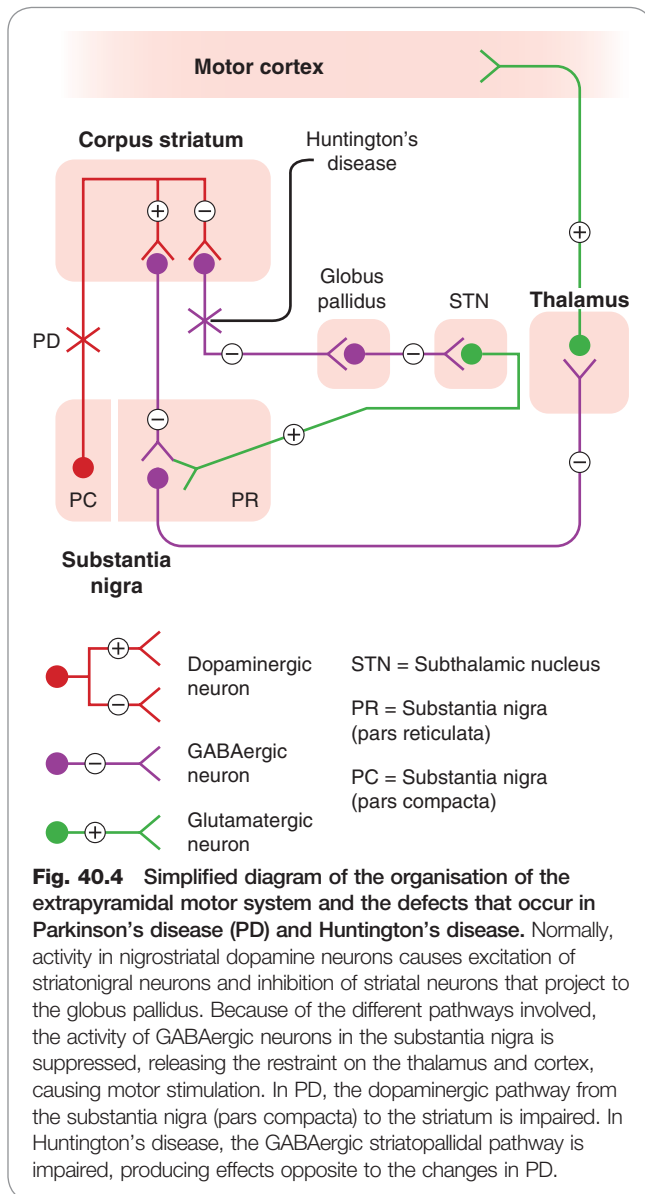
Parkinson's disease often occurs with no obvious underlying cause, but it may be the result of cerebral ischaemia, viral encephalitis or other types of pathological damage. The symptoms can also be drug-induced, the main drugs involved being those that reduce the amount of dopamine in the brain (e.g. **reserpine**; see Ch. 14) or block dopamine receptors (e.g. antipsychotic drugs such as **chlorpromazine**; see Ch. 46). There are rare instances of familial early-onset PD, and several gene mutations have been identified, including those encoding *synuclein* and *parkin* (see p. 492). Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) have also been associated with PD. Study of gene mutations has given some clues about the mechanism underlying the neurodegenerative process.

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. 39) in postmortem 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.[8] Neurons containing other monoamines such as noradrenaline and 5-hydroxytryptamine are also affected. 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 *bradykinesia*, 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

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[7]It is sometimes referred to as a 'medical food'.

[8]It is emerging that other types of neuron are also affected. Here we concentrate on the dopaminergic nigrostriatal pathway as it is the most important in relation to current therapies.

Fig. 40.4 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. 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.

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. 39), 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 40.4.

Cholinergic interneurons of the corpus striatum (not shown in Fig. 40.4) 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

As with other neurodegenerative disorders, the neuronal damage in PD is caused by protein misfolding and

aggregation, aided and abetted by other familiar villains, namely excitotoxicity, mitochondrial dysfunction, oxidative stress, inflammation and apoptosis. Aspects of the pathogenesis and animal models of PD are described by Duty & Jenner (2011).

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 the illegal preparation of 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 that is located in glial cells; see Chs 14 and 47). MPP^+ is then 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. It is also less effective in rats than in primates, yet mice show some susceptibility. **Selegiline**, a selective MAO-B inhibitor, prevents MPTP-induced neurotoxicity by blocking its conversion to MPP^+. Selegiline is also used in treating PD (see p. 495); 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. Recent evidence suggests that α-synuclein may act as a prion-like protein (see p. 496) and that PD is in fact a prion-like disease (Poewe et al., 2012). α-Synuclein normally exists in an α-helical conformation. However, under certain circumstances, such as genetic duplication or triplication or genetic mutation it can undergo a conformational change to a β-sheet-rich structure that polymerises to form toxic aggregates and amyloid plaques. Mutations occur in rare types of hereditary PD (see p. 493). It is believed that misfolding and aggregation renders the protein resistant to degradation within cells, causing it to pile up in Lewy bodies. In parkinsonian patients who received fetal dopaminergic neuron grafts (see p. 495) over time the grafted neurons developed Lewy bodies. Misfolded α-synuclein is thought to have migrated from the native tissue to the grafted tissue.

It is possible (see Lotharius & Brundin, 2002) that the normal function of α-synuclein is related to synaptic

vesicle recycling, and that the misfolded 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 overexpression, 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 certin 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 degeneration of dopaminergic nigrostriatal neurons that gives rise to the motor symptoms, as well as more general neurodegeneration resulting in dementia and depression.
- 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. 40.5) are:

- **levodopa** (often in combination with **carbidopa** and **entacapone**)
- dopamine agonists (e.g. **pramipexole**, **ropinirole**, **bromocriptine**)
- monoamine oxidase-B (MAO-B) inhibitors (e.g. **selegiline**, **rasagiline**)
- muscarinic ACh receptor antagonists (e.g. **orphenadrine, procyclidine** and **trihexyphenidyl**) are occasionally used.

None of the drugs used to treat PD affects the progression of the disease. For general reviews of current and future approaches, see Schapira (2009) and Poewe et al. (2012).

LEVODOPA

Levodopa is the first-line treatment for PD and is combined with a peripherally acting dopa decarboxylase inhibitor, such as **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). Oral and subcutaneous slow release preparations have been developed. 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 inhibitor. 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 p. 494) 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 a dopa decarboxylase inhibitor with a catechol-*O*-methyl transferase (COMT) inhibitor (e.g. **entacapone** or **tolcapone**, 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 bradykinesia, and about 20% are restored virtually to normal motor function. As time progresses, the effectiveness of levodopa gradually declines (Fig. 40.6). In a typical study of 100 patients treated 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. 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 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.

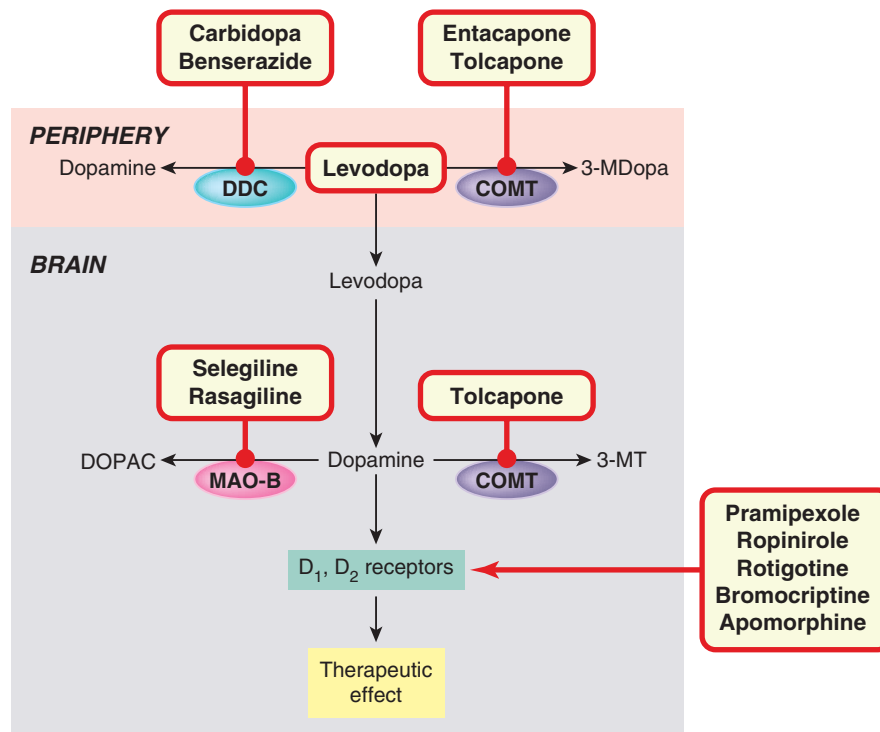2. Rapid fluctuations in clinical state, where bradykinesia and rigidity may suddenly worsen for

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Fig. 40.5 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.

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**, 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. 46) with delusions and hallucinations. More commonly, in about 20% of patients, it causes confusion, disorientation, insomnia or nightmares.

DOPAMINE AGONISTS

Bromocriptine, **pergolide** and **cabergoline** exhibit slight selectivity for $D_{2/3}$ over D_1 receptors (see Ch. 39). Bromocriptine, which inhibits the release of prolactin from the anterior pituitary gland, was first introduced for the treatment of galactorrhoea and gynaecomastia (Ch. 33). Though effective in controlling the symptoms of PD, their usefulness is limited by side effects, such as nausea and vomiting, and somnolence and a risk of fibrotic reactions in the lungs, retroperitoneum and pericardium. These disadvantages have led to the replacement of these drugs by **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,[9]

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[9]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.
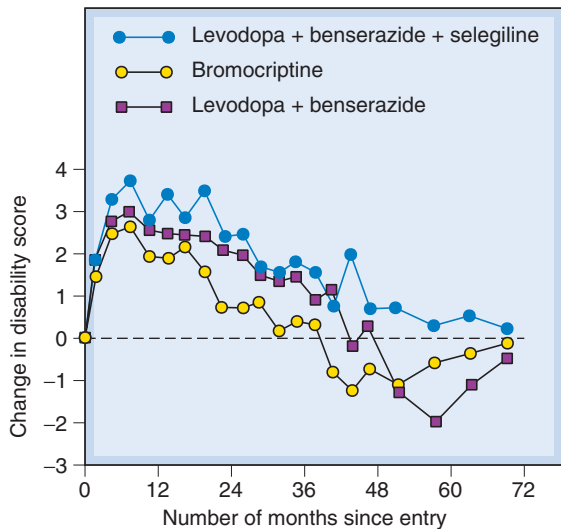
Fig. 40.6 Comparison of levodopa/benserazide, levodopa/benserazide/selegiline and bromocriptine on progression of Parkinson's disease symptoms. Patients (249–271 in each treatment group) were assessed on a standard disability rating score. Before treatment, the average rate of decline was 0.7 units/year. 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.)*

over-eating and sexual excess, related to the 'reward' functions of dopamine (see Ch. 49).

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[10] inhibitor, which lacks the unwanted peripheral effects of non-selective MAO inhibitors used to treat depression (Ch. 47) 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. Recogni-

tion of the role of MAO-B in neurotoxicity (see p. 492) 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. 40.6) 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 may somewhat retard disease progression, as well alleviating symptoms (Olanow et al., 2009). **Safinamide**, undergoing clinical trials, is a new drug that inhibits both MAO-B and dopamine reuptake.

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. More recently block of NMDA receptors by stabilising closed states of the channel has been described and this may be a novel target for antiparkinsonian drugs.

Amantadine is less effective than levodopa or bromocriptine in treating PD is but it is effective in reducing the dyskinesias induced by prolonged levodopa treatment (see p. 493).

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 levodopa; see Ch. 46).

NEW PHARMACOLOGICAL APPROACHES

▼ Potential new treatments for PD include adenosine A_{2A} receptor antagonists (e.g. **istradefylline** and **preladenant**), 5-HT$_{1A}$ antagonists (e.g. **sarizotan**) and glutamate receptor antagonists or negative allosteric modulators (acting at mGluR5, AMPA or NMDA receptors) as well as new, improved COMT inhibitors. For further information see Poewe et al. (2012).

NEURAL TRANSPLANTATION, GENE THERAPY 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 (Barker et al., 2013) have mainly involved injection of midbrain cells from aborted human fetuses. Although such transplants have been shown to survive and establish functional dopaminergic connections this approach has fallen out of favour recently. Some patients have gone on to develop serious dyskinesias, possibly due to dopamine overproduction. 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; Nishimura & Takahashi, 2013).

Gene therapy (see Ch. 59) for PD is aimed at increasing the synthesis of neurotransmitters and neurotrophic factors such as:

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[10]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).

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 affect 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)
  – **orphenadrine** (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.

- dopamine in the striatum – by expressing tyrosine hydroxylase or dopa decarboxylase
- GABA in the subthalamic nucleus – by overexpression of glutamic acid decarboxylase (to reduce the excitatory input to the substantia nigra (see Fig. 40.4)
- neurotrophic factors such as neurturin, a glial-derived neurotrophic factor (GDNF) analogue.

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 and does not stop the neurodegenerative process (see Okun, 2012).

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 choreiform (i.e. rapid, jerky involuntary) movements, especially of fingers, face or tongue. 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 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. 38). 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. 40.4).

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. 46) and the GABA agonist **baclofen** (Ch. 38). Other drug treatments include antidepressants, mood stabilisers (see Ch. 47) and benzodiazepines (see Ch. 44) to reduce the depression, mood swings and anxiety associated with the disorder. None of these drugs affects dementia or retards the course of the disease. It is possible that drugs that inhibit excitotoxicity, antisense to reduce mutant huntingtin expression, or possibly neural transplantation procedures when these become available, 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 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–Jakob 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 p. 482) 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. There was hope that **quinacrine** (an antimalarial drug), **chlorpromazine** or **pentosan polyphosphate** might inhibit disease progression, but clinical trials have proven negative. Interest has now turned to anti-prion antibodies and these are being investigated. Opioid drugs (see Ch. 42) are used to relieve pain, while clonazepam and sodium valproate (see Ch. 45) may help to relieve involuntary muscle jerks.

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