11 Individual variation, pharmacogenomics and personalised medicine

OVERVIEW

This chapter addresses sources of variation between individuals (inter-individual variation) in their responses to drugs. Important factors including ethnicity, age, pregnancy, disease and drug interaction (i.e. modification of the action of one drug by another) are described. The concept of individualising drug therapy in light of genomic information ('personalised medicine') – a rapidly developing area of clinical pharmacology – is introduced. We explain relevant elementary genetic concepts and describe briefly several single-gene pharmacogenetic disorders that affect drug responses. We then cover pharmacogenomic tests, including tests for variations in human leukocyte antigen (HLA) genes, in genes influencing drug metabolism, and encoding drug targets.

INTRODUCTION

Therapeutics would be a great deal easier if the same dose of drug always produced the same response. In reality, inter- and even intra-individual variation is often substantial. Physicians need to be aware of the sources of such variation to prescribe drugs safely and effectively. Variation can be caused by different concentrations at sites of drug action or by different responses to the same drug concentration. The first kind is called pharmacokinetic variation and can occur because of differences in absorption, distribution, metabolism or excretion (Chs 8 and 9). The second kind is called pharmacodynamic variation. Responses to some therapeutic agents, for example most vaccines and oral contraceptives (Ch. 35), are sufficiently predictable to permit a standard dose regimen, whereas treatment with **lithium** (Ch. 47), antihypertensive drugs (Ch. 22), anticoagulants (Ch. 24) and many other drugs is individualised, doses being adjusted on the basis of monitoring the drug concentration in the plasma or a response such as change in blood pressure, together with any adverse effects.

Inter-individual variation in response to some drugs is a serious problem; if not taken into account, it can result in lack of efficacy or unexpected adverse effects. Variation is partly caused by environmental factors but studies comparing identical with non-identical twins suggest that much of the variation in response to some drugs is genetically determined; for example, elimination half-lives of antipyrine, a probe of hepatic drug oxidation, and of **warfarin**, an oral anticoagulant (Ch. 24), differ much less between identical than between fraternal twins. However, even for drugs with a known genetic component such as **warfarin** (see p. 141 and Ch. 24) addition of pharmacogenetic information to a dosing algorithm incorporating other clinical sources of variation (age, sex and so on) does not improve outcome significantly, although when compared with a standardised (i.e. trial and error) loading dose strategy a genetically guided dose-initiation strategy does result in a greater fraction of time in the therapeutic range during the first weeks of treatment (see Zineh et al., 2013 for a discussion of recent randomised controlled trials of pharmacogenetics and warfarin dosing).

Genes influence pharmacokinetics by altering the expression of proteins involved in drug absorption, distribution, metabolism or excretion (ADME); pharmacodynamic variation reflects differences in drug targets, G proteins or other downstream pathways; and individual susceptibility to uncommon qualitatively distinct adverse reactions (Ch. 57) can result from genetically determined differences in enzymes or immune mechanisms. It is hoped that as our understanding of the human genome improves, together with the introduction of simpler methods to identify genetic differences between individuals, it will become possible to use genetic information specific to an individual patient to preselect a drug that will be effective and not cause toxicity, rather than relying on trial and error supported by physiological clues as at present – an aspiration referred to as '*personalised medicine*'. Thus far, this approach, which was initially overhyped, has yielded relatively little in the way of clinical benefit. Progress is being made, however, and the US Food and Drug Administration (FDA) has approved over 100 pharmacogenomic additions to drug labelling information – a doubling since the last edition of this book. The use of pharmacogenomic tests is not consistently supported by evidence of improved outcomes from clinical trials (Zineh et al., 2013) and indeed the FDA approach to pharmacogenetic labelling has been criticised (Shah & Shah, 2012). Nevertheless, pharmacogenetic testing seems likely ultimately to make an important contribution to therapeutics, though at a cost.

In this chapter we first describe the most important epidemiological sources of variation in drug responsiveness, before revisiting some elementary genetics as a basis for understanding genetic disorders characterised by abnormal responses to drugs, and conclude with a brief account of currently available pharmacogenomic tests and how these are beginning to be applied to individualise drug therapy (*pharmacogenomics*).

Variation is usually quantitative in the sense that the drug produces a larger or smaller effect, or acts for a longer or shorter time, while still exerting qualitatively the same effect. But importantly, the effect may be qualitatively different in susceptible individuals, often because of genetic or immunological differences. Examples include **primaquine**-induced haemolysis in individuals with glucose 6-phosphate dehydrogenase deficiency whose red blood cells are thereby more susceptible to the effect of oxidative stress (Ch. 57) or immune-mediated haemolytic anaemia caused by **methyldopa** – a

drug that commonly causes antidrug antibodies whereas only a few individuals expressing such antibodies develop haemolysis (Ch. 14).

Individual variation

- Variability is a serious problem; if not taken into account, it can result in:
  - lack of efficacy
  - unexpected harmful effects.
- Types of variability may be classified as:
  - pharmacokinetic
  - pharmacodynamic.
- The main causes of variability are:
  - age
  - genetic factors
  - immunological factors (Ch. 57)
  - disease (especially when this influences drug elimination or metabolism, e.g. kidney or liver disease)
  - drug interactions.

EPIDEMIOLOGICAL FACTORS AND INTER-INDIVIDUAL VARIATION OF DRUG RESPONSE

ETHNICITY

Ethnic means 'pertaining to race', and many anthropologists are sceptical as to the value of this concept (see, for example, Cooper et al., 2003). Citizens of several modern societies are asked to select their race or ethnicity from a list of options for census purposes (e.g. the UK 2011 National Census). Members of such self-defined groups share some characteristics on the basis of common genetic and cultural heritage, but there is obviously also enormous diversity within each group.

Despite the crudeness of such categorisation, it can give some pointers to drug responsiveness (Wood, 2001). One example is the evidence discussed in Chapter 22 that the life expectancy of African-Americans with heart failure is increased by treatment with a combination of **hydralazine** plus a nitrate, whereas that of white Americans may not be.

Some adverse effects may also be predicted on the basis of race; for example, many Chinese subjects differ from Europeans in the way that they metabolise ethanol, producing a higher plasma concentration of acetaldehyde, which can cause flushing and palpitations (Ch. 49). Chinese subjects are considerably more sensitive to the cardiovascular effects of **propranolol** (Ch. 14) than white Europeans, whereas Afro-Caribbean individuals are less sensitive. Despite their increased sensitivity to β-adrenoceptor antagonists, Chinese subjects metabolise propranolol faster than white people, implying that the difference relates to pharmacodynamic differences at or beyond the β adrenoceptors.

Overall effectiveness of **gefitinib** (Ch. 56) in treating patients with advanced lung tumours has been disappointing, but in about 10% of patients lung tumours shrink rapidly in response to this drug. Japanese patients are three times as likely as whites to respond in this way. The underlying difference is that patients who respond well have specific mutations in the receptor for epidermal growth factor (see Wadman, 2005). It is probable that many such ethnic differences are genetic in origin, but environmental factors, for example relating to distinctive dietary habits, may also contribute. It is important not to abandon the much more sophisticated search for ways to individualise medicine on the basis of pharmacogenomics (see p. 139-141) just because the much simpler and cheaper process of asking patients to define their ethnic group has had some success: this should rather act as a spur. If such a crude and imperfect approach has had some success, we ought surely to be able to do better with genomic testing!

AGE

The main reason that age affects drug action is that drug elimination is less efficient in newborn babies and in old people, so that drugs commonly produce greater and more prolonged effects at the extremes of life. Other age-related factors, such as variations in pharmacodynamic sensitivity, are also important with some drugs. Body composition changes with age, fat contributing a greater proportion to body mass in the elderly, with consequent changes in distribution volume of drugs. Elderly people consume more drugs than do younger adults, so the potential for drug interactions is also increased. For fuller accounts of drug therapy in paediatrics and in the elderly, see the chapters on renal and hepatic disease in Atkinson et al. (2006).

EFFECT OF AGE ON RENAL EXCRETION OF DRUGS

Glomerular filtration rate (GFR) in the newborn, normalised to body surface area, is only about 20% of the adult value. Accordingly, plasma elimination half-lives of renally eliminated drugs are longer in neonates than in adults (Table 11.1). In babies born at term, renal function increases to values similar to those in young adults in less than a week, and continues to increase to a maximum of approximately twice the adult value at 6 months of age. Improvement in renal function occurs more slowly in premature infants. Renal immaturity in premature infants can have a substantial effect on drug elimination. For example, in premature newborn babies, the antibiotic **gentamicin** (see Ch. 51) has a plasma half-life of ≥ 18 h, compared with 1–4 h for adults and approximately 10 h for babies born at term. It is therefore necessary to reduce and/or space out doses to avoid toxicity in premature babies.

Glomerular filtration rate declines slowly from about 20 years of age, falling by about 25% at 50 years and by 50% at 75 years. Figure 11.1 shows that the renal clearance of **digoxin** in young and old subjects is closely correlated with creatinine clearance, a measure of GFR. Consequently, chronic administration over the years of the same daily dose of digoxin to an individual as he or she ages leads to a progressive increase in plasma concentration, and this is a common cause of glycoside toxicity in elderly people (see Ch. 21).

▼ The age-related decline in GFR is not reflected by an increase in plasma creatinine concentration, as distinct from creatinine clearance. Plasma creatinine typically remains within the normal adult range in elderly persons despite substantially diminished GFR. This is because creatinine synthesis is reduced in elderly persons because of their reduced muscle mass. Consequently, a 'normal' plasma creatinine in an elderly person does not indicate that they have a normal GFR. Failure to recognise this and reduce the dose of drugs that are eliminated by renal excretion can lead to drug toxicity.

Table 11.1 Effect of age on plasma elimination half-lives of various drugs

Drug	Mean or range of half-life (h)		
	Term neonate[a]	*Adult*	*Elderly person*
Drugs that are mainly excreted unchanged in the urine			
Gentamicin	10	2	4
Lithium	120	24	48
Digoxin	200	40	80
Drugs that are mainly metabolised			
Diazepam	25–100	15–25	50–150
Phenytoin	10–30	10–30	10–30
Sulfamethoxypyridazine	140	60	100

[a]Even greater differences from mean adult values occur in premature babies.

Data from Reidenberg MM 1971 Renal Function and Drug Action. Saunders, Philadelphia; and Dollery CT 1991 Therapeutic Drugs. Churchill Livingstone, Edinburgh.

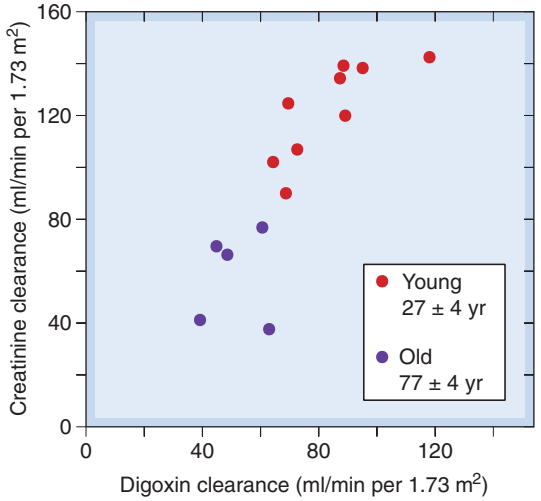

Fig. 11.1 Relationship between renal function (measured as creatinine clearance) and digoxin clearance in young and old subjects. *(From Ewy GA et al. 1969 Circulation 34, 452.)*

EFFECT OF AGE ON DRUG METABOLISM

Several important enzymes, including hepatic microsomal oxidase, glucuronyltransferase, acetyltransferase and plasma esterases, have low activity in neonates, especially if premature. These enzymes take 8 weeks or longer to reach the adult level of activity. The relative lack of conjugating activity in the newborn can have serious consequences, as in *kernicterus* caused by drug displacement of bilirubin from its binding sites on albumin (Ch. 8) and in the 'grey baby' syndrome caused by the antibiotic **chloramphenicol** (see Ch. 51). This sometimes fatal condition, at first thought to be a specific biochemical sensitivity to the drug in young babies, actually results simply from

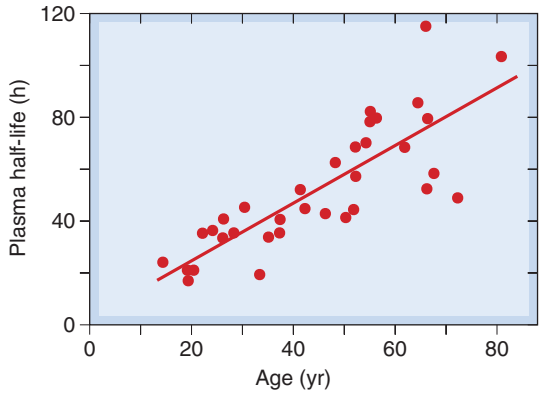

Fig. 11.2 Increasing plasma half-life for diazepam with age in 33 normal subjects. Note the increased variability as well as increased half-life with ageing. *(From Klotz U et al. 1975 J Clin Invest 55, 347.)*

accumulation of very high tissue concentrations of chloramphenicol because of slow hepatic conjugation. Chloramphenicol is no more toxic to babies than to adults provided the dose is reduced to make allowance for this. Slow conjugation is also one reason why **morphine** (which is excreted mainly as the glucuronide, see Ch. 42) is not used as an analgesic in labour, because drug transferred via the placenta has a long half-life in the newborn baby and can cause prolonged respiratory depression.

The activity of hepatic microsomal enzymes declines slowly (and very variably) with age, and the distribution volume of lipid-soluble drugs increases, because the proportion of the body that is fat increases with advancing age. The increasing half-life of the anxiolytic drug **diazepam** with advancing age (Fig. 11.2) is one consequence of this. Some other benzodiazepines and their active metabolites show even greater age-related increases in half-life. Because half-life determines the time course of drug accumulation during repeated dosing (Ch. 10), insidious effects, developing over days or weeks, can occur in elderly people and may be misattributed to age-related memory impairment rather than to drug accumulation. Even if the mean half-life of a drug is little affected, there is often a striking increase in the *variability* of half-life between individuals with increasing age (as in Fig. 11.2). This is important, because a population of old people will contain some individuals with grossly reduced rates of drug metabolism, whereas such extremes do not occur so commonly in young adult populations. Drug regulatory authorities therefore usually require studies in elderly persons as part of the evaluation of drugs likely to be used in older people.

AGE-RELATED VARIATION IN SENSITIVITY TO DRUGS

The same plasma concentration of a drug can cause different effects in young and old subjects. Benzodiazepines (Ch. 44) exemplify this, producing more confusion and less sedation in elderly than in young subjects; similarly, hypotensive drugs (Ch. 22) cause postural hypotension more commonly in elderly than in younger adult patients.

PREGNANCY

Pregnancy causes physiological changes that influence drug disposition (Ch. 8) in mother and fetus. Maternal

plasma albumin concentration is reduced, influencing drug protein binding. Cardiac output is increased, leading to increased renal blood flow and GFR, and increased renal elimination of drugs. Lipophilic molecules rapidly traverse the placental barrier, whereas transfer of hydrophobic drugs is slow, limiting fetal drug exposure following a single maternal dose. The placental barrier excludes some drugs (e.g. low-molecular-weight heparins; Ch. 24) so effectively that they can be administered chronically to the mother without causing effects in the fetus. However, drugs that are transferred to the fetus are eliminated more slowly than from the mother. The activity of most drug-metabolising enzymes in fetal liver is much less than in the adult. Furthermore, the fetal kidney is not an efficient route of elimination because excreted drug enters the amniotic fluid, which is swallowed by the fetus. For a fuller account see Atkinson et al. (2006).

DISEASE

Therapeutic drugs are prescribed to patients, so effects of disease on drug response are very important, especially disease of the major organs responsible for drug metabolism and drug (and drug metabolite) excretion. Detailed consideration is beyond the scope of this book, and interested readers should refer to a clinical text such as the chapters on renal and hepatic disease in Atkinson et al. (2006). Disease can cause pharmacokinetic or pharmacodynamic variation. Common disorders such as impaired renal or hepatic function predispose to toxicity by causing unexpectedly intense or prolonged drug effects as a result of increased drug concentration following a 'standard' dose. Drug absorption is slowed in conditions causing gastric stasis (e.g. *migraine*, *diabetic neuropathy*) and may be incomplete in patients with malabsorption owing to ileal or pancreatic disease or to oedema of the ileal mucosa caused by heart failure or nephrotic syndrome. *Nephrotic syndrome* (characterised by heavy proteinuria, oedema and a reduced concentration of albumin in plasma) alters drug absorption because of oedema of intestinal mucosa; alters drug disposition through changes in binding to plasma albumin; and causes insensitivity to diuretics such as **furosemide** that act on ion transport mechanisms in the luminal surface of tubular epithelium (Ch. 29), through drug binding to albumin in tubular fluid. *Hypothyroidism* is associated with increased sensitivity to several drugs (e.g. **pethidine**), for reasons that are poorly understood. *Hypothermia* (to which elderly persons, in particular, are predisposed) markedly reduces the clearance of many drugs.

Other disorders affect drug sensitivity by altering receptor or signal-transduction mechanisms (see Ch. 3). Examples include the following:

- Diseases that influence receptors:
  - *myasthenia gravis*, an autoimmune disease characterised by antibodies to nicotinic acetylcholine receptors (Ch. 13) and increased sensitivity to neuromuscular blocking agents (e.g. **vecuronium**) and other drugs that may influence neuromuscular transmission (e.g. *aminoglycoside antibiotics*, Ch. 51)
  - *X-linked nephrogenic diabetes insipidus*, characterised by abnormal antidiuretic hormone (ADH, vasopressin) receptors (Ch. 29) and insensitivity to ADH
  - *familial hypercholesterolaemia*, an inherited disease of low-density-lipoprotein receptors (Ch. 23); the homozygous form is relatively resistant to treatment with statins (which work mainly by increasing expression of these receptors), whereas the much commoner heterozygous form responds well to statins.
- Diseases that influence signal transduction mechanisms:
  - *pseudohypoparathyroidism*, which stems from impaired coupling of receptors with adenylyl cyclase
  - *familial precocious puberty* and *hyperthyroidism* caused by functioning thyroid adenomas, which are each caused by mutations in G protein-coupled receptors that result in the receptors remaining 'turned on' even in the absence of the hormones that are their natural agonists.

DRUG INTERACTIONS

Many patients, especially elderly ones, are treated continuously with one or more drugs for chronic diseases such as hypertension, heart failure, osteoarthritis and so on. Acute events (e.g. infections, myocardial infarction) are treated with additional drugs. The potential for drug interactions is therefore substantial, and drug interactions account for 5–20% of adverse drug reactions. These may be serious (approximately 30% of fatal adverse drug reactions are estimated to be the consequence of drug interaction). Drugs can also interact with chemical entities in other dietary constituents (e.g. grapefruit juice, which down-regulates expression of CYP3A4 in the gut) and herbal remedies (such as St John's wort; Ch. 47). The administration of one chemical entity (A) can alter the action of another (B) by one of two general mechanisms:[1]

1. Modifying the pharmacological effect of B without altering its concentration in the tissue fluid (pharmacodynamic interaction).
2. Altering the concentration of B at its site of action (pharmacokinetic interaction), as described in Chs 8 and 9.

PHARMACODYNAMIC INTERACTION

Pharmacodynamic interaction can occur in many different ways (including those discussed under *Drug antagonism* in Ch. 2). There are many mechanisms, and some examples of practical importance are probably more useful than attempts at classification.

- β-Adrenoceptor antagonists diminish the effectiveness of β-adrenoceptor agonists such as **salbutamol** (Ch. 14).
- Many diuretics lower plasma K^+ concentration (see Ch. 29), and thereby predispose to **digoxin** toxicity and to toxicity with *type III antidysrhythmic drugs* (Ch. 21).
- **Sildenafil** inhibits the isoform of phosphodiesterase (type V) that inactivates cGMP (Chs 20 and 35); consequently, it potentiates organic nitrates, which activate guanylyl cyclase, and can cause severe hypotension in patients taking these drugs.

[1]A third category of pharmaceutical interactions should be mentioned, in which drugs interact *in vitro* so that one or both are inactivated. No pharmacological principles are involved, just chemistry. An example is the formation of a complex between **thiopental** and **suxamethonium**, which must not be mixed in the same syringe. **Heparin** is highly charged and interacts in this way with many basic drugs; it is sometimes used to keep intravenous lines or cannulae open and can inactivate basic drugs if they are injected without first clearing the line with saline.

- *Monoamine oxidase inhibitors* increase the amount of noradrenaline stored in noradrenergic nerve terminals and interact dangerously with drugs, such as **ephedrine** or **tyramine**, that release stored noradrenaline. This can also occur with tyramine-rich foods – particularly fermented cheeses such as Camembert (see Ch. 47).
- **Warfarin** competes with vitamin K, preventing hepatic synthesis of various coagulation factors (see Ch. 24). If vitamin K production in the intestine is inhibited (e.g. by antibiotics), the anticoagulant action of warfarin is increased.
- The risk of bleeding, especially from the stomach, caused by warfarin is increased by drugs that cause bleeding by different mechanisms (e.g. **aspirin**, which inhibits platelet thromboxane A_2 biosynthesis and which can damage the stomach; Ch. 26).
- *Sulfonamides* prevent the synthesis of folic acid by bacteria and other microorganisms; **trimethoprim** inhibits its reduction to its active tetrahydrofolate form. Given together, the drugs have a synergistic action of value in treating *Pneumocystis* infection (Chs 53 and 54).
- *Non-steroidal anti-inflammatory drugs* (NSAIDs; Ch. 26), such as **ibuprofen** or **indometacin**, inhibit biosynthesis of prostaglandins, including renal vasodilator/natriuretic prostaglandins (prostaglandin E_2, prostaglandin I_2). If administered to patients receiving treatment for hypertension, they increase the blood pressure. If given to patients being treated with diuretics for chronic heart failure, they cause salt and water retention and hence cardiac decompensation.[2]
- Histamine H_1-receptor antagonists, such as **promethazine**, commonly cause drowsiness as an unwanted effect. This is more troublesome if such drugs are taken with alcohol, leading to accidents at work or on the road.

Pharmacokinetic interaction

All the four major processes that determine pharmacokinetics – absorption, distribution, metabolism and excretion – can be affected by drugs. Such interactions are covered in Chapters 8 and 9.

Drug interactions

- These are many and varied: if in doubt, look it up.
- Interactions may be pharmacodynamic or pharmacokinetic.
- Pharmacodynamic interactions are often predictable from the actions of the interacting drugs.
- Pharmacokinetic interactions can involve effects on:
  - absorption (Ch. 8)
  - distribution (e.g. competition for protein binding, Ch. 8)
  - hepatic metabolism (induction or inhibition, Ch. 9)
  - renal excretion (Ch. 9).

[2]The interaction with diuretics may involve a pharmacokinetic interaction in addition to the pharmacodynamic effect described here, because NSAIDs compete with weak acids, including diuretics, for renal tubular secretion; see Ch. 9.

GENETIC VARIATION IN DRUG RESPONSIVENESS

RELEVANT ELEMENTARY GENETICS

Genes are the fundamental units of heredity; they consist of ordered sequences of nucleotides (adenine, guanine, thymidine and cytosine – A, G, T, C) located in particular positions in a particular DNA strand. Genes are conventionally abbreviated as for the protein they code for, but are written in italics – for example 'CYP2D6' represents a protein while '*CYP2D6*' is the gene that encodes it. Most cellular DNA is located in the chromosomes in cell nuclei, but a small amount is present in mitochondria and is inherited from the mother (since the ovum contributes mitochondria to the gamete). DNA is *transcribed* to complementary messenger RNA (mRNA) which is *translated* in rough endoplasmic reticulum into a sequence of amino acids. The resulting peptide undergoes folding and often post-translational modification to form the final protein product. The DNA sequence of a gene that codes protein is known as the *exon*. *Introns* are DNA sequences that interrupt the exon; an intron is transcribed into mRNA but this sequence is excised from the message and not translated into protein. The rate of transcription is controlled by promoter regions in the DNA to which RNA polymerase binds to initiate transcription.

Mutations are heritable changes in the base sequence of DNA. This may, or may not,[3] result in a change in the amino acid sequence of the protein for which the gene codes. Most changes in protein structure are deleterious, and so the altered gene dies out in succeeding generations as a result of natural selection. A few changes may confer an advantage, however, at least under some environmental circumstances. A pharmacogenetically relevant example is the X-linked gene for *glucose 6-phosphate dehydrogenase* (G6PD); deficiency of this enzyme confers partial resistance to malaria (a considerable selective advantage in parts of the world where this disease is common) at the expense of susceptibility to haemolysis in response to oxidative stress in the form of exposure to various dietary constituents, including several drugs (e.g. the antimalarial drug **primaquine**; see Ch. 54). This ambiguity gives rise to the abnormal gene being preserved in future generations, at a frequency that depends on the balance of selective pressures in the environment. Thus the distribution of G6PD deficiency is similar to the geographical distribution of malaria. The situation where several functionally distinct forms of a gene are common in a population is called a 'balanced' polymorphism (balanced because a disadvantage, for example in a homozygote, is balanced by an advantage, for example in a heterozygote).

Polymorphisms are alternative sequences at a locus within the DNA strand (alleles) that persist in a population through several generations. They arise initially because of a mutation, and are stable if they are non-functional, or die out during subsequent generations if (as

[3]The genetic code is 'redundant', i.e. more than one set of nucleotide base triplets code for each amino acid. If a mutation results in a base change that leads to a triplet that codes for the same amino acid as the original, there is no change in the protein and consequently no change in function – a 'silent' mutation. Such mutations are neither advantageous nor disadvantageous, so they will neither be eliminated by natural selection nor accumulate in the population at the expense of the wild-type gene.

is usually the case) they are disadvantageous. However, if the prevailing selective pressures in the environment are favourable, leading to a selective advantage, a polymorphism may increase in frequency over successive generations. Now that genes can be sequenced readily, it has become apparent that *single nucleotide polymorphisms* (SNPs, DNA sequence variations that occur when a single nucleotide in the genome sequence is altered) are very common. They may entail substitution of one nucleotide for another (usually substitution of C for T), or deletion or insertion of a nucleotide. Insertions and deletions result in a 'frame shift' in translation – for example, after an insertion, the first element of the next triplet in the code becomes the second and all subsequent bases are shifted one 'to the right'. The result can be loss of protein synthesis, abnormal protein synthesis or an abnormal rate of protein synthesis.

SNPs occur every 100–300 bases along the 3 billion base human genome. Approximately two-thirds of SNPs involve C for T substitution. SNPs can occur in coding (gene) and non-coding regions of the genome. A single SNP can be an important determinant of disease – for example, a common genetic variant due to an SNP in one of the coagulation factors, known as factor V Leiden, is the commonest form of inherited thrombophilia (Ch. 24). This confers an increased risk of venous thrombosis in response to environmental factors such as prolonged immobility, but might perhaps have been an advantage to ancestors more at risk of haemorrhage than of thrombosis. Alternatively, predisposition to disease may depend on a combination of several SNPs in or near a gene. Such combinations are known as *haplotypes* and are inherited from each parent.

SINGLE-GENE PHARMACOKINETIC DISORDERS

Where a mutation disrupts gene function profoundly this may result in a 'single-gene disorder', which is inherited in Mendelian fashion. This was recognised for albinism (albinos lack an enzyme that is needed to synthesise the brown pigment melanin) and other 'inborn errors of metabolism' in the early part of the 20th century by Archibald Garrod, a British physician who initiated the study of biochemical genetics. Investigation of this large group of individually rare diseases has contributed disproportionately to our understanding of molecular pathology – familial hypercholesterolaemia and the mechanism of action of statins (Ch. 23) is one example.

PLASMA CHOLINESTERASE DEFICIENCY

In the 1950s Walter Kalow discovered that **suxamethonium** sensitivity is due to genetic variation in the rate of drug metabolism as a result of a Mendelian autosomal recessive trait. This short-acting neuromuscular-blocking drug is widely used in anaesthesia and is normally rapidly hydrolysed by plasma cholinesterase (Ch. 13). About 1 in 3000 individuals fail to inactivate suxamethonium rapidly and experience prolonged neuromuscular block if treated with it; this is because a recessive gene gives rise to an abnormal type of plasma cholinesterase. The abnormal enzyme has a modified pattern of substrate and inhibitor specificity. It is detected by a blood test that measures the effect of **dibucaine**, which inhibits the abnormal enzyme less than the normal enzyme. Heterozygotes hydrolyse suxamethonium at a more or less normal rate, but their plasma cholinesterase has reduced sensitivity to dibucaine, intermediate between normal subjects and homozygotes. Only homozygotes express the disease: they appear completely healthy unless exposed to suxamethonium (or, presumably, closely related chemicals) but experience prolonged paralysis if exposed to a dose that would cause neuromuscular block for only a few minutes in a healthy person.[4] There are other reasons why responses to suxamethonium may be abnormal in an individual patient, notably *malignant hyperpyrexia* (Ch. 13), a genetically determined idiosyncratic adverse drug reaction involving the ryanodine receptor (Ch. 4). It is important to test family members who may be affected, but the disorder is so rare that it is currently impractical to screen for it routinely before therapeutic use of suxamethonium.

ACUTE INTERMITTENT PORPHYRIA

The hepatic *porphyrias* are prototypic pharmacogenetic disorders in which patients may be symptomatic even if they are not exposed to a drug, but where many drugs can provoke very severe worsening of the course of the disease. They are inherited disorders involving the biochemical pathway of porphyrin haem biosynthesis. *Acute intermittent porphyria* is the least uncommon and most severe form. It is autosomal dominant and is due to one of many different mutations in the gene coding *porphobilinogen deaminase* (PBGD), a key enzyme in haem biosynthesis in red cell precursors, hepatocytes and other cells. All of these mutations reduce activity of this enzyme, and clinical features are caused by the resulting build-up of haem precursors including porphyrins. There is a strong interplay with the environment through exposure to drugs, hormones and other chemicals. The use of sedative, anticonvulsant or other drugs in patients with undiagnosed porphyria can be lethal, though with appropriate supportive management most patients recover completely.[5] Many drugs, especially but not exclusively those that induce CYP enzymes (e.g. barbiturates, **griseofulvin**, **carbamazepine**, oestrogens – see Ch. 9), can precipitate acute attacks in susceptible individuals. Porphyrins are synthesised from δ-amino laevulinic acid (ALA) which is formed by ALA synthase in the liver. This enzyme is induced by drugs such as barbiturates, resulting in increased ALA production and, hence, increased porphyrin accumulation. As mentioned above, the genetic trait is inherited as an autosomal dominant, but frank disease is approximately five times more common in women than in men, because hormonal fluctuations precipitate acute attacks.

[4] An apparently healthy middle-aged man saw one of the authors over several months because of hypertension; he also saw a psychiatrist because of depression. This failed to improve with other treatment and he underwent electroconvulsive therapy (ECT). Suxamethonium was used to prevent injury caused by convulsions; this usually results in short-lived paralysis but this poor man recovered consciousness some 2 days later to find himself being weaned from artificial ventilation in an intensive care unit. Subsequent analysis showed him to be homozygous for an ineffective form of plasma cholinesterase.

[5] Life expectancy, obtained from parish records, of patients with porphyria diagnosed retrospectively within large kindreds in Scandinavia was normal until the advent and widespread use of barbiturates and other sedative and anticonvulsant drugs in the 20th century, when it plummeted. There is a long and useful list of drugs to avoid in the *British National Formulary*, together with the warning that drugs not on the list may not necessarily be safe in such patients!

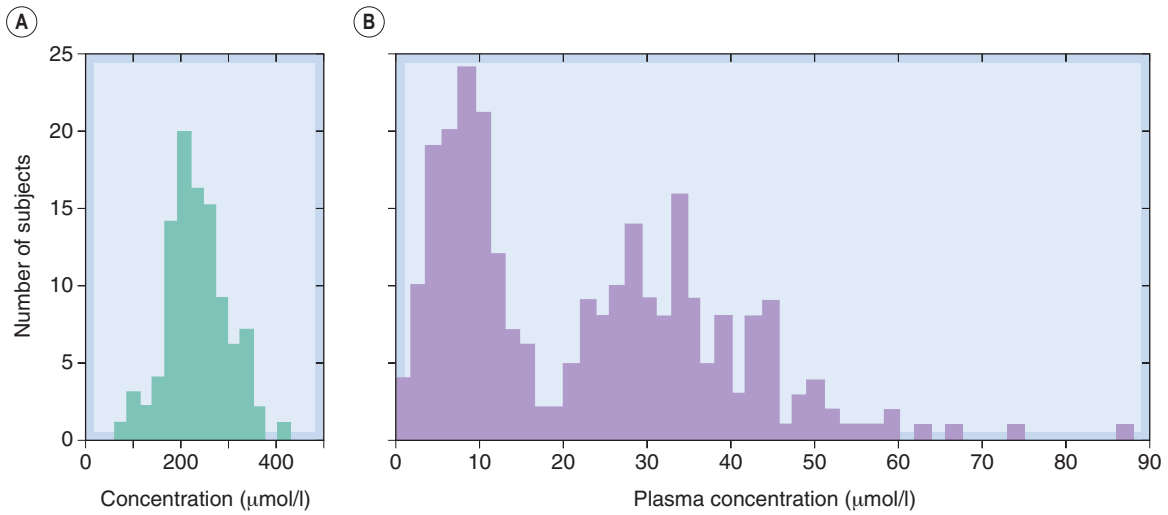

Fig. 11.3 Distribution of individual plasma concentrations for two drugs in humans. [**A**] Plasma salicylate concentration 3 h after oral dosage with sodium salicylate. [**B**] Plasma isoniazid concentration 6 h after oral dosage. Note the normally distributed values for salicylate, compared with the bimodal distribution of isoniazid. *(Panel [A] from Evans DA, Clarke CA 1961 Br Med Bull 17, 234–280; panel [B] from Price-Evans DA 1963 Am J Med 3, 639.)*

DRUG ACETYLATION DEFICIENCY

Both examples considered so far are uncommon diseases. However, in the 1960s Price-Evans demonstrated that the rate of drug acetylation varied in different populations as a result of balanced polymorphism. Figure 11.3 contrasts the approximately Gaussian distribution of plasma concentrations achieved 3 h after administration of a dose of **salicylate** with the bimodal distribution of plasma concentrations after a dose of **isoniazid**. The isoniazid concentration was <20 μmol/l in about half the population, and in this group the mode was approximately 9 μmol/l. In the other half of the population (plasma concentration >20 μmol/l), the mode was approximately 30 μmol/l. Elimination of isoniazid depends mainly on acetylation, catalysed by an acetyltransferase enzyme (Ch. 9). White populations contain roughly equal numbers of 'fast acetylators' and 'slow acetylators'. The characteristic of fast or slow acetylation is controlled by a single recessive gene associated with low hepatic acetyltransferase activity. Other ethnic groups have different proportions of fast and slow acetylators. Isoniazid causes two distinct forms of toxicity. One is peripheral neuropathy, which is produced by isoniazid itself and is commoner in slow acetylators. The other is hepatotoxicity, caused by the acetylated metabolite and is commoner in fast acetylators, at least in some populations. This genetic variation thus produces a qualitative change in the pattern of toxicity caused by the drug in different populations.

Acetyltransferase is also important in the metabolism of other drugs, including **hydralazine** (Ch. 22), **procainamide** (Ch. 21), **dapsone** and various other sulfonamides (Ch. 51) and acetylator status influences drug-induced *lupus* (an autoimmune disorder affecting many organs including skin, joints and kidneys) caused by several of these agents. However, neither phenotyping (by measuring kinetics of drug transformation) nor genotyping for acetyltransferase has found a way into routine clinical practice, probably because these drugs are relatively little used and there are several alternative treatments available that are usually preferred.

AMINOGLYCOSIDE OTOTOXICITY

In the examples above, variations in chromosomal genes cause variations in drug response. Increased susceptibility to hearing loss caused by aminoglycoside antibiotics (see Ch. 51) is, in some families, inherited quite differently, namely exclusively through the mother to all her children. This is the pattern expected of a mitochondrial gene, and indeed the most common predisposing mutation is *m.1555A>G*, a mitochondrial DNA mutation. This mutation accounts for 30–60% of aminoglycoside ototoxicity in China, where aminoglycoside use is common. Aminoglycosides work by binding to bacterial ribosomes (Ch. 51), which share properties with human mitochondrial ribosomes (mitochondria are believed to have evolved from symbiotic bacteria); aminoglycosides cause ototoxity in all individuals exposed to too high a dose. The *m.1555A>G* mutation makes mitochondrial ribosomes even more similar to their bacterial counterpart, increasing the affinity of the drug which remains bound to ribosomes in the hair cells in the ear for several months following a single dose in susceptible individuals. Screening for this variant may be appropriate in children who are likely to require treatment with aminoglycosides (Bitner-Glindzicz & Rahman, 2007).

THERAPEUTIC DRUGS AND CLINICALLY AVAILABLE PHARMACOGENOMIC TESTS

Clinical tests to predict drug responsiveness were anticipated to be one of the first applications of sequencing the human genome, but their development has been slowed by various scientific, commercial, political and educational barriers (Flockhart et al., 2009). Reimbursement for expensive drugs, whether provided by the state or by insurance schemes, depends increasingly on evidence of

cost-effectiveness. New tests need to improve demonstrably on our current ability to prescribe optimally, and must lead to a clear-cut change in prescribing, such as using a different drug or a different dosing regimen. So far the evidence in support of any pharmacogenetic test is less convincing than the ideal of a randomised controlled trial of a pharmacogenomics-informed prescribing strategy versus current best practice, but several of the tests mentioned below are increasingly used in clinical practice. They include tests for (a) variants of different human leukocyte antigens (HLAs) that have been strongly linked to susceptibilities to several severe qualitatively distinct off-target harmful drug reactions; (b) genes controlling aspects of drug metabolism; and (c) genes encoding drug targets. For one drug (**warfarin**), a test combines genetic information about metabolism with information about its target. The genetic susceptibility of Collie dogs to neurotoxic effects of **ivermectin** mentioned in Chapter 8 is of importance in veterinary medicine. It results from a variant of P-glycoprotein that alters the properties of the blood–brain barrier of dogs with Collie ancestry, and in future genes coding for proteins influencing drug distribution in man may also be fertile territory for new tests.

Methodology: Mutations in the germline are passed to the next generation where they are present in all cells; in practice, tests for such germline mutations are usually made on venous blood samples that contain chromosomal and mitochondrial DNA in white blood cells. Somatic cell mutations underlie the pathogenesis of some tumours (Ch. 5), and the presence or absence of such somatic cell mutations guides drug selection. The genomic tests are performed on DNA from samples of the tumour obtained surgically. The tests themselves involve amplification of the relevant sequence(s) and molecular biological methods, often utilising chip technology, to identify the various polymorphisms.

HLA GENE TESTS

ABACAVIR AND *HLAB*5701*

▼ **Abacavir** (Ch. 52) is a reverse transcriptase inhibitor that is highly effective in treating HIV infection. Its use has been limited by severe rashes. Susceptibility to this adverse effect is closely linked to the human leukocyte antigen (HLA) variant *HLAB*5701*, and testing for this variant is used widely and supported by prospective trials; see Figure 11.4 (Lai-Goldman & Faruki, 2008).

ANTICONVULSANTS AND *HLAB*1502*

▼ **Carbamazepine** (Ch. 45) can also cause severe (life-threatening) rashes including *Stevens–Johnson syndrome* (in which a multiform rash with blistering and other lesions extends into the gastrointestinal tract) and *toxic epidermal necrolysis* (in which the outer layer of the skin peels away from the dermis as though it has been scalded). These are associated with a particular HLA allele, *HLAB*1502*, which occurs almost exclusively in people with Asian ancestry (Man et al., 2007); the FDA recommends that Chinese patients should be screened for this allele before starting treatment. People who develop such a reaction to carbamazepine may develop a similar problem if treated with **phenytoin**, and the same allele has been associated with hypersensitivity reactions to this drug too.

CLOZAPINE AND *HLA-DQB1*0201*

▼ **Clozapine** is a uniquely effective antipsychotic drug with a different pattern of adverse effects from classical antipsychotic drugs (Ch. 46); its use is limited by agranulocytosis which occurs in approximately 1% of treated patients. This has been associated with *HLA-DQB1*0201*, but so far studies have been small and the specificity and sensitivity of the test remain to be established.

Pharmacogenetics and pharmacogenomics

- Several inherited disorders influence responses to drugs, including:
  - glucose-6-phosphatase deficiency, a sex-linked disorder in which affected men (or rare homozygous women) experience haemolysis if exposed to various chemicals including the antimalarial drug **primaquine**
  - plasma cholinesterase deficiency, an autosomal recessive disorder that confers sensitivity to the neuromuscular blocker suxamethonium
  - acute intermittent porphyria, an autosomal dominant disease more severe in women and in which severe attacks are precipitated by drugs or endogenous sex hormones that induce CYP enzymes
  - drug acetylator deficiency, a balanced polymorphism
  - increased susceptibility to ototoxicity from aminoglycosides, which is conferred by a mutation in mitochondrial DNA.
- These pharmacogenetic disorders prove that drug responses can be genetically determined in individuals.
- Single nucleotide polymorphisms (SNPs) and combinations of SNPs (haplotypes) in genes coding for proteins involved in drug disposition or drug action are common and may predict drug response. Pharmacogenomic tests in blood or tissue removed surgically have established associations between several such variants and individual drug response, and several such tests are available for clinical use although their status in individualising drug treatment is still being established.
- Such tests are available for:
  - several HLA variants that predict toxicity of **abacavir**, **carbamazepine** and **clozapine**
  - genes for several enzymes in drug metabolism including CYP2D6 and CYP2C9, and thiopurine-*S*-methyltransferase (TPMT)
  - germline and somatic mutations in growth-factor receptors that predict responsiveness to cancer treatments including **imatinib** and **trastuzumab**.

DRUG METABOLISM-RELATED GENE TESTS

THIOPURINES AND TPMT

▼ Thiopurine drugs (**tioguanine**, **mercaptopurine** and its prodrug **azathioprine**; Ch. 56) have been used for the past 50 years to treat leukaemias, including acute lymphoblastic leukaemia (ALL, which accounts for approximately one-fifth of all childhood malignancies), and more recently to cause immunosuppression, e.g. in treating inflammatory bowel disease. All of these drugs cause bone marrow and liver toxicity, and are detoxified by thiopurine-*S*-methyltransferase (TPMT), which is present in blood cells, as well as by xanthine oxidase. There are large inherited variations in TPMT activity with a trimodal frequency distribution (Weinshilboum & Sladek, 1980); low TPMT activity in blood is associated with high concentrations of active 6-thioguanine nucleotides (TGN) in blood and with bone marrow toxicity whereas high TPMT activity is associated with lower concentrations of TGN and reduced efficacy. Before starting treatment, phenotyping (by a blood test for TPMT activity) or genotyping of *TMPT* alleles *TPMT*3A*, *TPMT*3C*, *TPMT*2*, is

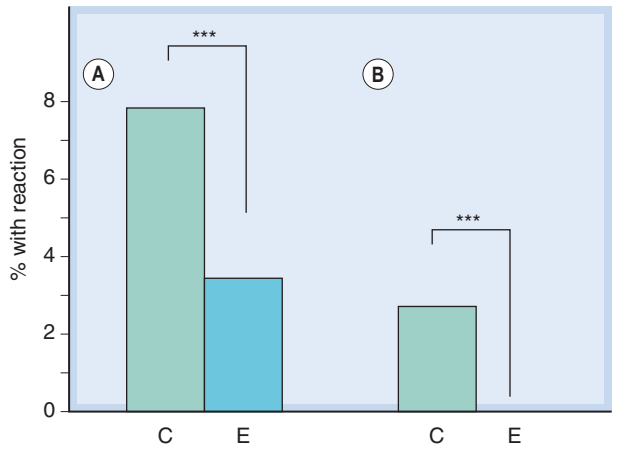

Fig. 11.4 Incidence of abacavir hypersensitivity is reduced by pharmacogenetic screening. In the PREDICT-1 study (Mallal et al., 2008), patients were randomised to standard care (control group, C) or prospective pharmacogenetic screening (experimental group, E). All the control subjects were treated with abacavir, but only those experimental subjects who were *HLA-B*5701* negative were treated with abacavir. There were two prespecified end points: clinically suspected hypersensitivity reactions [**A**] and clinically suspected reactions that were immunologically confirmed by a positive patch test [**B**]. Both end points favoured the experimental group ($P < 0.0001$). *(Figure redrawn from Hughes AR et al. 2008 Pharmacogenet J 8, 365–374.)*

recommended. Even with such testing, careful monitoring of the white blood cell count is needed because of environmental susceptibility factors (e.g. drug interaction with **allopurinol** via inhibition of xanthine oxidase).

5-FLUOROURACIL (5-FU) AND DPYD

▼ **5-FU** (Ch. 56, Fig. 56.6) is used extensively to treat solid tumours, but has variable efficacy and unpredictable mucocutaneous toxicity. It is detoxified by dihydropyrimidine dehydrogenase (DPYD), which has multiple clinically identifiable functional genetic variants. Currently available genetic information is neither highly sensitive nor specific, but the FDA recommends that the drug not be given to patients with DPYD deficiency.

TAMOXIFEN AND CYP2D6

▼ **Tamoxifen** (Chs 35 and 56) is metabolised to an oestrogen antagonist endoxifen by CYP2D6, an enzyme that is subject to marked polymorphic variation; several small association studies have suggested a link between *CYP2D6* genotype and efficacy. Genotyping tests for *CYP2D6* are available, but genetic results from larger comparative trials of tamoxifen versus aromatase inhibitors are awaited. Treatment with other CYP2D6 substrates, for example **tetrabenazine**, used to treat Huntington's disease (Ch. 40) may also be influenced by knowledge of *CYP2D6* genotype: the FDA recommends that patients who are CYP2D6 poor metabolisers should not be prescribed more than 50 mg daily because of the risk of severe depression.

IRINOTECAN AND UGT1A1*28

▼ **Irinotecan**, a topoisomerase I inhibitor (Ch. 56) has marked activity against colorectal and lung cancers in a minority of patients, but toxicity (diarrhoea and bone marrow suppression) can be severe. It works through an active metabolite (SN-38) which is detoxified by glucuronidation by UDP-glucuronyltransferase (UGT; Ch. 9, Fig. 9.3). Reduced activity of this enzyme is common and gives rise to the inherited benign condition of hyperbilirubinaemia known as *Gilbert's syndrome* in which unconjugated bilirubin accumulates in plasma. UGT1A1 genetic testing is clinically available and predicts irinotecan pharmacokinetics and clinical outcomes. The best way to use information from the test is still uncertain, however.

DRUG TARGET-RELATED GENE TESTS

TRASTUZUMAB AND HER2

▼ **Trastuzumab** ('Herceptin'; Ch. 56) is a monoclonal antibody that antagonises epidermal growth factor (EGF) by binding to one of its receptors (human epidermal growth factor receptor 2 – HER2) which can occur in tumour tissue as a result of somatic mutation. It is used in patients with breast cancer whose tumour tissue overexpresses this receptor. Other patients do not benefit from it.

DASATINIB, IMATINIB AND BCR-ABL1

▼ **Dasatinib** is a tyrosine kinase inhibitor used in haematological malignancies characterised by the presence of a Philadelphia chromosome, namely chronic myeloid leukaemia (CML) and some adults with acute lymphoblastic leukaemia (ALL). The Philadelphia chromosome results from a translocation defect when parts of two chromosomes (9 and 22) swap places; part of a 'breakpoint cluster region' (BCR) in chromosome 22 links to the 'Abelson-1' (ABL) region of chromosome 9. A mutation (T315I) in BCR/ABL confers resistance to the inhibitory effect of dasatinib and patients with this variant do not benefit from this drug. Pharmacogenetic testing is also being evaluated for **imatinib** (Ch. 56), another tyrosine kinase inhibitor used in patients with CML and other myelodysplastic disorders associated with rearrangements in the gene for platelet-derived growth factor receptor or for BCR-ABL.

COMBINED (METABOLISM AND TARGET) GENE TESTS

WARFARIN AND CYP2C9 + VKORC1 GENOTYPING

▼ **Warfarin** is *par excellence* a drug where dosing must be individualised. This is done by measuring the *international normalised ratio* (INR), a measure of its effect on blood coagulability (Ch. 24), but thrombotic events despite treatment (lack of efficacy) and serious adverse effects (usually bleeding) remain all too common. Warfarin is the most widely used drug for which pharmacogenetic testing has been proposed, based on a study showing that polymorphisms in its key target, vitamin K epoxide reductase (VKOR; see Fig. 24.5) and in CYP2C9, involved in its metabolism, are associated with outcomes. Figure 11.5 shows the effects of VKOR haplotype and of CYP2C9 genotype on the mean dose of warfarin needed to achieve therapeutic INR. Dosing algorithms have been proposed based on the results of testing for polymorphisms of these genes (Schwarz et al., 2008). A randomised trial favoured this strategy for initiating treatment versus a standard loading dose approach, but genetic testing did not improve on an individualised algorithm for dose initiation based on other clinical variables (Zineh et al., 2013).

CONCLUSIONS

Twin studies as well as several well-documented single-gene disorders (including Mendelian chromosomal – autosomal recessive, autosomal dominant and X-linked – and maternally inherited mitochondrial disorders) prove the concept that susceptibility to adverse drug effects can be genetically determined. Pharmacogenomic testing offers the possibility of more precise 'personalised' therapeutics for several drugs and disorders. This is a field of intense research activity, rapid progress and high expectations, but proving that these tests add to present best practice and improve outcomes remains a challenge.

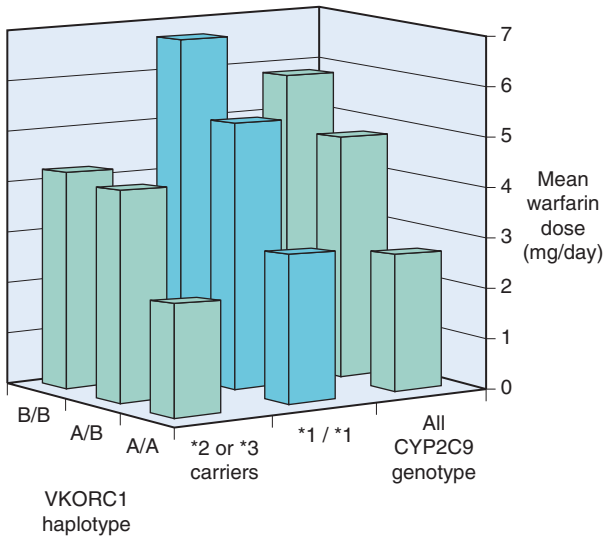

Fig. 11.5 Effect of *VKOR* haplotype and *CYP2C9* genotype on warfarin dose. A series of 186 patients on long-term warfarin treatment who had already been studied for *CYP2C9* were studied retrospectively for genetic variants of *VKOR* (Rieder et al., 2005). *VKOR* haplotype as well as *CYP2C9* genotype influenced the mean warfarin dose (which had been adjusted to achieve therapeutic INR). A, Haplotypes 1 and 2; B, haplotypes 7, 8 and 9. A/A, A/B and B/B represent haplotype combinations. *1/*1 represents CYP2C9 wild-type homozygotes; *2 and *3 represent CYP2C9 variants. *(Figure redrawn from Beitelshees AL, McLeod HL 2006 Applying pharmacogenomics to enhance the use of biomarkers for drug effect and drug safety. TIPS 27, 498–502.)*

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Chemical mediators and the autonomic nervous system

12

OVERVIEW

The network of chemical signals and associated receptors by which cells in the body communicate with one another provides many targets for drug action, and has always been a focus of attention for pharmacologists. Chemical transmission in the peripheral autonomic nervous system, and the various ways in which the process can be pharmacologically subverted, is the main focus of this chapter, but the mechanisms described operate also in the central nervous system. In addition to neurotransmission, we also consider briefly the less clearly defined processes, collectively termed neuromodulation, by which many mediators and drugs exert control over the function of the nervous system. The relative anatomical and physiological simplicity of the peripheral nervous system has made it the proving ground for many important discoveries about chemical transmission, and the same general principles apply to the central nervous system (see Ch. 37). For more detail than is given here, see Robertson (2004), Burnstock (2009) and Iversen et al. (2009).

HISTORICAL ASPECTS

▼ Studies initiated on the peripheral nervous system have been central to the understanding and classification of many major types of drug action, so it is worth recounting a little history. Excellent accounts are given by Bacq (1975) and Valenstein (2005).

Experimental physiology became established as an approach to the understanding of the function of living organisms in the middle of the 19th century. The peripheral nervous system, and particularly the autonomic nervous system, received a great deal of attention. The fact that electrical stimulation of nerves could elicit a whole variety of physiological effects – from blanching of the skin to arrest of the heart – presented a real challenge to comprehension, particularly of the way in which the signal was passed from the nerve to the effector tissue. In 1877, Du Bois-Reymond was the first to put the alternatives clearly: 'Of known natural processes that might pass on excitation, only two are, in my opinion, worth talking about – either there exists at the boundary of the contractile substance a stimulatory secretion … or the phenomenon is electrical in nature.' The latter view was generally favoured. In 1869, it had been shown that an exogenous substance, **muscarine**, could mimic the effects of stimulating the vagus nerve, and that **atropine** could inhibit the actions both of muscarine and of nerve stimulation. In 1905, Langley showed the same for **nicotine** and **curare** acting at the neuromuscular junction. Most physiologists interpreted these phenomena as stimulation and inhibition of the nerve endings, respectively, rather than as evidence for chemical transmission. Hence the suggestion of T.R. Elliott, in 1904, that **adrenaline** (**epinephrine**) might act as a chemical transmitter mediating the actions of the sympathetic nervous system was coolly received, until Langley, the Professor of Physiology at Cambridge and a powerful figure at that time, suggested, a year later, that transmission to skeletal muscle involved the secretion by the nerve terminals of a substance related to nicotine.

One of the key observations for Elliott was that degeneration of sympathetic nerve terminals did not abolish the sensitivity of smooth muscle preparations to adrenaline (which the electrical theory predicted) but actually enhanced it. The hypothesis of chemical transmission was put to direct test in 1907 by Dixon, who tried to show that vagus nerve stimulation released from a dog's heart into the blood a substance capable of inhibiting another heart. The experiment failed, and the atmosphere of scepticism prevailed.

It was not until 1921, in Germany, that Loewi showed that stimulation of the vagosympathetic trunk connected to an isolated and cannulated frog's heart could cause the release into the cannula of a substance ('*Vagusstoff*') that, if the cannula fluid was transferred from the first heart to a second, would inhibit the second heart. This is a classic and much-quoted experiment that proved extremely difficult for even Loewi to perform reproducibly. In an autobiographical sketch, Loewi tells us that the idea of chemical transmission arose in a discussion that he had in 1903, but no way of testing it experimentally occurred to him until he dreamed of the appropriate experiment one night in 1920. He wrote some notes of this very important dream in the middle of the night, but in the morning could not read them. The dream obligingly returned the next night and, taking no chances, he went to the laboratory at 3 a.m. and carried out the experiment successfully. Loewi's experiment may be, and was, criticised on numerous grounds (it could, for example, have been potassium rather than a neurotransmitter that was acting on the recipient heart), but a series of further experiments proved him to be right. His findings can be summarised as follows:

- Stimulation of the vagus caused the appearance in the perfusate of the frog heart of a substance capable of producing, in a second heart, an inhibitory effect resembling vagus stimulation.
- Stimulation of the sympathetic nervous system caused the appearance of a substance capable of accelerating a second heart. By fluorescence measurements, Loewi concluded later that this substance was adrenaline.
- Atropine prevented the inhibitory action of the vagus on the heart but did not prevent release of Vagusstoff. Atropine thus prevented the effects, rather than the release, of the transmitter.
- When Vagusstoff was incubated with ground-up heart muscle, it became inactivated. This effect is now known to be due to enzymatic destruction of acetylcholine by cholinesterase.
- **Physostigmine**, which potentiated the effect of vagus stimulation on the heart, prevented destruction of Vagusstoff by heart muscle, providing evidence that the potentiation is due to inhibition of cholinesterase, which normally destroys the transmitter substance acetylcholine.

A few years later, in the early 1930s, Dale showed convincingly that acetylcholine was also the transmitter substance at the neuromuscular junction of striated muscle and at autonomic ganglia. One of the keys to Dale's success lay in the use of highly sensitive bioassays, especially the leech dorsal muscle, for measuring acetylcholine release. Chemical transmission at sympathetic nerve terminals was demonstrated at about the same time as cholinergic transmission and by very similar methods. Cannon and his colleagues at Harvard first showed unequivocally the phenomenon of chemical transmission at sympathetic nerve endings, by experiments *in vivo* in which tissues made supersensitive to adrenaline by prior sympathetic denervation were shown to respond, after a delay, to the transmitter released by stimulation of the sympathetic nerves to other parts of the body. The chemical identity of the transmitter, tantalisingly like adrenaline but not identical to it, caused confusion for many years, until in 1946 von Euler showed it to be the non-methylated derivative **noradrenaline** (**norepinephrine**).

THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system for a long time occupied centre stage in the pharmacology of chemical transmission.

BASIC ANATOMY AND PHYSIOLOGY

The autonomic nervous system (see Robertson, 2004) consists of three main anatomical divisions: *sympathetic, parasympathetic* and *enteric* nervous systems. The sympathetic and parasympathetic systems (Fig. 12.1) provide a link between the central nervous system and peripheral organs. The enteric nervous system comprises the intrinsic nerve plexuses of the gastrointestinal tract, which are closely interconnected with the sympathetic and parasympathetic systems.

The autonomic nervous system conveys all the outputs from the central nervous system to the rest of the body, except for the motor innervation of skeletal muscle. The enteric nervous system has sufficient integrative capabilities to allow it to function independently of the central nervous system, but the sympathetic and parasympathetic systems are agents of the central nervous system and cannot function without it. The autonomic nervous system is largely outside the influence of voluntary control. The main processes that it regulates, to a greater or lesser extent, are:

- contraction and relaxation of vascular and visceral smooth muscle
- all exocrine and certain endocrine secretions
- the heartbeat
- energy metabolism, particularly in liver and skeletal muscle.

A degree of autonomic control also affects many other systems, including the kidney, immune system and somatosensory system. The autonomic efferent pathway consists of two neurons arranged in series, whereas in the somatic motor system a single motor neuron connects the central nervous system to the skeletal muscle fibre (see Fig. 12.2). The two neurons in the autonomic pathway are known, respectively, as *preganglionic* and *postganglionic*. In the sympathetic nervous system, the intervening synapses lie in *autonomic* ganglia, which are outside the central nervous system, and contain the nerve endings of preganglionic fibres and the cell bodies of postganglionic neurons. In parasympathetic pathways, the postganglionic cells are mainly found in the target organs, discrete parasympathetic ganglia (e.g. the ciliary ganglion) being found only in the head and neck.

The cell bodies of the sympathetic preganglionic neurons lie in the *lateral horn* of the grey matter of the thoracic and lumbar segments of the spinal cord, and the fibres leave the spinal cord in the spinal nerves as the *thoracolumbar sympathetic outflow*. The preganglionic fibres synapse in the *paravertebral chains* of sympathetic ganglia, lying on either side of the spinal column. These ganglia contain the cell bodies of the postganglionic sympathetic

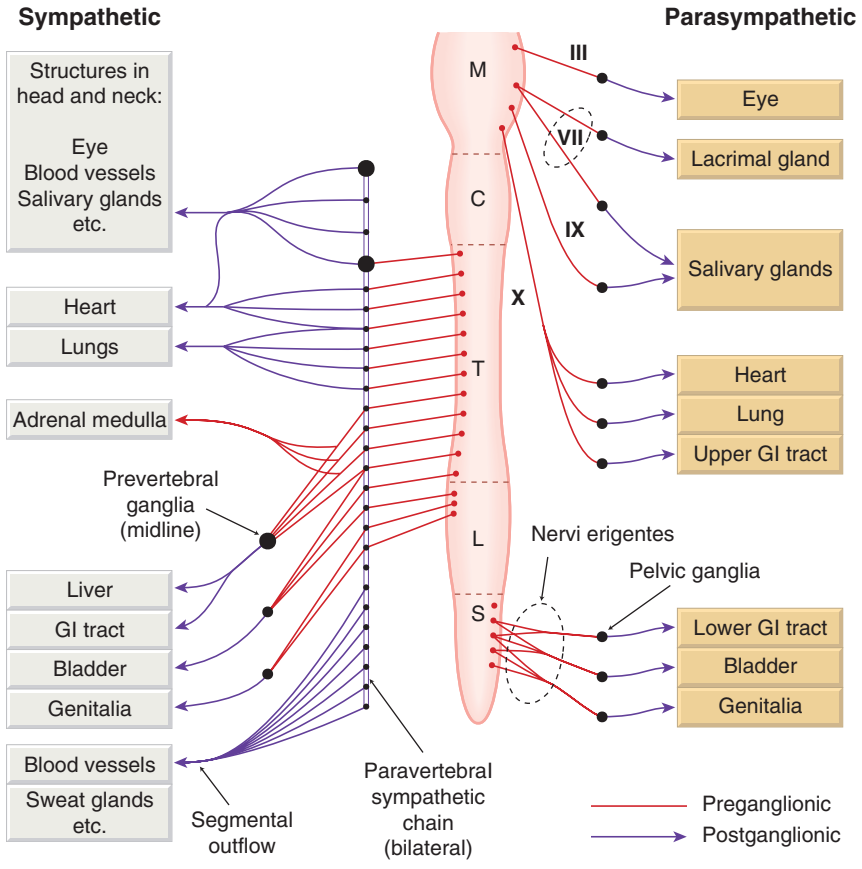


Fig. 12.1 Basic plan of the mammalian autonomic nervous system. C, cervical; GI, gastrointestinal; L, lumbar; M, medullary; S, sacral; T, thoracic.

neurons, the axons of which rejoin the spinal nerve. Many of the postganglionic sympathetic fibres reach their peripheral destinations via the branches of the spinal nerves. Others, destined for abdominal and pelvic viscera, have their cell bodies in a group of unpaired *prevertebral ganglia* in the abdominal cavity. The only exception to the two-neuron arrangement is the innervation of the adrenal medulla. The catecholamine-secreting cells of the adrenal medulla are, in effect, modified postganglionic sympathetic neurons, and the nerves supplying the gland are equivalent to preganglionic fibres.

The parasympathetic nerves emerge from two separate regions of the central nervous system. The *cranial outflow* consists of preganglionic fibres in certain cranial nerves, namely the *oculomotor nerve* (carrying parasympathetic fibres destined for the eye), the *facial* and *glossopharyngeal nerves* (carrying fibres to the salivary glands and the nasopharynx), and the *vagus nerve* (carrying fibres to the thoracic and abdominal viscera). The ganglia lie scattered in close relation to the target organs; the postganglionic neurons are very short compared with those of the sympathetic system. Parasympathetic fibres destined for the pelvic and abdominal viscera emerge as the *sacral outflow* from the spinal cord in a bundle of nerves known as the *nervi erigentes* (because stimulation of these nerves evokes genital erection – a fact of some importance to those responsible for artificial insemination of livestock). These fibres synapse in a group of scattered *pelvic ganglia*, whence the short postganglionic fibres run to target tissues such as the bladder, rectum and genitalia. The pelvic ganglia carry both sympathetic and parasympathetic fibres, and the two divisions are not anatomically distinct in this region.

The enteric nervous system (reviewed by Goyal & Hirano, 1996) consists of the neurons whose cell bodies lie in the intramural plexuses in the wall of the intestine. It is estimated that there are more cells in this system than in the spinal cord, and functionally they do not fit simply into the sympathetic/parasympathetic classification. Incoming nerves from both the sympathetic and the parasympathetic systems terminate on enteric neurons, as well as running directly to smooth muscle, glands and blood vessels. Some enteric neurons function as mechanoreceptors or chemoreceptors, providing local reflex pathways that can control gastrointestinal function without external inputs. The enteric nervous system is pharmacologically more complex than the sympathetic or parasympathetic systems, involving many neuropeptide and other transmitters (such as 5-hydroxytryptamine, nitric oxide and ATP; see Ch. 30).

In some places (e.g. in the visceral smooth muscle of the gut and bladder, and in the heart), the sympathetic and the parasympathetic systems produce opposite effects, but there are others where only one division of the autonomic system operates. The *sweat glands* and most *blood vessels*, for example, have only a sympathetic innervation, whereas the *ciliary muscle* of the eye has only a parasympathetic innervation. *Bronchial smooth muscle* has only a parasympathetic (constrictor) innervation (although its tone is highly sensitive to circulating adrenaline – acting probably to inhibit the constrictor innervation rather than on the smooth muscle directly). *Resistance arteries* (see Ch. 22) have a sympathetic vasoconstrictor innervation but no parasympathetic innervation; instead, the constrictor tone is opposed by a background release of nitric oxide from the endothelial cells (see Ch. 20). There are other examples, such as the *salivary glands*, where the two systems produce similar, rather than opposing, effects.

It is therefore a mistake to think of the sympathetic and parasympathetic systems simply as physiological opponents. Each serves its own physiological function and can be more or less active in a particular organ or tissue according to the need of the moment. Cannon rightly emphasised the general role of the sympathetic system in evoking 'fight or flight' reactions in an emergency, but emergencies are rare for most animals. In everyday life, the autonomic nervous system functions continuously to control specific local functions, such as adjustments to postural changes, exercise or ambient temperature (see Jänig & McLachlan, 1992). The popular concept of a continuum from the extreme 'rest and digest' state (parasympathetic active, sympathetic quiescent) to the extreme emergency fight or flight state (sympathetic active, parasympathetic quiescent) is an oversimplification, albeit one that provides the student with a generally reliable *aide memoire*.

Table 12.1 lists some of the more important autonomic responses in humans.

TRANSMITTERS IN THE AUTONOMIC NERVOUS SYSTEM

The two main neurotransmitters that operate in the autonomic system are **acetylcholine** and **noradrenaline**, whose sites of action are shown diagrammatically in Figure 12.2. This diagram also shows the type of postsynaptic receptor with which the transmitters interact at the different sites (discussed more fully in Chs 13 and 14). Some general rules apply:

- All autonomic nerve fibres leaving the central nervous system release acetylcholine, which acts on *nicotinic receptors* (although in autonomic ganglia a minor component of excitation is due to activation of *muscarinic receptors*; see Ch. 13).

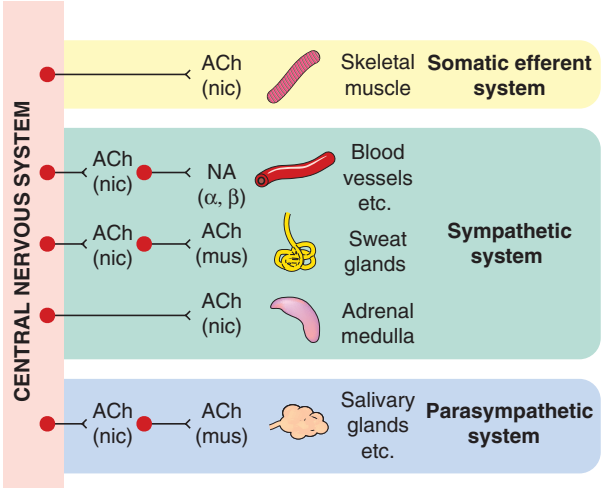


Fig. 12.2 Acetylcholine and noradrenaline as transmitters in the peripheral nervous system. The two main types of acetylcholine (ACh) receptor, nicotinic (nic) and muscarinic (mus) (see Ch. 13) and two types of adrenoceptor, α and β (Ch. 14), are indicated. NA, noradrenaline (norepinephrine).

Table 12.1 The main effects of the autonomic nervous system

Organ	Sympathetic effect	Adrenoceptor type[a]	Parasympathetic effect	Cholinoceptor type[a]
Heart				
Sinoatrial node	Rate ↑	β_1	Rate ↓	M_2
Atrial muscle	Force ↑	β_1	Force ↓	M_2
Atrioventricular node	Automaticity ↑	β_1	Conduction velocity ↓	M_2
			Atrioventricular block	M_2
Ventricular muscle	Automaticity ↑ Force ↑	β_1	No effect	M_2
Blood vessels				
ARTERIOLES				
Coronary	Constriction	α	No effect	–
Muscle	Dilatation	β_2	No effect	–
Viscera, skin, brain	Constriction	α	No effect	–
Erectile tissue	Constriction	α	Dilatation	M_3[b]
Salivary gland	Constriction	α	Dilatation	M_3[b]
VEINS	Constriction	α	No effect	–
	Dilatation	β_2	No effect	–
Viscera				
BRONCHI				
Smooth muscle	No sympathetic innervation, but dilated by circulating adrenaline (epinephrine)	β_2	Constriction	M_3
Glands	No effect	–	Secretion	M_3
GASTROINTESTINAL TRACT				
Smooth muscle	Motility ↓	α_1, α_2, β_2	Motility ↑	M_3
Sphincters	Constriction	α_1, α_2, β_2	Dilatation	M_3
Glands	No effect	–	Secretion	M_3
			Gastric acid secretion	M_1
BLADDER	Relaxation	β_2	Contraction	M_3
	Sphincter contraction	α_1	Sphincter relaxation	M_3
UTERUS				
Pregnant	Contraction	α	Variable	–
Non-pregnant	Relaxation	β_2		
MALE SEX ORGANS	Ejaculation	α	Erection	M_3[b]
Eye				
Pupil	Dilatation	α	Constriction	M_3
Ciliary muscle	Relaxation (slight)	β	Contraction	M_3
Skin				
Sweat glands	Secretion (mainly cholinergic via M_3 receptors)	–	No effect	–
Pilomotor	Piloerection	α	No effect	–
Salivary glands	Secretion	α, β	Secretion	M_3
Lacrimal glands	No effect	—	Secretion	M_3
Kidney	Renin secretion	β_1	No effect	–
Liver	Glycogenolysis Gluconeogenesis	α, β_2	No effect	–
Adipose tissue[c]	Lipolysis Thermogenesis	β_3	No effect	–
Pancreatic islets[c]	Insulin secretion ↓	α_2	No effect	–

[a]The adrenoceptor and cholinoceptor types shown are described more fully in Chapters 13 and 14. Transmitters other than acetylcholine and noradrenaline contribute to many of these responses (see Table 12.2).
[b]Vasodilator effects of M_3 receptors are due to nitric oxide release from endothelial cells (see Ch. 20).
[c]No direct innervation. Effect mediated by circulating adrenaline released from the adrenal medulla.

Basic anatomy and physiology of the autonomic nervous system

Anatomy

- The autonomic nervous system comprises three divisions: *sympathetic*, *parasympathetic* and *enteric*.
- The basic (two-neuron) pattern of the sympathetic and parasympathetic systems consists of a *preganglionic* neuron with a cell body in the central nervous system (CNS) and a *postganglionic* neuron with a cell body in an autonomic ganglion.
- The parasympathetic system is connected to the CNS via:
  - cranial nerve outflow (III, VII, IX, X)
  - sacral outflow.
- Parasympathetic ganglia usually lie close to or within the target organ.
- Sympathetic outflow leaves the CNS in thoracic and lumbar spinal roots. Sympathetic ganglia form two paravertebral chains, plus some midline ganglia.
- The enteric nervous system consists of neurons lying in the intramural plexuses of the gastrointestinal tract. It receives inputs from sympathetic and parasympathetic systems, but can act on its own to control the motor and secretory functions of the intestine.

Physiology

- The autonomic system controls smooth muscle (visceral and vascular), exocrine (and some endocrine) secretions, rate and force of contraction of the heart, and certain metabolic processes (e.g. glucose utilisation).
- Sympathetic and parasympathetic systems have opposing actions in some situations (e.g. control of heart rate, gastrointestinal smooth muscle), but not in others (e.g. salivary glands, ciliary muscle).
- Sympathetic activity increases in stress ('fight or flight' response), whereas parasympathetic activity predominates during satiation and repose. Both systems exert a continuous physiological control of specific organs under normal conditions, when the body is at neither extreme.

- All postganglionic parasympathetic fibres release acetylcholine, which acts on muscarinic receptors.
- All postganglionic sympathetic fibres (with one important exception) release noradrenaline, which may act on either α or β *adrenoceptors* (see Ch. 14). The exception is the sympathetic innervation of sweat glands, where transmission is due to acetylcholine acting on muscarinic receptors. In some species, but not humans, vasodilatation in skeletal muscle is produced by cholinergic sympathetic nerve fibres.

Acetylcholine and noradrenaline are the grandees among autonomic transmitters, and are central to understanding autonomic pharmacology. However, many other chemical mediators are also released by autonomic neurons (see p. 149-151), and their functional significance is gradually becoming clearer.

Transmitters of the autonomic nervous system

- The principal transmitters are **acetylcholine** (ACh) and **noradrenaline**.
- Preganglionic neurons are cholinergic, and ganglionic transmission occurs via nicotinic ACh receptors (although excitatory muscarinic ACh receptors are also present on postganglionic cells).
- Postganglionic parasympathetic neurons are cholinergic, acting on muscarinic receptors in target organs.
- Postganglionic sympathetic neurons are mainly noradrenergic, although a few are cholinergic (e.g. sweat glands).
- Transmitters other than noradrenaline and acetylcholine (NANC transmitters) are also abundant in the autonomic nervous system. The main ones are nitric oxide and vasoactive intestinal peptide (parasympathetic), ATP and neuropeptide Y (sympathetic). Others, such as 5-hydroxytryptamine, GABA and dopamine, also play a role.
- Co-transmission is a general phenomenon.

SOME GENERAL PRINCIPLES OF CHEMICAL TRANSMISSION

The essential processes in chemical transmission – the release of mediators, and their interaction with receptors on target cells – are described in Chapters 4 and 3, respectively. Here we consider some general characteristics of chemical transmission of particular relevance to pharmacology. Many of these principles apply also to the central nervous system and are taken up again in Chapter 37.

DALE'S PRINCIPLE

▼ Dale's principle, advanced in 1934, states, in its modern form: 'A mature neuron releases the same transmitter (or transmitters) at all of its synapses.' Dale considered it unlikely that a single neuron could store and release different transmitters at different nerve terminals, and his view was supported by physiological and neurochemical evidence. It is now known, however, that there are situations where different transmitters are released from different terminals of the same neuron. Further, most neurons release more than one transmitter (see co-transmission, p. 149) and may change their transmitter repertoire, for example during development or in response to injury. Moreover (see Fig. 4.12), the balance of the cocktail of mediators released by a nerve terminal can vary with stimulus conditions, and in response to presynaptic modulators. Dale's principle was, of course, framed long before these complexities were discovered, and it has probably now outlived its usefulness, although purists seem curiously reluctant to let it go.

DENERVATION SUPERSENSITIVITY

It is known, mainly from the work of Cannon on the sympathetic system, that if a nerve is cut and its terminals

allowed to degenerate, the structure supplied by it becomes supersensitive to the transmitter substance released by the terminals. Thus skeletal muscle, which normally responds to injected acetylcholine only if a large dose is given directly into the arterial blood supply, will, after denervation, respond by contracture to much smaller amounts. Other organs, such as salivary glands and blood vessels, show similar supersensitivity to acetylcholine and noradrenaline when the postganglionic nerves degenerate, and there is evidence that pathways in the central nervous system show the same phenomenon.

▼ Several mechanisms contribute to denervation supersensitivity, and the extent and mechanism of the phenomenon varies from organ to organ. Reported mechanisms include the following (see Luis & Noel, 2009).

- *Proliferation of receptors.* This is particularly marked in skeletal muscle, in which the number of acetylcholine receptors increases 20-fold or more after denervation; the receptors, normally localised to the endplate region of the fibres (Ch. 13), spread over the whole surface. Elsewhere, increases in receptor number are much smaller, or absent altogether.
- *Loss of mechanisms for transmitter removal.* At noradrenergic synapses, the loss of neuronal uptake of noradrenaline (see Ch. 14) contributes substantially to denervation supersensitivity. At cholinergic synapses, a partial loss of cholinesterase occurs (see Ch. 13).
- *Increased postjunctional responsiveness.* Smooth muscle cells become partly depolarised and hyperexcitable after denervation (due in part to reduced Na^+-K^+-ATPase activity; see Ch. 4) and this phenomenon contributes appreciably to their supersensitivity. Increased Ca^{2+} signalling, resulting in enhanced excitation–contraction coupling, may also occur.

Supersensitivity can occur, but is less marked, when transmission is interrupted by processes other than nerve section. Pharmacological block of ganglionic transmission, for example, if sustained for a few days, causes some degree of supersensitivity of the target organs, and long-term blockade of postsynaptic receptors also causes receptors to proliferate, leaving the cell supersensitive when the blocking agent is removed. Phenomena such as this are of importance in the central nervous system, where such supersensitivity can cause 'rebound' effects when drugs that impair synaptic transmission are given for some time and then discontinued.

PRESYNAPTIC MODULATION

The presynaptic terminals that synthesise and release transmitter in response to electrical activity in the nerve fibre are often themselves sensitive to transmitter substances and to other substances that may be produced locally in tissues (for review see Boehm & Kubista, 2002). Such presynaptic effects most commonly act to inhibit transmitter release, but may enhance it. Figure 12.3A shows the inhibitory effect of adrenaline on the release of acetylcholine (evoked by electrical stimulation) from the postganglionic parasympathetic nerve terminals of the intestine. The release of noradrenaline from nearby sympathetic nerve terminals can also inhibit release of acetylcholine. Noradrenergic and cholinergic nerve terminals often lie close together in the myenteric plexus, so the opposing effects of the sympathetic and parasympathetic systems result not only from the opposite effects of the two transmitters on the smooth muscle cells, but also from the inhibition of acetylcholine release by noradrenaline acting on the parasympathetic nerve terminals. A similar situation of mutual presynaptic inhibition exists in the heart, where noradrenaline inhibits acetylcholine release, as in the myenteric plexus, and acetylcholine also inhibits noradrenaline release. These are examples of *heterotropic interactions*, where one neurotransmitter affects the release of another. *Homotropic interactions* also occur, where the transmitter, by

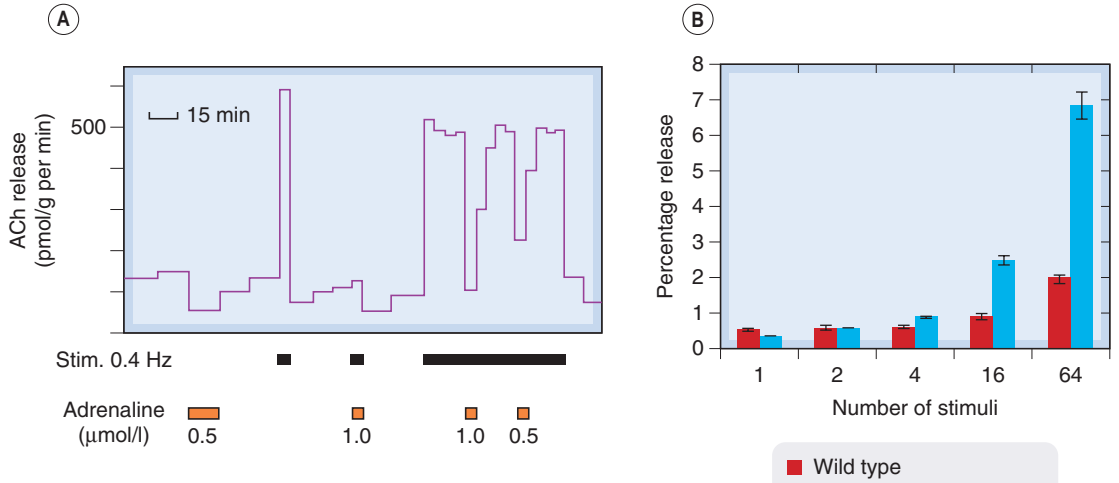


Fig. 12.3 Examples of presynaptic inhibition. [**A**] Inhibitory effect of adrenaline on acetylcholine (ACh) release from postganglionic parasympathetic nerves in the guinea pig ileum. The intramural nerves were stimulated electrically where indicated, and the ACh released into the bathing fluid determined by bioassay. Adrenaline strongly inhibits ACh release. [**B**] Noradrenaline release from mouse hippocampal slices in response to trains of electrical stimuli. Blue bars show normal (wild type) mice. Red bars show α_2-adrenoceptor knockout mice. The lack of presynaptic autoinhibition in the knockout mice results in a large increase in release with a long stimulus train, but does not affect release by fewer than four stimuli, because the autoinhibition takes a few seconds to develop. This example is taken from a study of brain noradrenergic nerves, but similar findings have been made on sympathetic nerves. *(Panel [A] from Vizi ES 1979 Prog Neurobiol 12, 181; panel [B] redrawn from Trendelenburg et al. 2001 Naunyn Schmiedeberg's Arch Pharmacol 364, 117–130.)*

binding to presynaptic autoreceptors, affects the nerve terminals from which it is being released. This type of *autoinhibitory feedback* acts powerfully at noradrenergic nerve terminals (see Starke et al., 1989). Figure 12.3B shows that in normal mice, noradrenaline release increases only slightly as the number of stimulus trains increases from 1 to 64. In transgenic mice lacking a specific type of presynaptic α_2 adrenoceptor (see Ch. 14), the amount released by the longer stimulus train is greatly increased, though the amount released by a single stimulus is unaffected. This is because with one or a few stimuli, there is no opportunity for autoinhibitory feedback to develop, whereas with longer trains the inhibition operates powerfully. A similar autoinhibitory feedback occurs with many transmitters, including acetylcholine and 5-hydroxytryptamine.

In both the noradrenergic and cholinergic systems, the presynaptic autoreceptors are pharmacologically distinct from the postsynaptic receptors (see Fig. 12.4 and Chs 13 and 14), and there are drugs that act selectively, as agonists or antagonists, on the pre- or postsynaptic receptors.

Cholinergic and noradrenergic nerve terminals respond not only to acetylcholine and noradrenaline, as described above, but also to other substances that are released as co-transmitters, such as ATP and neuropeptide Y (NPY), or derived from other sources, including nitric oxide, prostaglandins, adenosine, dopamine, 5-hydroxytryptamine, GABA, opioid peptides, endocannabinoids and many other substances. The physiological role and pharmacological significance of these various interactions is still unclear (see review by Vizi, 2001), but the description of the autonomic nervous system represented in Figure 12.2 is undoubtedly oversimplified. Figure 12.4 shows some of the main presynaptic interactions between autonomic neurons, and summarises the many chemical influences that regulate transmitter release from noradrenergic neurons.

Presynaptic receptors regulate transmitter release mainly by affecting Ca^{2+} entry into the nerve terminal (see Ch. 4), but also by other mechanisms (see Kubista & Boehm, 2006). Most presynaptic receptors are of the G protein-coupled type (see Ch. 3), which control the function of calcium channels and potassium channels either through second messengers that regulate the state of phosphorylation of the channel proteins, or by a direct interaction of G proteins with the channels. Transmitter release is inhibited when calcium channel opening is inhibited, or when potassium channel opening is increased (see Ch. 4); in many cases, both mechanisms operate simultaneously. Presynaptic regulation by receptors linked directly to ion channels (ionotropic receptors; see Ch. 3) rather than to G proteins also occurs (see Kubista & Boehm, 2006). Nicotinic acetylcholine receptors (nAChRs) are particularly important in this respect. They can either facilitate or inhibit the release of other transmitters, such as glutamate (see Ch. 38), and most of the nAChRs expressed in the central nervous system are located presynaptically. Another example is the $GABA_A$ receptor, whose action is to inhibit transmitter release (see Chs 4 and 37). Other ionotropic receptors, such as those activated by ATP and 5-hydroxytryptamine (Chs 15, 16 and 38), may have similar effects on transmitter release.

POSTSYNAPTIC MODULATION

Chemical mediators often act on postsynaptic structures, including neurons, smooth muscle cells, cardiac muscle cells, etc., in such a way that their excitability or spontaneous firing pattern is altered. In many cases, as with presynaptic modulation, this is caused by changes in calcium and/or potassium channel function mediated by a second messenger. We give only a few examples here.

- The slow excitatory effect produced by various mediators, including acetylcholine and peptides such as **substance P** (see Ch. 17), results mainly from a decrease in K^+ permeability. Conversely, the inhibitory effect of various opioids in the gut is mainly due to increased K^+ permeability.
- **Neuropeptide Y** (**NPY**), which is released as a co-transmitter with noradrenaline at many sympathetic nerve endings and acts on smooth muscle cells to enhance the vasoconstrictor effect of noradrenaline, thus greatly facilitating transmission.

The pre- and postsynaptic effects described above are often described as *neuromodulation*, because the mediator acts to increase or decrease the efficacy of synaptic transmission without participating directly as a transmitter. Many neuropeptides, for example, affect membrane ion channels in such a way as to increase or decrease excitability and thus control the firing pattern of the cell. Neuromodulation[1] is loosely defined but, in general, involves slower processes (taking seconds to days) than neurotransmission (which occurs in milliseconds), and operates through cascades of intracellular messengers (Ch. 3) rather than directly on ligand-gated ion channels.

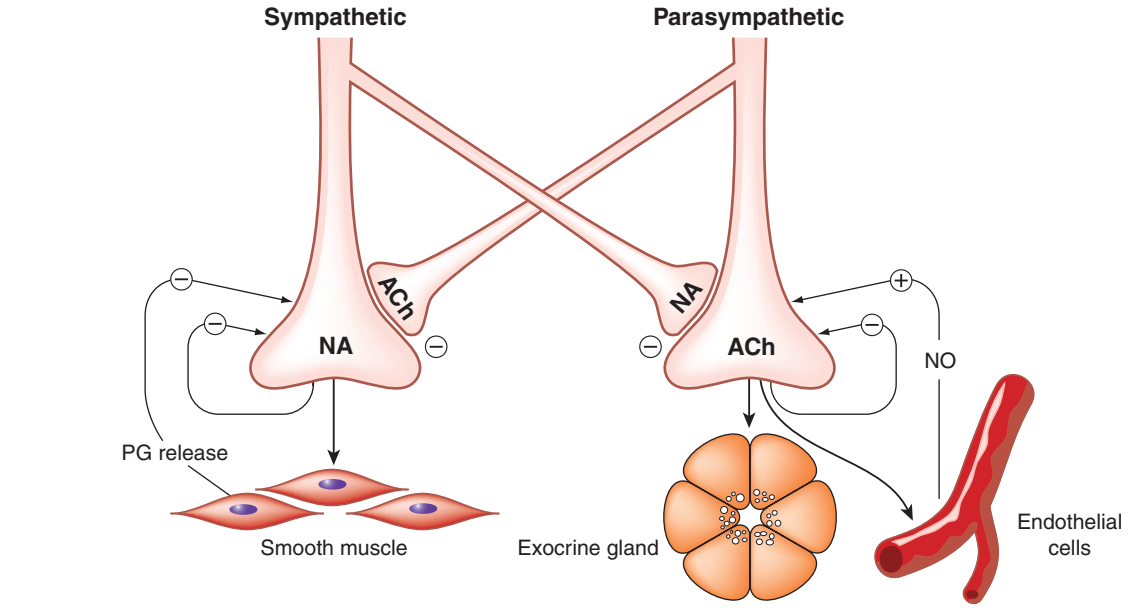
TRANSMITTERS OTHER THAN ACETYLCHOLINE AND NORADRENALINE

As mentioned above, acetylcholine or noradrenaline are not the only autonomic transmitters. The rather grudging realisation that this was so dawned many years ago when it was noticed that autonomic transmission in many organs could not be completely blocked by drugs that abolish responses to these transmitters. The dismal but tenacious term *non-adrenergic non-cholinergic* (NANC) transmission was coined. Later, fluorescence and immunocytochemical methods showed that neurons, including autonomic neurons, contain many potential transmitters, often several in the same cell. Compounds now known to function as NANC transmitters include ATP, vasoactive intestinal peptide (VIP), NPY and nitric oxide (see Fig. 12.5 and Table 12.2), which function at postganglionic nerve terminals, as well as substance P, 5-hydroxytryptamine, GABA and dopamine, which play a role in ganglionic transmission (see Lundberg, 1996, for a comprehensive review).

CO-TRANSMISSION

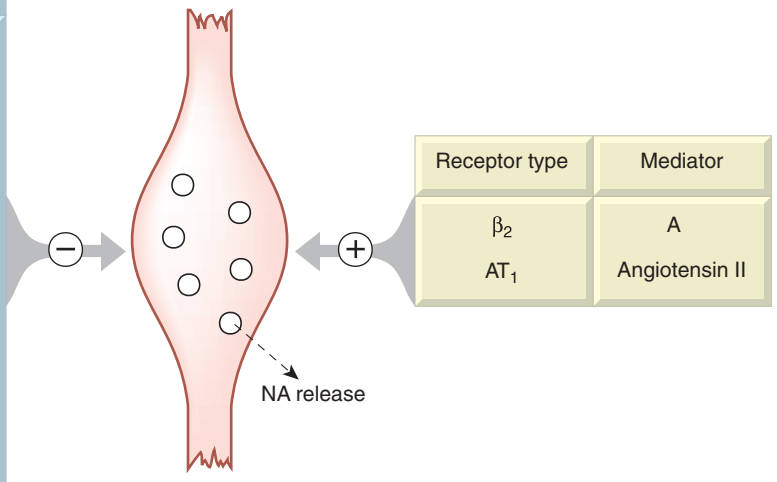
It is the rule rather than the exception that neurons release more than one transmitter or modulator (see Kupfermann, 1991; Lundberg, 1996), each of which interacts with specific receptors and produces effects, often both pre- and postsynaptically. The example of noradrenaline/ATP co-transmission at sympathetic nerve endings is shown in Figure 12.5, and the best-studied examples and

[1]Confusingly, the same term has been used to embrace a range of experimental therapeutic approaches based on nerve stimulation techniques, which have been claimed to be effective in a variety of neurological disorders such as bladder dysfunction, epilepsy and depression.



B

Mediator	Receptor type
ACh	Musc.
NA/A	α_2
5-HT	5-HT$_1$
Adenosine	A_1
PGE	EP
Histamine	H_2
Enkephalin	μ, δ
Dopamine	D_2
ATP	P2X / P2Y
Endocannabinoids	CB_1



Receptor type	Mediator
β_2	A
AT_1	Angiotensin II

Fig. 12.4 Presynaptic regulation of transmitter release from noradrenergic and cholinergic nerve terminals. [**A**] Postulated homotropic and heterotropic interactions between sympathetic and parasympathetic nerves. [**B**] Some of the known inhibitory and facilitatory influences on noradrenaline release from sympathetic nerve endings. 5-HT, 5-hydroxytryptamine; A, adrenaline; ACh, acetylcholine; NA, noradrenaline; NO, nitric oxide; PG, prostaglandin; PGE, prostaglandin E.

mechanisms are summarised in Table 12.2 and Figures 12.6 and 12.7.

What, one might well ask, could be the functional advantage of co-transmission, compared with a single transmitter acting on various different receptors? The possible advantages include the following.

- One constituent of the cocktail (e.g. a peptide) may be removed or inactivated more slowly than the other (e.g. a monoamine), and therefore reach targets further from the site of release and produce longer-lasting effects. This appears to be the case, for example, with acetylcholine and gonadotrophin-releasing hormone in sympathetic ganglia (Jan & Jan, 1983).
- The balance of the transmitters released may vary under different conditions. At sympathetic nerve terminals, for example, where noradrenaline and NPY are stored in separate vesicles, NPY is

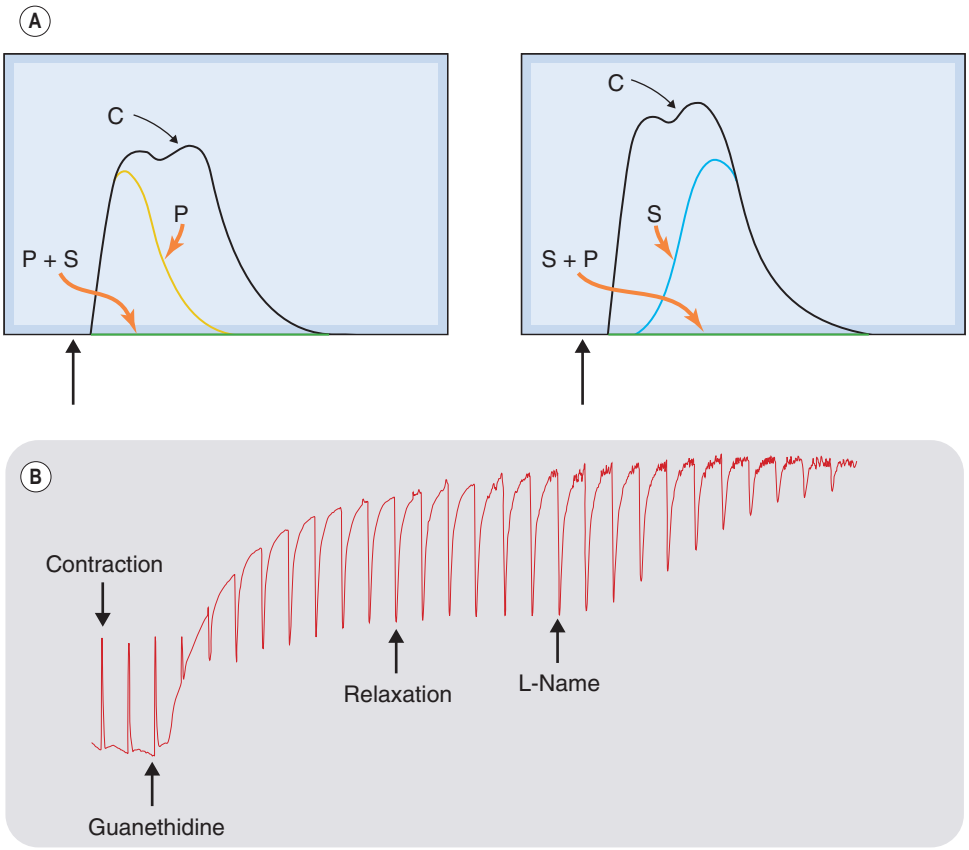


Fig. 12.5 **ATP and nitric oxide as neurotransmitters.** [**A**] Noradrenaline and ATP are co-transmitters released from the same nerves in the guinea pig vas deferens. Contractions of the tissue are shown in response to a single electrical stimulus causing excitation of sympathetic nerve endings. With no blocking drugs present, a twin-peaked response is produced (C). The early peak is selectively abolished by the ATP antagonist suramin (S), while the late peak is blocked by the α1-adrenoceptor antagonist prazosin (P). The response is completely eliminated when both drugs are present. [**B**] Noradrenaline and nitric oxide are neurotransmitters in the rat anococcygeus muscle but are probably released from different nerves. The nerves innervating the muscle were stimulated with brief trains of pulses. Initially nerve stimulation evoked rapid contractions by releasing noradrenaline. Application of guanethidine blocked stimulus-evoked noradrenaline release and raised the tone of the preparation revealing nerve-evoked relaxations that were blocked by L-NAME an inhibitor of nitric oxide synthesis. *(Panel [A] reproduced with permission from von Kugelglen I, Starke K 1991 Trends Pharmacol Sci 12, 319–324; data in panel [B] are from a student practical class at Glasgow Caledonian University, courtesy of A Corbett.)*

preferentially released at high stimulation frequencies, so that differential release of one or other mediator may result from varying impulse patterns. Differential effects of presynaptic modulators are also possible; for example, activation of β adrenoceptors inhibits ATP release while enhancing noradrenaline release from sympathetic nerve terminals (Gonçalves et al., 1996).

TERMINATION OF TRANSMITTER ACTION

Chemically transmitting synapses other than the peptidergic variety (Ch. 17) invariably incorporate a mechanism for disposing rapidly of the released transmitter, so that its action remains brief and localised. At cholinergic synapses (Ch. 13), the released acetylcholine is inactivated very rapidly in the synaptic cleft by *acetylcholinesterase*. In most other cases (see Fig. 12.8), transmitter action is terminated by active reuptake into the presynaptic nerve, or into supporting cells such as glia. Such reuptake depends on transporter proteins (see Ch. 4), each being specific for a particular transmitter. The major class (Na^+/Cl^- co-transporters), whose molecular structure and function are well understood (see Nelson, 1998; Torres et al., 2003; Gether et al., 2006), consists of a family of membrane proteins, each possessing 12 transmembrane helices. Different members of the family show selectivity for each of the main monoamine transmitters (e.g. the noradrenaline [norepinephrine] transporter, NET, the serotonin transporter, SERT, which transports 5-hydroxytryptamine and the dopamine transporter, DAT). These transporters are important targets for psychoactive drugs, particularly antidepressants (Ch. 47), anxiolytic drugs (Ch. 44) and stimulants (Ch. 48). Transporters for glycine and GABA belong to the same family.

Vesicular transporters (Ch. 4), which load synaptic vesicles with transmitter molecules, are closely related to the membrane transporters. Membrane transporters usually act as co-transporters of Na^+, Cl^- and transmitter molecules, and it is the inwardly directed 'downhill' gradient for Na^+ that provides the energy for the inward 'uphill' movement of the transmitter. The simultaneous transport of ions along with the transmitter means that the process generates a net current across the membrane, which can

Table 12.2 Examples of non-adrenergic non-cholinergic transmitters and co-transmitters in the peripheral nervous system

Transmitter	Location	Function
Non-peptides		
ATP	Postganglionic sympathetic neurons	Fast depolarisation/contraction of smooth muscle cells (e.g. blood vessels, vas deferens)
GABA, 5-HT	Enteric neurons	Peristaltic reflex
Dopamine	Some sympathetic neurons (e.g. kidney)	Vasodilatation
Nitric oxide	Pelvic nerves Gastric nerves	Erection Gastric emptying
Peptides		
Neuropeptide Y	Postganglionic sympathetic neurons	Facilitates constrictor action of noradrenaline; inhibits noradrenaline release (e.g. blood vessels)
Vasoactive intestinal peptide (VIP)	Parasympathetic nerves to salivary glands NANC innervation of airways smooth muscle	Vasodilatation; co-transmitter with acetylcholine Bronchodilatation
Gonadotrophin-releasing hormone	Sympathetic ganglia	Slow depolarisation; co-transmitter with acetylcholine
Substance P	Sympathetic ganglia, enteric neurons	Slow depolarisation; co-transmitter with acetylcholine
Calcitonin gene-related peptide	Non-myelinated sensory neurons	Vasodilatation; vascular leakage; neurogenic inflammation

5-HT, 5-hydroxytryptamine; ATP, adenosine triphosphate; GABA, gamma-aminobutyric acid; NANC, non-adrenergic non-cholinergic.

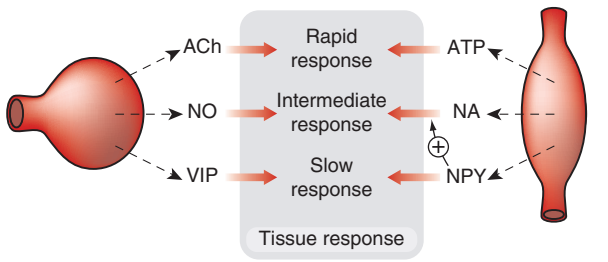


Fig. 12.6 The main co-transmitters at postganglionic parasympathetic and sympathetic neurons. The different mediators generally give rise to fast, intermediate and slow responses of the target organ. ACh, acetylcholine; ATP, adenosine triphosphate; NA, noradrenaline; NO, nitric oxide; NPY, neuropeptide Y; VIP, vasoactive intestinal peptide.

Neuromodulation and presynaptic interactions

- As well as functioning directly as neurotransmitters, chemical mediators may regulate:
  - presynaptic transmitter release
  - neuronal excitability.
- Both are examples of *neuromodulation* and generally involve second messenger regulation of membrane ion channels.
- Presynaptic receptors may inhibit or increase transmitter release, the former being more important.
- Inhibitory *presynaptic autoreceptors* occur on noradrenergic and cholinergic neurons, causing each transmitter to inhibit its own release (*autoinhibitory feedback*).
- Many endogenous mediators (e.g. GABA, prostaglandins, opioid and other peptides), as well as the transmitters themselves, exert presynaptic control (mainly inhibitory) over autonomic transmitter release.

be measured directly and used to monitor the transport process. Very similar mechanisms are responsible for other physiological transport processes, such as glucose uptake (Ch. 31) and renal tubular transport of amino acids. Because it is the electrochemical gradient for sodium that drives the inward transport of transmitter molecules, a reduction of this gradient can reduce or even reverse the flow of transmitter. This is probably not important under normal conditions, but when the nerve terminals are depolarised or abnormally loaded with sodium (e.g. in ischaemic conditions), the resulting non-vesicular release of transmitter (and inhibition of the normal synaptic reuptake mechanism) may play a significant role in the effects of ischaemia on tissues such as heart and brain (see Chs 21 and 40). Studies with transgenic 'knockout' mice (see Torres et al., 2003) show that the store of releasable transmitter is substantially depleted in animals lacking the membrane transporter, showing that synthesis is unable to maintain the store if the recapture mechanism is disabled. As with receptors (see Ch. 3), many genetic polymorphisms of transporter genes occur in humans, which raised hopes of finding associations with various neurological, cardiovascular and psychiatric disorders. But despite intensive research efforts, the links remain elusive (see Lin & Madras, 2006).

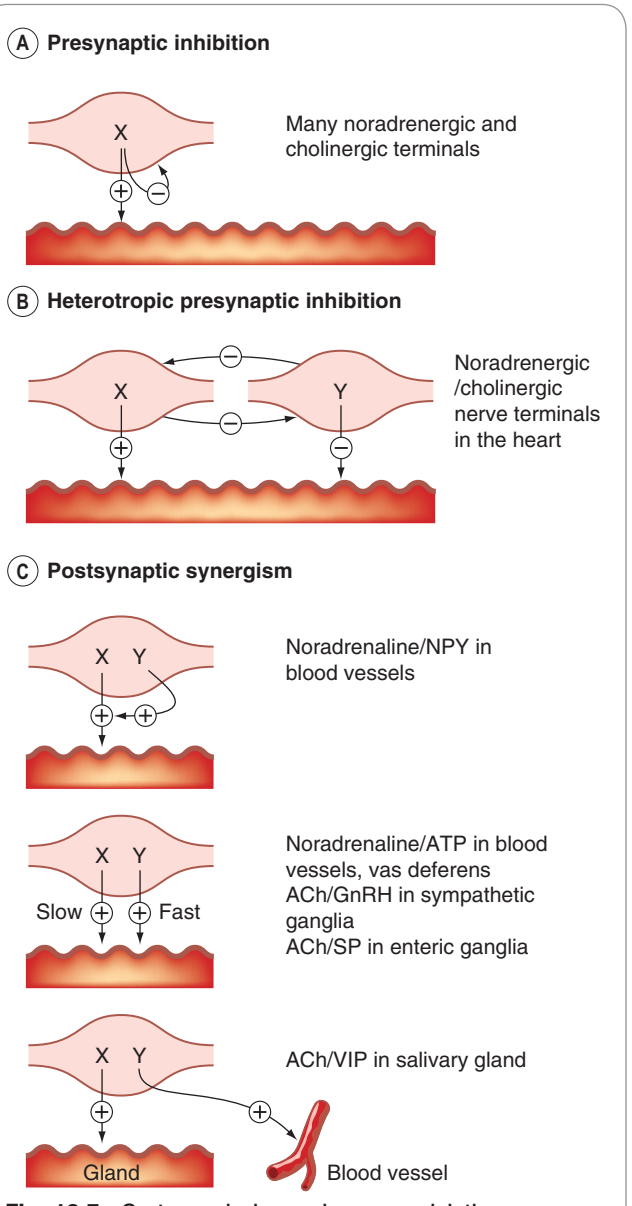


Fig. 12.7 Co-transmission and neuromodulation – some examples. [**A**] Presynaptic inhibition. [**B**] Heterotropic presynaptic inhibition. [**C**] Postsynaptic synergism. ACh, acetylcholine; ATP, adenosine triphosphate; GnRH, gonadotrophin-releasing hormone (luteinising hormone-releasing hormone); NPY, neuropeptide Y; SP, substance P; VIP, vasoactive intestinal peptide.

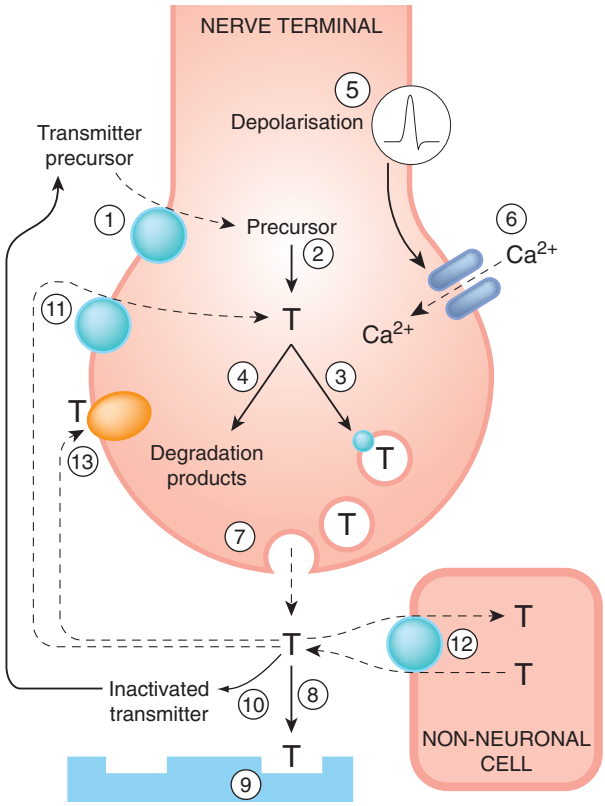


Fig. 12.8 The main processes involved in synthesis, storage and release of amine and amino acid transmitters. 1, Uptake of precursors; 2, synthesis of transmitter; 3, uptake/transport of transmitter into vesicles; 4, degradation of surplus transmitter; 5, depolarisation by propagated action potential; 6, influx of Ca^{2+} in response to depolarisation; 7, release of transmitter by exocytosis; 8, diffusion to postsynaptic membrane; 9, interaction with postsynaptic receptors; 10, inactivation of transmitter; 11, reuptake of transmitter or degradation products by nerve terminals; 12, uptake and release of transmitter by non-neuronal cells; and 13, interaction with presynaptic receptors. The transporters (11 and 12) can release transmitter under certain conditions by working in reverse. These processes are well characterised for many transmitters (e.g. acetylcholine, monoamines, amino acids, ATP). Peptide mediators (see Ch. 17) differ in that they may be synthesised and packaged in the cell body rather than the terminals.

As we shall see in subsequent chapters, both membrane and vesicular transporters are targets for various drug effects, and defining the physiological role and pharmacological properties of these molecules is the focus of much current research.

BASIC STEPS IN NEUROCHEMICAL TRANSMISSION: SITES OF DRUG ACTION

Figure 12.8 summarises the main processes that occur in a classical chemically transmitting synapse, and provides a useful basis for understanding the actions of the many different classes of drug, discussed in later chapters, that act by facilitating or blocking neurochemical transmission.

All the steps shown in Figure 12.8 (except for transmitter diffusion, step 8) can be influenced by drugs. For example, the enzymes involved in synthesis or inactivation of the transmitter can be inhibited, as can the transport systems responsible for the neuronal and vesicular uptake of the transmitter or its precursor. The actions of the great majority of drugs that act on the peripheral nervous system (Chs 13 and 14) and the central nervous system fit into this general scheme.

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13 Cholinergic transmission

OVERVIEW

This chapter is concerned mainly with cholinergic transmission in the periphery, and the ways in which drugs affect it. Here we describe the different types of acetylcholine (ACh) receptors and their functions, as well as the synthesis and release of ACh. Drugs that act on ACh receptors, many of which have clinical uses, are described in this chapter. Cholinergic mechanisms in the central nervous system (CNS) and their relevance to dementia are discussed in Chapters 39 and 40.

MUSCARINIC AND NICOTINIC ACTIONS OF ACETYLCHOLINE

▼ The discovery of the pharmacological action of ACh came, paradoxically, from work on adrenal glands, extracts of which were known to produce a rise in blood pressure owing to their content of adrenaline (epinephrine). In 1900, Reid Hunt found that after adrenaline had been removed from such extracts, they produced a fall in blood pressure instead of a rise. He attributed the fall to the presence of choline, but later concluded that a more potent derivative of choline must be responsible. With Taveau, he tested a number of choline derivatives and discovered that ACh was some 100 000 times more active than choline in lowering the rabbit's blood pressure. The physiological role of ACh was not apparent at that time, and it remained a pharmacological curiosity until Loewi and Dale and their colleagues discovered its transmitter role in the 1930s.

Analysing the pharmacological actions of ACh in 1914, Dale distinguished two types of activity, which he designated as *muscarinic* and *nicotinic* because they mimicked, respectively, the effects of injecting **muscarine**, the active principle of the poisonous mushroom *Amanita muscaria*, and of injecting **nicotine**. Muscarinic actions closely resemble the effects of parasympathetic stimulation, as shown in Table 12.1. After the muscarinic effects have been blocked by **atropine**, larger doses of ACh produce nicotine-like effects, which include:

- stimulation of all autonomic ganglia
- stimulation of voluntary muscle
- secretion of adrenaline from the adrenal medulla.

The muscarinic and nicotinic actions of ACh are demonstrated in Figure 13.1. Small and medium doses of ACh produce a transient fall in blood pressure due to arteriolar vasodilatation and slowing of the heart – muscarinic effects that are abolished by atropine. A large dose of ACh given after atropine produces nicotinic effects: an initial rise in blood pressure due to a stimulation of sympathetic ganglia and consequent vasoconstriction, and a secondary rise resulting from secretion of adrenaline.

Dale's pharmacological classification corresponds closely to the main physiological functions of ACh in the body. The muscarinic actions correspond to those of ACh released at postganglionic parasympathetic nerve endings, with two significant exceptions:

1. Acetylcholine causes generalised vasodilatation, even though most blood vessels have no parasympathetic innervation. This is an indirect effect: ACh (like many other mediators) acts on vascular endothelial cells to release **nitric oxide** (see Ch. 20), which relaxes smooth muscle. The physiological function of this is uncertain, because ACh is not normally present in circulating blood.
2. Acetylcholine evokes secretion from sweat glands, which are innervated by cholinergic fibres of the sympathetic nervous system (see Table 12.1).

The nicotinic actions correspond to those of ACh acting on autonomic ganglia of the sympathetic and parasympathetic systems, the motor endplate of voluntary muscle and the secretory cells of the adrenal medulla.

ACETYLCHOLINE RECEPTORS

Although Dale himself dismissed the concept of receptors as sophistry rather than science, his functional classification provided the basis for distinguishing the two major classes of ACh receptor (see Ch. 3).

NICOTINIC RECEPTORS

Nicotinic ACh receptors (nAChRs) fall into three main classes – the muscle, ganglionic and CNS types – whose subunit compositions are summarised in Table 13.1. Muscle receptors are confined to the skeletal neuromuscular junction; ganglionic receptors are responsible for transmission at sympathetic and parasympathetic ganglia; and CNS-type receptors are widespread in the brain, and are heterogeneous with respect to their molecular composition and location (see Ch. 39). Most of the CNS-type nAChRs are located presynaptically and serve to facilitate or inhibit the release of other mediators, such as glutamate and dopamine.

▼ All nAChRs are pentameric structures that function as ligand-gated ion channels (see Ch. 3). The five subunits that form the receptor–channel complex are similar in structure, and so far 17 different members of the family have been identified and cloned, designated α (10 types), β (four types), γ, δ and ε (one of each). The five subunits each possess four membrane-spanning helical domains, and one of these helices (M_2) from each subunit defines the central pore (see Ch. 3). nAChR subtypes generally contain both α and β subunits, the exception being the homomeric $(\alpha7)_5$ subtype found mainly in the brain (Ch. 39). The adult muscle receptor has the composition $(\alpha1)_2/\beta1\varepsilon\delta$, while the main ganglionic subtype is $(\alpha3)_2(\beta2)_3$. The two binding sites for ACh (both of which need to be occupied to cause the channel to open) reside at the interface between the extracellular domain of each of the α subunits and its neighbour. The diversity of the nAChR family (for details see Kalamida et al., 2007), which emerged from cloning studies in the

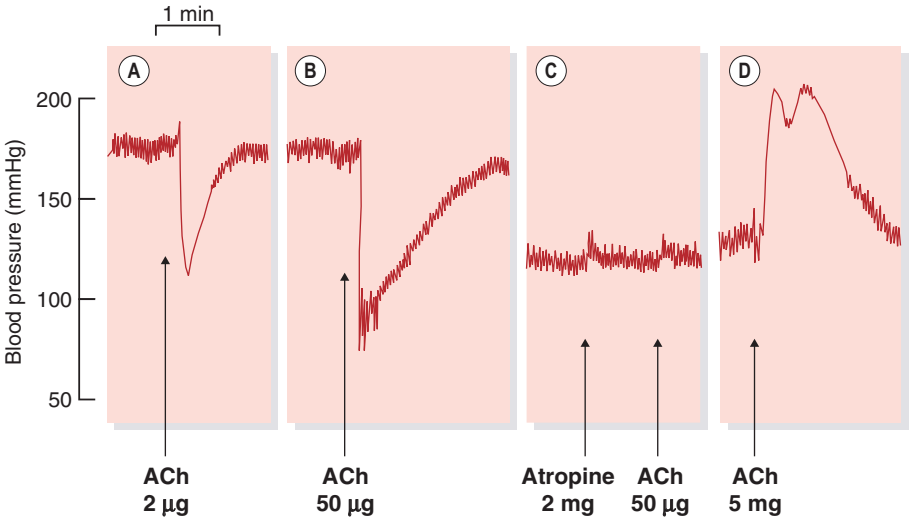


Fig. 13.1 Dale's experiment showing that acetylcholine (ACh) produces two kinds of effect on the cat's blood pressure. Arterial pressure was recorded with a mercury manometer from a spinal cat. [**A**] ACh causes a fall in blood pressure due to vasodilatation. [**B**] A larger dose also produces bradycardia. Both [**A**] and [**B**] are muscarinic effects. [**C**] After atropine (muscarinic antagonist), the same dose of ACh has no effect. [**D**] Still under the influence of atropine, a much larger dose of ACh causes a rise in blood pressure (due to stimulation of sympathetic ganglia), accompanied by tachycardia, followed by a secondary rise (due to release of adrenaline from the adrenal gland). These effects result from its action on nicotinic receptors. *(From Burn JH 1963 Autonomic Pharmacology. Blackwell, Oxford.)*

Table 13.1 Nicotinic receptor subtypes[a]

	Muscle type	Ganglion type	CNS types		Notes
Main molecular form	$(\alpha1)_2\beta1\delta\varepsilon$ (adult form)	$(\alpha3)_2(\beta2)_3$	$(\alpha4)_2(\beta2)_3$	$(\alpha7)_5$	–
Main synaptic location	Skeletal neuromuscular junction: mainly postsynaptic	Autonomic ganglia: mainly postsynaptic	Many brain regions: pre- and postsynaptic	Many brain regions: pre- and postsynaptic	–
Membrane response	Excitatory Increased cation permeability (mainly Na^+, K^+)	Excitatory Increased cation permeability (mainly Na^+, K^+)	Pre- and postsynaptic excitation Increased cation permeability (mainly Na^+, K^+)	Pre- and postsynaptic excitation Increased cation permeability	$(\alpha7)_5$ receptor produces large Ca^{2+} entry, evoking transmitter release
Agonists	Acetylcholine Carbachol Succinylcholine	Acetylcholine Carbachol Nicotine Epibatidine Dimethylphenyl-piperazinium	Nicotine Epibatidine Acetylcholine Cytosine Varenicline[b]	Epibatidine Dimethylphenyl-piperazinium Varenicline[b]	$(\alpha4)_2(\beta2)_3$ is main brain 'nicotine receptor'. See Ch. 39
Antagonists	Tubocurarine Pancuronium Atracurium Vecuronium α-Bungarotoxin α-Conotoxin	Mecamylamine Trimetaphan Hexamethonium α-Conotoxin	Mecamylamine Methylaconitine	α-Bungarotoxin α-Conotoxin Methylaconitine	

[a]This table shows only the main subtypes expressed in mammalian tissues. Several other subtypes are expressed in selected brain regions, and also in the peripheral nervous system and in non-neuronal tissues. For further details, see Ch. 39 and review by Kalamida et al. (2007).

[b]Varenicline is a recently introduced drug for smoking cessation. It acts as a partial agonist on $(\alpha4)_2(\beta2)_3$ receptors and a full agonist on $(\alpha7)_5$ receptors (see Ch. 49).

1980s, took pharmacologists somewhat by surprise. Although they knew that the neuromuscular and ganglionic synapses differed pharmacologically, and suspected that cholinergic synapses in the CNS might be different again, the molecular diversity goes far beyond this, and its functional significance is only slowly emerging.

The different action of agonists and antagonists on neuromuscular, ganglionic and brain synapses is of practical importance and mainly reflects the differences between the muscle and neuronal nAChRs (Table 13.1).

MUSCARINIC RECEPTORS

Muscarinic receptors (mAChRs) are typical G protein-coupled receptors (see Ch. 3), and five molecular subtypes (M_1–M_5) are known. The odd-numbered members of the group (M_1, M_3, M_5) couple with G_q to activate the inositol phosphate pathway (Ch. 3), while the even-numbered receptors (M_2, M_4) open potassium (K_{ATP}) channels causing membrane hyperpolarisation as well as acting through G_i to inhibit adenylyl cyclase and thus reduce intracellular cAMP. Both groups activate the MAP kinase pathway. The location and pharmacology of these subtypes are summarised in Table 13.2.

M_1 receptors ('neural') are found mainly on CNS and peripheral neurons and on gastric parietal cells. They mediate excitatory effects, for example the slow muscarinic excitation mediated by ACh in sympathetic ganglia (Ch. 12) and central neurons. This excitation is produced by a decrease in K^+ conductance, which causes membrane depolarisation. Deficiency of this kind of ACh-mediated effect in the brain is possibly associated with dementia (see Ch. 40), although transgenic M_1-receptor knockout mice show only slight cognitive impairment (see Wess et al., 2007). M_1 receptors are also involved in the increase of gastric acid secretion following vagal stimulation (see Ch. 30).

Table 13.2 Muscarinic receptor subtypes[a]

	M_1 ('neural')	M_2 ('cardiac')	M_3 ('glandular/ smooth muscle')	M_4	M_5
Main locations	Autonomic ganglia (including intramural ganglia in stomach) Glands: salivary, lacrimal, etc. Cerebral cortex	Heart: atria CNS: widely distributed	Exocrine glands: gastric (acid-secreting parietal cells), salivary, etc. Smooth muscle: gastrointestinal tract, eye, airways, bladder Blood vessels: endothelium	CNS	CNS: very localised expression in substantia nigra Salivary glands Iris/ciliary muscle
Cellular response	↑ IP_3, DAG Depolarisation Excitation (slow epsp) ↓ K^+ conductance	↓ cAMP Inhibition ↓ Ca^{2+} conductance ↑ K^+ conductance	↑ IP_3 Stimulation ↑ $[Ca^{2+}]_i$	↓ cAMP Inhibition	↑ IP_3 Excitation
Functional response	CNS excitation (? improved cognition) Gastric secretion	Cardiac inhibition Neural inhibition Central muscarinic effects (e.g. tremor, hypothermia)	Gastric, salivary secretion Gastrointestinal smooth muscle contraction Ocular accommodation Vasodilatation	Enhanced locomotion	Not known
Non-selective agonists (see also Table 13.3)	Acetylcholine Carbachol Oxotremorine Pilocarpine **Bethanechol**				
Selective agonists	McNA343		**Cevimeline**		
Non-selective antagonists (see also Table 13.5)	**Atropine** **Dicycloverine** **Tolterodine** **Oxybutynin** **Ipratropium**				
Selective antagonists	**Pirenzepine** Mamba toxin MT7	Gallamine (see p. 158)	**Darifenacin**	Mamba toxin MT3	

[a]This table shows only the predominant subtypes expressed in mammalian tissues. For further details, see Ch. 39 and review by and Kalamida et al. (2007).

CNS, central nervous system; DAG, diacylglycerol; epsp, excitatory postsynaptic potential; IP_3, inositol trisphosphate.

Drugs in clinical use are shown in **bold**.

M_2 receptors (*'cardiac'*) occur in the heart, and also on the presynaptic terminals of peripheral and central neurons. They exert inhibitory effects, mainly by increasing K^+ conductance and by inhibiting calcium channels (see Ch. 4). M_2-receptor activation is responsible for cholinergic inhibition of the heart, as well as presynaptic inhibition in the CNS and periphery (Ch. 12). They are also co-expressed with M_3 receptors in visceral smooth muscle, and contribute to the smooth-muscle-stimulating effect of muscarinic agonists in several organs.

M_3 receptors (*'glandular/smooth muscle'*) produce mainly excitatory effects, i.e. stimulation of glandular secretions (salivary, bronchial, sweat, etc.) and contraction of visceral smooth muscle. M_3 receptors also mediate relaxation of smooth muscle (mainly vascular), which results from the release of nitric oxide from neighbouring endothelial cells (Ch. 20). M_3 receptors occur also in specific locations in the CNS (see Ch. 39).

M_4 and M_5 receptors are largely confined to the CNS, and their functional role is not well understood, although mice lacking these receptors do show behavioural changes (Wess et al., 2007). Recently it has been discovered that cytokine secretion from lymphocytes and other cells is regulated by M_1 and M_3 receptors, while M_2 and M_4 receptors affect cell proliferation in various situations, opening up the possibility of new therapeutic roles for muscarinic receptor ligands (see Wessler & Kirkpatrick, 2008).

The agonist binding region is highly conserved between the different subtypes, so attempts to develop selective agonists and antagonists have had limited success. Most known agonists are non-selective, though two experimental compounds, **McNA343** and **oxotremorine**, are selective for M_1 receptors, on which **carbachol** is relatively inactive. **Cevimeline,** a selective M_3-receptor agonist, is used to improve salivary and lacrimal secretion in Sjögren's syndrome, an autoimmune disorder characterised by dryness of mouth and eyes. It is possible that new allosteric mAChR ligands, targeted at sites outside the agonist binding domain (see Ch. 3, Fig. 3.7), will allow better subtype selectivity for drugs acting on this important class of receptors (see Conn et al., 2009).

There is more selectivity among antagonists. Although most of the classic muscarinic antagonists (e.g. **atropine**, **hyoscine**) are non-selective, **pirenzepine** (previously used for peptic ulcer disease) is selective for M_1 receptors, and **darifenacin** (used for urinary incontinence in adults with detrusor muscle instability, known as 'overactive bladder') is selective for M_3 receptors. **Gallamine**, once used as a neuromuscular-blocking drug, is also a selective, although weak, M_2 receptor antagonist.[1] Toxins from the venom of the green mamba have been discovered to be highly selective mAChR antagonists (see Table 13.2).

PHYSIOLOGY OF CHOLINERGIC TRANSMISSION

The physiology of cholinergic neurotransmission is described in detail by Nicholls et al. (2012). The main ways in which drugs can affect cholinergic transmission are shown in Figure 13.2.

[1]Unlike most other antagonists, gallamine acts *allosterically* (i.e. at a site distinct from the ACh binding site).

Acetylcholine receptors

- Main subdivision is into nicotinic (nAChR) and muscarinic (mAChR) subtypes.
- nAChRs are directly coupled to cation channels, and mediate fast excitatory synaptic transmission at the neuromuscular junction, autonomic ganglia and various sites in the central nervous system (CNS). Muscle and neuronal nAChRs differ in their molecular structure and pharmacology.
- mAChRs and nAChRs occur presynaptically as well as postsynaptically, and function to regulate transmitter release.
- mAChRs are G protein-coupled receptors causing:
  - activation of phospholipase C (hence formation of inositol trisphosphate and diacylglycerol as second messengers)
  - inhibition of adenylyl cyclase
  - activation of potassium channels or inhibition of calcium channels.
- mAChRs mediate acetylcholine effects at postganglionic parasympathetic synapses (mainly heart, smooth muscle and glands), and contribute to ganglionic excitation. They occur in many parts of the CNS.
- Three main types of mAChR occur:
  - M_1 receptors ('neural') producing slow excitation of ganglia. They are selectively blocked by **pirenzepine**.
  - M_2 receptors ('cardiac') causing decrease in cardiac rate and force of contraction (mainly of atria). They are selectively blocked by **gallamine**. M_2 receptors also mediate presynaptic inhibition.
  - M_3 receptors ('glandular') causing secretion, contraction of visceral smooth muscle, vascular relaxation. **Cevimeline** is a selective M_3 agonist.
- Two further molecular mAChR subtypes, M_4 and M_5, occur mainly in the CNS.
- All mAChRs are activated by acetylcholine and blocked by **atropine**. There are also subtype-selective agonists and antagonists.

ACETYLCHOLINE SYNTHESIS AND RELEASE

ACh is synthesised within the nerve terminal from choline, which is taken up into the nerve terminal by a specific transporter (Ch. 12), similar to those that operate for many transmitters. The difference is that it transports the precursor, choline, not ACh, so it is not important in terminating the action of the transmitter. The concentration of choline in the blood and body fluids is normally about 10 µmol/l, but in the immediate vicinity of cholinergic nerve terminals it increases, probably to about 1 mmol/l, when the released ACh is hydrolysed, and more than 50% of this choline is normally recaptured by the nerve terminals. Free choline within the nerve terminal is acetylated by a cytosolic enzyme, *choline acetyltransferase* (CAT), which transfers the acetyl group from acetyl coenzyme A. The rate-limiting process in ACh synthesis appears to be choline transport, which is determined by the extracellular choline concentration and hence is linked to the rate at which ACh is being released (Fig. 13.2). *Cholinesterase*

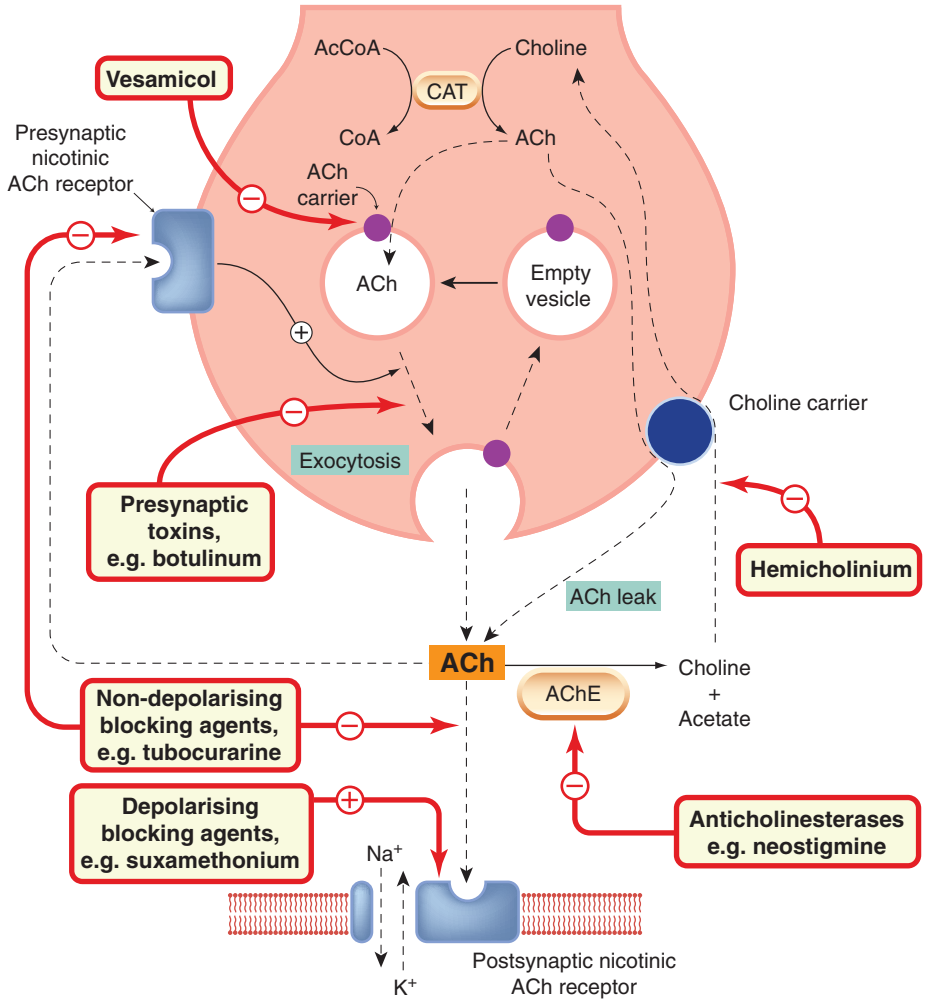


Fig. 13.2 **Events and sites of drug action at a nicotinic cholinergic synapse.** Acetylcholine (ACh) is shown acting postsynaptically on a nicotinic receptor controlling a cation channel (e.g. at the neuromuscular or ganglionic synapse), and also on a presynaptic nicotinic receptor that acts to facilitate ACh release during sustained synaptic activity. The nerve terminal also contains acetylcholinesterase (not shown); when this is inhibited, the amount of free ACh, and the rate of leakage of ACh via the choline carrier, is increased. Under normal conditions, this leakage of ACh is insignificant. At muscarinic cholinergic junctions (e.g. heart, smooth muscle and exocrine glands), both postsynaptic and presynaptic (inhibitory) receptors are of the muscarinic type. AcCoA, acetyl coenzyme A; AChE, acetylcholinesterase; CAT, choline acetyltransferase; CoA, coenzyme A.

is present in the presynaptic nerve terminals, and ACh is continually being hydrolysed and resynthesised. Inhibition of the nerve terminal cholinesterase causes the accumulation of 'surplus' ACh in the cytosol, which is not available for release by nerve impulses (although it is able to leak out via the choline carrier). Most of the ACh synthesised, however, is packaged into synaptic vesicles, in which its concentration is extraordinarily high (about 100 mmol/l), and from which release occurs by exocytosis triggered by Ca^{2+} entry into the nerve terminal (see Ch. 4).

Cholinergic vesicles accumulate ACh actively, by means of a specific transporter belonging to the family of amine transporters described in Chapter 12. Accumulation of ACh is coupled to the large electrochemical gradient for protons that exists between acidic intracellular organelles and the cytosol; it is blocked selectively by the experimental drug **vesamicol**. Following its release, ACh diffuses across the synaptic cleft to combine with receptors on the postsynaptic cell. Some of it succumbs on the way to hydrolysis by *acetylcholinesterase* (AChE), an enzyme that is bound to the basement membrane that lies between the pre- and postsynaptic membranes. At fast cholinergic synapses (e.g. the neuromuscular and ganglionic synapses), but not at slow ones (smooth muscle, gland cells, heart, etc.), the released ACh is hydrolysed very rapidly (within 1 ms), so that it acts only very briefly.

▼ At the neuromuscular junction, which is a highly specialised synapse, a single nerve impulse releases about 300 synaptic vesicles (altogether about 3 million ACh molecules) from the nerve terminals supplying a single muscle fibre, which contain altogether about 3 million synaptic vesicles. Approximately two million ACh molecules combine with receptors, of which there are about 30 million on each muscle fibre, the rest being hydrolysed without reaching a receptor. The ACh molecules remain bound to receptors for, on average, about 2 ms, and are quickly hydrolysed after dissociating, so that they cannot combine with a second receptor. The result is that transmitter action is very rapid and very brief, which is important for a synapse that has to initiate speedy muscular responses, and that may have to transmit signals faithfully at high frequency. Muscle cells are much larger than neurons and require much more synaptic current to generate an action potential. Thus all the

chemical events happen on a larger scale than at a neuronal synapse; the number of transmitter molecules in a quantum, the number of quanta released, and the number of receptors activated by each quantum are all 10–100 times greater. Our brains would be huge, but not very clever, if their synapses were built on the industrial scale of the neuromuscular junction.

PRESYNAPTIC MODULATION

Acetylcholine release is regulated by mediators, including ACh itself, acting on presynaptic receptors, as discussed in Chapter 12. At postganglionic parasympathetic nerve endings, inhibitory M_2 receptors participate in autoinhibition of ACh release; other mediators, such as noradrenaline, also inhibit the release of ACh (see Ch. 12). At the neuromuscular junction, on the other hand, presynaptic nAChRs facilitate ACh release, a mechanism that may allow the synapse to function reliably during prolonged high-frequency activity whereas, as mentioned above, presynaptic CNS-type nAChRs either facilitate or inhibit the release of other mediators.

ELECTRICAL EVENTS IN TRANSMISSION AT FAST CHOLINERGIC SYNAPSES

Acetylcholine, acting on the postsynaptic membrane of a nicotinic (neuromuscular or ganglionic) synapse, causes a large increase in its permeability to cations, particularly to Na^+ and K^+, and to a lesser extent Ca^{2+}. The resulting inflow of Na^+ depolarises the postsynaptic membrane. This transmitter-mediated depolarisation is called an *end-plate potential* (*epp*) in a skeletal muscle fibre, or a *fast excitatory postsynaptic potential* (*fast epsp*) at the ganglionic synapse. In a muscle fibre, the localised epp spreads to adjacent, electrically excitable parts of the muscle fibre; if its amplitude reaches the threshold for excitation, an action potential is initiated, which propagates to the rest of the fibre and evokes a contraction (Ch. 4).

In a nerve cell, depolarisation of the soma or a dendrite by the fast epsp causes a local current to flow. This depolarises the axon hillock region of the cell, where, if the epsp is large enough, an action potential is initiated. Figure 13.3 shows that **tubocurarine**, a drug that blocks postsynaptic ACh receptors, reduces the amplitude of the fast epsp until it no longer initiates an action potential, although the cell is still capable of responding when it is stimulated electrically. Most ganglion cells are supplied by several presynaptic axons, and it requires simultaneous activity in more than one to make the postganglionic cell fire (integrative action). At the neuromuscular junction, only one nerve fibre supplies each muscle fibre – like a relay station in a telegraph line the synapse ensures faithful 1:1 transmission despite the impedance mismatch between the fine nerve fibre and the much larger muscle fibre. The amplitude of the epp is normally more than enough to initiate an action potential – indeed, transmission still occurs when the epp is reduced by 70–80%, showing a large margin of safety so that fluctuations in transmitter release (e.g. during repetitive stimulation) do not affect transmission.

▼ Transmission at the ganglionic synapse is more complex than at the neuromuscular junction. Although the primary event at both is the epp or fast epsp produced by ACh acting on nAChRs, this is followed in the ganglion by a succession of much slower postsynaptic responses:

- A *slow inhibitory (hyperpolarising) postsynaptic potential (slow ipsp)*, lasting 2–5 s. This mainly reflects a muscarinic (M_2)-receptor-mediated increase in K^+ conductance, but other transmitters, such as dopamine and adenosine, also contribute.
- A *slow epsp*, which lasts for about 10 s. This is produced by ACh acting on M_1 receptors, which close K^+ channels.
- A *late slow epsp*, lasting for 1–2 min. This is thought to be mediated by a peptide co-transmitter, which may be substance P in some ganglia, and a gonadotrophin-releasing hormone-like peptide in others (see Ch. 12). Like the slow epsp, it is produced by a decrease in K^+ conductance.

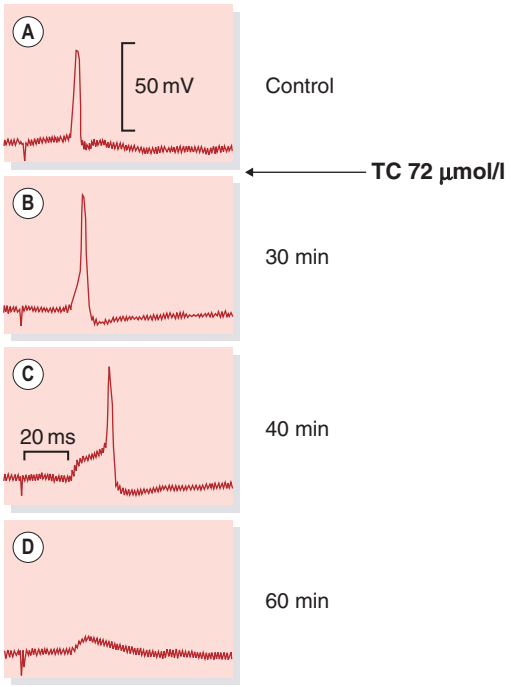


Fig. 13.3 Cholinergic transmission in an autonomic ganglion cell. Records were obtained with an intracellular microelectrode from a guinea pig parasympathetic ganglion cell. The artefact at the beginning of each trace shows the moment of stimulation of the preganglionic nerve. Tubocurarine (TC), an acetylcholine antagonist, causes the epsp to become smaller. In record [**C**], it only just succeeds in triggering the action potential, and in [**D**] it has fallen below the threshold. Following complete block, antidromic stimulation (not shown) will still produce an action potential (cf. depolarisation block, Fig. 13.4). *(From Blackman JG et al. 1969 J Physiol 201, 723.)*

DEPOLARISATION BLOCK

▼ Depolarisation block occurs at cholinergic synapses when the excitatory nAChRs are persistently activated, and it results from a decrease in the electrical excitability of the postsynaptic cell. This is shown in Figure 13.4. Application of nicotine to a sympathetic ganglion activates nAChRs, causing a depolarisation of the cell, which at first initiates action potential discharge. After a few seconds, this discharge ceases and transmission is blocked. The loss of electrical excitability at this time is shown by the fact that electrical stimuli also fail to produce an action potential. The main reason for the loss of electrical excitability during a period of maintained depolarisation is that the voltage-sensitive sodium channels (see Ch. 4) become inactivated (i.e. refractory) and no longer able to open in response to a brief depolarising stimulus.

A second type of effect is also seen in the experiment shown in Figure 13.4. After nicotine has acted for several minutes, the cell partially repolarises and its electrical excitability returns but, despite this, transmission remains blocked. This type of secondary, *non-depolarising block* occurs also at the neuromuscular junction if

Cholinergic transmission

- Acetylcholine (ACh) synthesis:
  - requires choline, which enters the neuron via carrier-mediated transport
  - choline is acetylated to form ACh by choline acetyl transferase, a cytosolic enzyme found only in cholinergic neurons. Acetyl coenzyme A is the source of acetyl groups.
- ACh is packaged into synaptic vesicles at high concentration by carrier-mediated transport.
- ACh release occurs by Ca^{2+}-mediated exocytosis. At the neuromuscular junction, one presynaptic nerve impulse releases 100–500 vesicles.
- At the neuromuscular junction, ACh acts on nicotinic receptors to open cation channels, producing a rapid depolarisation (endplate potential), which normally initiates an action potential in the muscle fibre. Transmission at other 'fast' cholinergic synapses (e.g. ganglionic) is similar.
- At 'fast' cholinergic synapses, ACh is hydrolysed within about 1 ms by acetylcholinesterase, so a presynaptic action potential produces only one postsynaptic action potential.
- Transmission mediated by muscarinic receptors is much slower in its time course, and synaptic structures are less clearly defined. In many situations, ACh functions as a modulator rather than as a direct transmitter.
- Main mechanisms of pharmacological block: inhibition of choline uptake, inhibition of ACh release, block of postsynaptic receptors or ion channels, persistent postsynaptic depolarisation.

repeated doses of the depolarising drug **suxamethonium**[2] (see below) are used. The main factor responsible for the secondary block (known clinically as *phase II block*) appears to be receptor desensitisation (see Ch. 2). This causes the depolarising action of the blocking drug to subside, but transmission remains blocked because the receptors are desensitised to ACh.

EFFECTS OF DRUGS ON CHOLINERGIC TRANSMISSION

As shown in Figure 13.2, drugs can influence cholinergic transmission either by acting on postsynaptic ACh receptors as agonists or antagonists (Tables 13.1 and 13.2), or by affecting the release or destruction of endogenous ACh.

In the rest of this chapter, we describe the following groups of drugs, subdivided according to their site of action:

- muscarinic agonists
- muscarinic antagonists
- ganglion-stimulating drugs
- ganglion-blocking drugs
- neuromuscular-blocking drugs
- anticholinesterases and other drugs that enhance cholinergic transmission.

[2]Known in the USA as **succinylcholine**.

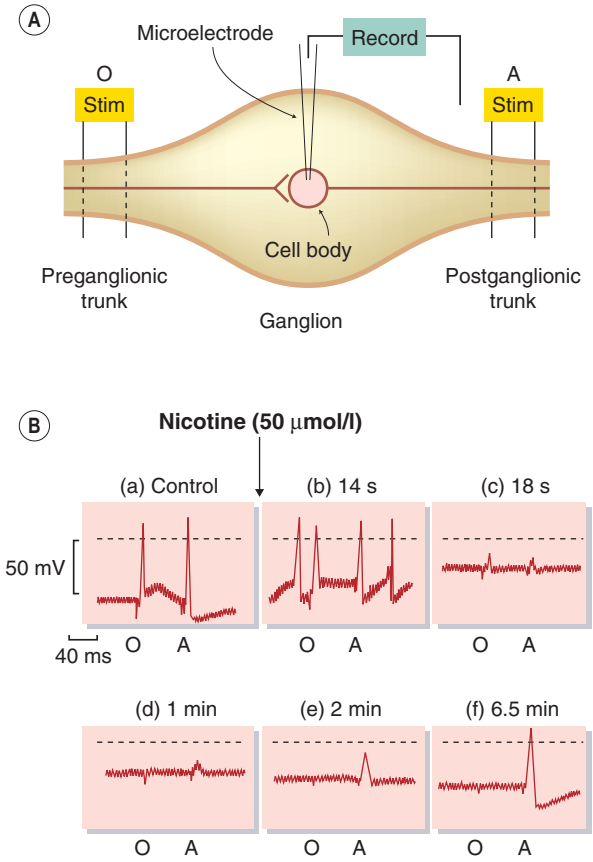


Fig. 13.4 Depolarisation block of ganglionic transmission by nicotine. [**A**] System used for intracellular recording from sympathetic ganglion cells of the frog, showing the location of orthodromic (O) and antidromic (A) stimulating (stim) electrodes. Stimulation at O excites the cell via the cholinergic synapse, whereas stimulation at A excites it by electrical propagation of the action potential. [**B**] The effect of nicotine. (a) Control records. The membrane potential is –55 mV (dotted line = 0 mV), and the cell responds to both O and A. (b) Shortly after adding nicotine, the cell is slightly depolarised and spontaneously active, but still responsive to O and A (c and d). The cell is further depolarised, to –25 mV, and produces only a vestigial action potential. The fact that it does not respond to A shows that it is electrically inexcitable (e and f). In the continued presence of nicotine, the cell repolarises and regains its responsiveness to A, but it is still unresponsive to O because the ACh receptors are desensitised by nicotine. *(From Ginsborg BL, Guerrero S 1964 J Physiol 172, 189.)*

DRUGS AFFECTING MUSCARINIC RECEPTORS

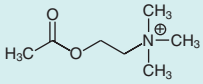
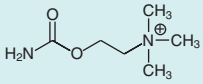
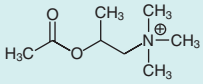
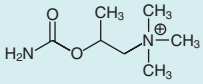
MUSCARINIC AGONISTS

Structure–activity relationships

Muscarinic agonists, as a group, are often referred to as *parasympathomimetic*, because the main effects that they produce in the whole animal resemble those of parasympathetic stimulation. The structures of acetylcholine and related choline esters are given in Table 13.3. They are agonists at both mAChRs and nAChRs, but act more potently on mAChRs (see Fig. 13.1). **Bethanechol**, **pilocarpine** and **cevimeline** are the only ones used clinically.

The key features of the ACh molecule that are important for its activity are the quaternary ammonium group, which

Table 13.3 Muscarinic agonists

Compound	Structure	Receptor specificity		Hydrolysis by cholinesterase	Clinical uses
		Muscarinic	*Nicotinic*		
Acetylcholine		+++	+++	+++	None
Carbachol		++	+++	–	None
Methacholine		+++	+	++	None
Bethanechol		+++	–	–	Treatment of bladder and gastrointestinal hypotonia[a]
Muscarine		+++	–	–	None[b]
Pilocarpine		++	–	–	Glaucoma
Oxotremorine		++	–	–	None
Cevimeline		++[c]	–	–	Sjögren's syndrome (to increase salivary and lacrimal secretion)

[a]Essential to check that bladder neck is not obstructed.
[b]Cause of one type of mushroom poisoning.
[c]Selective for M_3 receptors.

bears a positive charge, and the ester group, which bears a partial negative charge and is susceptible to rapid hydrolysis by cholinesterase. Variants of the choline ester structure (Table 13.3) have the effect of reducing the susceptibility of the compound to hydrolysis by cholinesterase, and altering the relative activity on mAChRs and nAChRs.

Carbachol and **methacholine** are used as experimental tools. Bethanechol, which is a hybrid of these two molecules, is stable to hydrolysis and selective for mAChRs, and is occasionally used clinically (see clinical box, p. 164). Pilocarpine is a partial agonist and shows some selectivity in stimulating secretion from sweat, salivary, lacrimal and bronchial glands, and contracting iris smooth muscle (see below), with weak effects on gastrointestinal smooth muscle and the heart.

Effects of muscarinic agonists

The main actions of muscarinic agonists are readily understood in terms of the parasympathetic nervous system.

Cardiovascular effects. These include cardiac slowing and a decrease in cardiac output due both to the reduced heart rate and to a decreased force of contraction of the atria (the ventricles have only a sparse parasympathetic innervation and a low sensitivity to muscarinic agonists). Generalised vasodilatation also occurs (mediated by nitric oxide, NO; see Ch. 20) and, combined with the reduced cardiac output, produces a sharp fall in arterial pressure (Fig. 13.1). The mechanism of action of muscarinic agonists on the heart is discussed in Chapter 21 (see Fig. 21.7).

Smooth muscle. Smooth muscle generally *contracts* in direct response to muscarinic agonists, in contrast to the indirect effect via NO on vascular smooth muscle. Peristaltic activity of the gastrointestinal tract is increased, which can cause colicky pain, and the bladder and bronchial smooth muscle also contract.

Sweating, lacrimation, salivation and bronchial secretion. These result from stimulation of exocrine glands. The combined effect of bronchial secretion and constriction can interfere with breathing.

Effects on the eye. These effects are clinically important. The parasympathetic nerves to the eye supply the constrictor pupillae muscle, which runs circumferentially in the iris, and the ciliary muscle, which adjusts the curvature of the lens (Fig. 13.5). Contraction of the ciliary muscle in response to activation of mAChRs pulls the ciliary body forward and inward, thus relaxing the tension on the suspensory ligament of the lens, allowing the lens to bulge more and reducing its focal length. This parasympathetic reflex is thus necessary to accommodate the eye for near vision. The constrictor pupillae is important not only for adjusting the pupil in response to changes in light intensity, but also in regulating the intraocular pressure. Aqueous humour is secreted slowly and continuously by the cells of the epithelium covering the ciliary body, and it drains into the *canal of Schlemm* (Fig. 13.5), which runs around the eye close to the outer margin of the iris. The intraocular pressure is normally 10–15 mmHg above atmospheric, which keeps the eye slightly distended. Abnormally raised intraocular pressure (which leads to

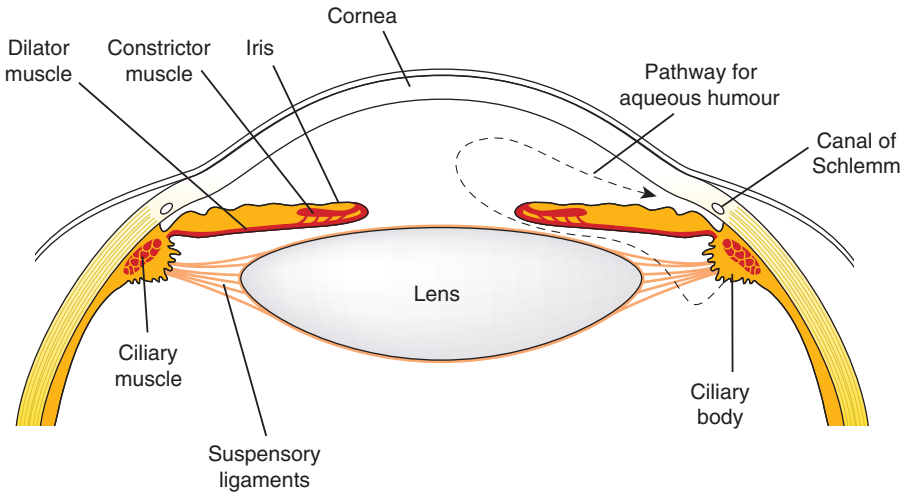


Fig. 13.5 The anterior chamber of the eye, showing the pathway for secretion and drainage of the aqueous humour.

Table 13.4 Drugs that lower intraocular pressure

Drug[a]	Mechanism	Notes	See Chapter
Timolol, carteolol	β-adrenoceptor antagonist	Given as eye drops but may still cause systemic side effects: bradycardia, bronchoconstriction	14
Acetazolamide, dorzolamide	Carbonic anhydrase inhibitor	Acetazolamide is given systemically Side effects include diuresis, loss of appetite, tingling, neutropenia Dorzolamide is used as eye drops Side effects include bitter taste and burning sensation	29
Clonidine, apraclonidine	α_2-adrenoceptor agonist	Used as eye drops	14
Latanoprost	Prostaglandin analogue	Can alter iris pigmentation	17
Pilocarpine	Muscarinic agonist	Used as eye drops	This chapter
Ecothiophate	Anticholinesterase	Used as eye drops Can cause muscle spasm and systemic effects	This chapter

[a]The most important drugs are shown in **bold**.

the pathological condition of *glaucoma*) damages the eye and is one of the commonest preventable causes of blindness. In acute glaucoma, drainage of aqueous humour becomes impeded when the pupil is dilated, because folding of the iris tissue occludes the drainage angle, causing the intraocular pressure to rise. Activation of the constrictor pupillae muscle by muscarinic agonists in these circumstances lowers the intraocular pressure, although in a normal individual it has little effect. The increased tension in the ciliary muscle produced by these drugs may also play a part in improving drainage by realigning the connective tissue trabeculae through which the canal of Schlemm passes. Drugs used in the treatment of glaucoma are summarised in Table 13.4.

In addition to these peripheral effects, muscarinic agonists that are able to penetrate the blood–brain barrier produce marked central effects due to activation mainly of M_1 receptors in the brain. These include tremor, hypothermia and increased locomotor activity, as well as improved cognition (see Ch. 40).

Clinical use

Currently there are few important uses for muscarinic agonists (though there are still hopes that new, more selective agents may prove useful in various CNS disorders). Current clinical uses are summarised in the clinical box (p. 164).

MUSCARINIC ANTAGONISTS

Muscarinic receptor antagonists (*parasympatholytic drugs*; Table 13.5) are competitive antagonists whose chemical structures usually contain ester and basic groups in the same relationship as ACh, but they have a bulky aromatic group in place of the acetyl group. The two naturally occurring compounds, **atropine** and **hyoscine** (also known as **scopolamine**), are alkaloids found in

Table 13.5 Muscarinic antagonists[a]

Compound	Pharmacological properties		Notes
Atropine	Non-selective antagonist Well absorbed orally CNS stimulant		Belladonna alkaloid Main side effects: urinary retention, dry mouth, blurred vision Dicycloverine (dicyclomine) is similar and used mainly as an antispasmodic agent
Hyoscine	Similar to atropine CNS depressant		Belladonna alkaloid (also known as scopolamine) Causes sedation; other side effects as atropine
Hyoscine butylbromide	Similar to atropine but poorly absorbed and lacks CNS effects Significant ganglion-blocking activity		Quaternary ammonium derivative Similar drugs include atropine methonitrate, propantheline
Tiotropium	Similar to atropine methonitrate Does not inhibit mucociliary clearance from bronchi		Quaternary ammonium compound Ipratropium similar
Tropicamide	Similar to atropine May raise intraocular pressure		–
Cyclopentolate	Similar to tropicamide		–
Darifenacin	Selective for M_3 receptors	Urinary incontinence	Few side effects

Other non-selective muscarinic antagonists in clinical use, with very similar actions and side effects, include oxybutynin, tolterodine, fesoterodine, solifenacin and trospium – an example of me-too development by pharmaceutical companies.

[a]For chemical structures, see Brunton L et al. 2006. Goodman and Gilman's Pharmacological Basis of Therapeutics, 11th edn. McGraw–Hill, New York.

Clinical uses of muscarinic agonists and related drugs

- **Pilocarpine** eye drops cause constriction of the pupils (miosis) and have been used to treat glaucoma (raised pressure within the eye).
- **Pilocarpine** or **cevimeline**, a selective M_3 agonist, can be used to increase salivation and lacrimal secretion in patients with dry mouth or dry eyes (e.g. following irradiation, or in patients with autoimmune damage to the salivary or lacrimal glands as in Sjögren's syndrome).
- **Bethanechol** or **distigmine** (a cholinesterase inhibitor) are now seldom used as stimulant laxatives or to stimulate bladder emptying.

solanaceous plants. The deadly nightshade (*Atropa belladonna*) contains mainly atropine, whereas the thorn apple (*Datura stramonium*) contains mainly hyoscine. These are tertiary ammonium compounds that are sufficiently lipid-soluble to be readily absorbed from the gut or conjunctival sac and, importantly, to penetrate the blood–brain barrier. Quaternary ammonium compounds, which have peripheral actions very similar to those of atropine but, because of their exclusion from the brain, lack central actions, include **hyoscine butylbromide** and **propantheline**. **Ipratropium**, another quaternary ammonium compound, is used by inhalation as a bronchodilator. **Cyclopentolate** and **tropicamide** are tertiary amines developed for ophthalmic use and administered as eye drops. **Oxybutynin**, **tolterodine** and **darifenacin** (M_3-selective) are drugs that act on the bladder to inhibit micturition, and are used for treating urinary incontinence. They produce unwanted effects typical of muscarinic antagonists, such as dry mouth, constipation and blurred vision, but these are less severe than with earlier drugs.

Effects of muscarinic antagonists

All the muscarinic antagonists produce basically similar peripheral effects, although some show a degree of selectivity, for example for the heart or bladder, reflecting heterogeneity among mAChRs.

The main effects of atropine are:

Inhibition of secretions. Salivary, lacrimal, bronchial and sweat glands are inhibited by very low doses of atropine, producing an uncomfortably dry mouth and skin. Gastric secretion is only slightly reduced. Mucociliary clearance in the bronchi is inhibited, so that residual secretions tend to accumulate in the lungs. Ipratropium lacks this effect.

Effects on heart rate. Atropine causes tachycardia through block of cardiac mAChRs. The tachycardia is modest, up to 80–90 beats/min in humans. This is because there is no effect on the sympathetic system, but only inhibition of tonic parasympathetic tone. Tachycardia is most pronounced in young people, in whom vagal tone at rest is highest; it is often absent in the elderly. At very low doses, atropine causes a paradoxical bradycardia, possibly due to a central action. Arterial blood pressure and the response of the heart to exercise are unaffected.

Effects on the eye. The pupil is dilated (*mydriasis*) by atropine administration, and becomes unresponsive to light. Relaxation of the ciliary muscle causes paralysis of accommodation (*cycloplegia*), so that near vision is impaired. Intraocular pressure may rise; although this is unimportant in normal individuals, it can be dangerous in patients suffering from narrow-angle glaucoma.

Effects on the gastrointestinal tract. Gastrointestinal motility is inhibited by atropine, although this requires larger doses than the other effects listed, and is not complete since excitatory transmitters other than ACh are important in normal function of the myenteric plexus (see Ch. 12). Atropine is used in pathological conditions in which there is increased gastrointestinal motility. Pirenzepine, owing to its selectivity for M_1 receptors, inhibits gastric acid secretion in doses that do not affect other systems.

Effects on other smooth muscle. Bronchial, biliary and urinary tract smooth muscle are all relaxed by atropine. Reflex bronchoconstriction (e.g. during anaesthesia) is prevented by atropine, whereas bronchoconstriction caused by local mediators, such as histamine and leukotrienes (e.g. in asthma; Ch. 28) is unaffected. Biliary and urinary tract smooth muscle are only slightly affected in normal individuals, probably because transmitters other than ACh (see Ch. 12) are important in these organs; nevertheless, atropine and similar drugs commonly precipitate urinary retention in elderly men with prostatic enlargement. Incontinence due to bladder overactivity is reduced by muscarinic antagonists.

Effects on the CNS. Atropine produces mainly excitatory effects on the CNS. At low doses, this causes mild restlessness; higher doses cause agitation and disorientation. In atropine poisoning, which occurs mainly in young children who eat deadly nightshade berries, marked excitement and irritability result in hyperactivity and a considerable rise in body temperature, which is accentuated by the loss of sweating. These central effects are the result of blocking mAChRs in the brain, and they are opposed by anticholinesterase drugs such as **physostigmine**, which have been used to treat atropine poisoning. Hyoscine in low doses causes marked sedation, but has similar effects in high dosage. Hyoscine also has a useful antiemetic effect and is used to prevent motion sickness. Muscarinic antagonists also affect the extrapyramidal system, reducing the involuntary movement and rigidity of patients with Parkinson's disease (Ch. 40) and counteracting the extrapyramidal side effects of many antipsychotic drugs (Ch. 46).

Clinical use

The main uses of muscarinic antagonists are summarised in the clinical box (p. 166).

DRUGS AFFECTING AUTONOMIC GANGLIA

GANGLION STIMULANTS

Most nAChR agonists act on either neuronal (ganglionic and CNS) nACh receptors or on striated muscle (motor endplate) receptors but not, apart from nicotine and ACh, on both (Table 13.6).

Nicotine and **lobeline** are tertiary amines found in the leaves of tobacco and lobelia plants, respectively. Nicotine belongs in pharmacological folklore, as it was the substance on the tip of Langley's paintbrush causing stimulation of muscle fibres when applied to the endplate region, leading him to postulate in 1905 the existence of a 'receptive substance' on the surface of the fibres (Ch. 12). **Epibatidine**, found in the skin of poisonous frogs, is a highly potent nicotinic agonist selective for ganglionic and CNS receptors. It was found, unexpectedly, to be a powerful analgesic (see Ch. 42), though its autonomic side effects ruled out its clinical use. **Varenicline**, a synthetic agonist relatively selective for CNS receptors, is used (as is nicotine itself) to treat nicotine addiction (Ch. 49). Otherwise these drugs are used only as experimental tools.

They cause complex peripheral responses associated with generalised stimulation of autonomic ganglia. The effects of nicotine on the gastrointestinal tract and sweat glands are familiar to neophyte smokers (see Ch. 49), although usually insufficient to act as an effective deterrent.

Drugs acting on muscarinic receptors

Muscarinic agonists

- Important compounds include **acetylcholine**, **carbachol**, **methacholine**, **muscarine** and **pilocarpine**. They vary in muscarinic/nicotinic selectivity, and in susceptibility to cholinesterase.
- Main effects are bradycardia and vasodilatation (endothelium-dependent), leading to fall in blood pressure; contraction of visceral smooth muscle (gut, bladder, bronchi, etc.); exocrine secretions, pupillary constriction and ciliary muscle contraction, leading to decrease of intraocular pressure.
- Main use is in treatment of glaucoma (especially **pilocarpine**).
- Most agonists show little receptor subtype selectivity, but more selective compounds are in development.

Muscarinic antagonists

- Most important compounds are **atropine**, **hyoscine**, **ipratropium** and **pirenzepine**.
- Main effects are inhibition of secretions; tachycardia, pupillary dilatation and paralysis of accommodation; relaxation of smooth muscle (gut, bronchi, biliary tract, bladder); inhibition of gastric acid secretion (especially **pirenzepine**); central nervous system effects (mainly excitatory with **atropine**; depressant, including amnesia, with **hyoscine**), including antiemetic effect and antiparkinsonian effect.

GANGLION-BLOCKING DRUGS

Ganglion blocking drugs are used experimentally to study autonomic function, but their clinical use is obsolete. Ganglion block can occur by several mechanisms:

- By interference with ACh release, as at the neuromuscular junction (Ch. 12).
- By prolonged depolarisation. Nicotine (see Fig. 13.4) can block ganglia, after initial stimulation, in this way, as can ACh itself if cholinesterase is inhibited so that it can exert a continuing action on the postsynaptic membrane.
- By interference with the postsynaptic action of ACh. The few ganglion-blocking drugs of practical

Table 13.6 Nicotinic receptor agonists and antagonists

Drug	Main site	Type of action	Notes
Agonists			
Nicotine	Autonomic ganglia CNS	Stimulation then block Stimulation	See Ch. 49
Lobeline	Autonomic ganglia Sensory nerve terminals	Stimulation Stimulation	–
Epibatidine	Autonomic ganglia, CNS	Stimulation	Isolated from frog skin Highly potent No clinical use
Varenicline	CNS, autonomic ganglia	Stimulation	Used for nicotine addiction (see Ch. 49)
Suxamethonium	Neuromuscular junction	Depolarisation block	Used clinically as muscle relaxant
Decamethonium	Neuromuscular junction	Depolarisation block	No clinical use
Antagonists			
Hexamethonium	Autonomic ganglia	Transmission block	No clinical use
Trimetaphan	Autonomic ganglia	Transmission block	Blood pressure-lowering in surgery (rarely used)
Tubocurarine	Neuromuscular junction	Transmission block	Now rarely used
Pancuronium Atracurium Vecuronium	Neuromuscular junction	Transmission block	Widely used as muscle relaxants in anaesthesia

Clinical uses of muscarinic antagonists

Cardiovascular

- Treatment of sinus bradycardia (e.g. after myocardial infarction; see Ch. 21): for example **atropine**.

Ophthalmic

- To dilate the pupil: for example **tropicamide** or **cyclopentolate** eye drops.

Neurological

- Prevention of motion sickness: for example **hyoscine** (orally or transdermally).
- Parkinsonism (see Ch. 40), especially to counteract movement disorders caused by antipsychotic drugs (see Ch. 46): for example, **benzhexol**, **benztropine**.

Respiratory

- Asthma and chronic obstructive pulmonary disease (see Ch. 28): **ipratropium** or **tiotropium** by inhalation.

Anaesthetic premedication

- To dry secretions: for example **atropine**, **hyoscine**. (Current anaesthetics are relatively non-irritant, see Ch. 41, so this use is now less important.)

Gastrointestinal

- To facilitate endoscopy and gastrointestinal radiology by relaxing gastrointestinal smooth muscle (antispasmodic action; see Ch. 30): for example, **hyoscine**.
- As an antispasmodic in irritable bowel syndrome or colonic diverticular disease: for example **dicycloverine** (**dicyclomine**).

importance act by blocking neuronal nAChRs or the associated ion channels.

▼ Sixty-five years ago, Paton and Zaimis investigated a series of linear bisquaternary compounds. Compounds with five or six carbon atoms (**hexamethonium**, no longer used clinically but famous as the first effective antihypertensive agent) in the methylene chain linking the two quaternary groups produced ganglionic block.[3]

Effects of ganglion-blocking drugs

The effects of ganglion-blocking drugs are numerous and complex, as would be expected, because both divisions of the autonomic nervous system are blocked indiscriminately. The description by Paton of 'hexamethonium man' cannot be bettered:

▼ He is a pink-complexioned person, except when he has stood in a queue for a long time, when he may get pale and faint. His handshake is warm and dry. He is a placid and relaxed companion; for instance he may laugh but he can't cry because the tears cannot come. Your rudest story will not make him blush, and the most unpleasant circumstances will fail to make him turn pale. His collars and socks stay very clean and sweet. He wears corsets and may, if you meet him out, be rather fidgety (corsets to compress his splanchnic vascular pool, fidgety to keep the venous return going from his legs). He dislikes speaking much unless helped with something to moisten his dry mouth and throat. He is long-sighted and easily blinded by bright light. The redness of his eyeballs may suggest irregular habits and in fact his head is rather weak. But he always behaves like a gentleman and never belches or hiccups. He tends to get cold and keeps well wrapped up. But his health is good; he does not have chilblains and those diseases of modern civilisation, hypertension and peptic ulcer, pass him by. He gets thin because his appetite is modest; he never feels hunger pains and his stomach never rumbles.

[3]Based on their structural similarity to ACh, these compounds were originally assumed to compete with ACh for its binding site. However, they are now known to act mainly by blocking the ion channel rather than the receptor itself.

He gets rather constipated so that his intake of liquid paraffin is high. As old age comes on, he will suffer from retention of urine and impotence, but frequency, precipitancy and strangury (i.e. an intensely painful sensation of needing to pass urine coupled with an inability to do so) will not worry him. One is uncertain how he will end, but perhaps if he is not careful, by eating less and less and getting colder and colder, he will sink into a symptomless, hypoglycaemic coma and die, as was proposed for the universe, a sort of entropy death.

(From Paton WDM 1954 The principles of ganglion block. Lectures on the scientific basis of medicine, vol. 2.)

In practice, the main effect is a marked fall in arterial blood pressure resulting mainly from block of sympathetic ganglia, which causes arteriolar vasodilatation, and the block of cardiovascular reflexes. In particular, the venoconstriction, which occurs normally when a subject stands up and which is necessary to prevent the central venous pressure (and hence cardiac output) from falling sharply, is reduced. Standing thus causes a sudden fall in arterial pressure (*postural hypotension*) that can cause fainting. Similarly, the vasodilatation of skeletal muscle during exercise is normally accompanied by vasoconstriction elsewhere (e.g. splanchnic area) produced by sympathetic activity. If this adjustment is prevented, the overall peripheral resistance falls and the blood pressure also falls (*post-exercise hypotension*).

Drugs acting on autonomic ganglia

Ganglion-stimulating drugs

- Compounds include **nicotine**, **dimethylphenylpiperazinium** (**DMPP**).
- Both sympathetic and parasympathetic ganglia are stimulated, so effects are complex, including tachycardia and increase of blood pressure; variable effects on gastrointestinal motility and secretions; increased bronchial, salivary and sweat secretions. Additional effects result from stimulation of other neuronal structures, including sensory and noradrenergic nerve terminals.
- Ganglion stimulation may be followed by depolarisation block.
- **Nicotine** also has important central nervous system effects.
- No therapeutic uses, except for **nicotine** to assist giving up smoking.

Ganglion-blocking drugs

- Compounds include **hexamethonium**, **tubocurarine** (also **nicotine**; see p. 160).
- Block all autonomic ganglia and enteric ganglia. Main effects: hypotension and loss of cardiovascular reflexes, inhibition of secretions, gastrointestinal paralysis, impaired micturition.
- Clinically obsolete.

NEUROMUSCULAR-BLOCKING DRUGS

Drugs can block neuromuscular transmission either by acting presynaptically to inhibit ACh synthesis or release, or by acting postsynaptically.

Neuromuscular block is an important adjunct to anaesthesia (Ch. 40), when artificial ventilation is available. The drugs used for this purpose all work postsynaptically, either (a) by blocking ACh receptors (or in some cases the ion channel) or (b) by activating ACh receptors and thus causing persistent depolarisation of the motor endplate. Apart from **suxamethonium** (see p. 169-170) all of the drugs used clinically are *non-depolarising agents*.

NON-DEPOLARISING BLOCKING AGENTS

In 1856, Claude Bernard, in a famous experiment, showed that 'curare' causes paralysis by blocking neuromuscular transmission, rather than by abolishing nerve conduction or muscle contractility. Curare is a mixture of naturally occurring alkaloids found in various South American plants and used as arrow poisons by South American Indians. The most important component is **tubocurarine**, itself now rarely used in clinical medicine, being superseded by synthetic drugs with improved properties. The most important are **pancuronium**, **vecuronium**, **cisatracurium** and **mivacurium** (Table 13.7), which differ mainly in their duration of action. These substances are all quaternary ammonium compounds, so are poorly absorbed (they are administered intravenously) and generally are efficiently excreted by the kidneys. They do not cross the placenta, which is important in relation to their use in obstetric anaesthesia.

Mechanism of action

Non-depolarising blocking agents act as competitive antagonists (see Ch. 2) at the ACh receptors of the endplate.

▼ The amount of ACh released by a nerve impulse normally exceeds by several-fold what is needed to elicit an action potential in the muscle fibre. It is therefore necessary to block 70–80% of the receptor sites before transmission actually fails. In any individual muscle fibre, transmission is all or nothing, so graded degrees of block represent a varying proportion of muscle fibres failing to respond. In this situation, where the amplitude of the epp in all the fibres is close to threshold (just above in some, just below in others), small variations in the amount of transmitter released, or in the rate at which it is destroyed, will have a large effect on the proportion of fibres contracting, so the degree of block is liable to vary according to various physiological circumstances (e.g. stimulation frequency, temperature and cholinesterase inhibition), which normally have relatively little effect on the efficiency of transmission.

Non-depolarising blocking agents also block facilitatory presynaptic autoreceptors, and thus inhibit the release of ACh during repetitive stimulation of the motor nerve, resulting in the phenomenon of 'tetanic fade', which is often used by anaesthetists to monitor postoperative recovery of neuromuscular transmission.

Effects of non-depolarising blocking drugs

The effects of non-depolarising neuromuscular-blocking agents are mainly due to motor paralysis, although some of the drugs also produce clinically significant autonomic effects.

▼ The first muscles to be affected are the extrinsic eye muscles (causing double vision), reminiscent of the disease myasthenia gravis, which is caused by autoantibodies directed against nAChR (see p. 175-176), and the small muscles of the face, limbs and pharynx (causing difficulty in swallowing). Respiratory muscles are the last to be affected and the first to recover. An experiment in 1947 in which a heroic volunteer was fully curarised while conscious under artificial ventilation established this orderly paralytic march, and showed that consciousness and awareness of pain were quite normal even when paralysis was complete.[4]

[4]The risk of patients waking up paralysed during surgery is a serious worry for anaesthetists.

Table 13.7 Characteristics of neuromuscular-blocking drugs[a]

Drug	Speed of onset	Duration of action	Main side effects	Notes
Tubocurarine	Slow (>5 min)	Long (1–2 h)	Hypotension (ganglion block plus histamine release) Bronchoconstriction (histamine release)	Plant alkaloid, now rarely used Alcuronium is a semisynthetic derivative with similar properties but fewer side effects
Pancuronium	Intermediate (2–3 min)	Long (1–2 h)	Slight tachycardia Hypertension	The first steroid-based compound Better side effect profile than tubocurarine Widely used Pipecuronium is similar
Vecuronium	Intermediate	Intermediate (30–40 min)	Few side effects	Widely used Occasionally causes prolonged paralysis, probably owing to active metabolite Rocuronium is similar, with faster onset
Atracurium	Intermediate	Intermediate (<30 min)	Transient hypotension (histamine release)	Unusual mechanism of elimination (spontaneous non-enzymic chemical degradation in plasma); degradation slowed by acidosis Widely used Doxacurium is chemically similar but stable in plasma, giving it long duration of action Cisatracurium is the pure active isomeric constituent of atracurium, more potent but with less histamine release
Mivacurium	Fast (~2 min)	Short (~15 min)	Transient hypotension (histamine release)	Chemically similar to atracurium but rapidly inactivated by plasma cholinesterase (therefore longer acting in patients with liver disease or with genetic cholinesterase deficiency [see p. 172 and Ch. 11])
Suxamethonium	Fast	Short (~10 min)	Bradycardia (muscarinic agonist effect) Cardiac dysrhythmias (increased plasma K^+ concentration – avoid in patients with burns or severe trauma) Raised intraocular pressure (nicotinic agonist effect on extraocular muscles) Postoperative muscle pain	Acts by depolarisation of endplate (nicotinic agonist effect) – the only drug of this type still in use Paralysis is preceded by transient muscle fasciculations Short duration of action owing to hydrolysis by plasma cholinesterase (prolonged action in patients with liver disease or genetic deficiency of plasma cholinesterase) Used for brief procedures (e.g. tracheal intubation, electroconvulsive shock therapy) Rocuronium has similar speed of onset and recovery, with fewer unwanted effects

[a]For chemical structures, see Hardman JG, Limbird LE, Gilman AG, Goodman-Gilman A et al. 2001 Goodman and Gilman's Pharmacological Basis of Therapeutics, tenth ed. McGraw–Hill, New York.

Unwanted effects

The main side effect of tubocurarine is a fall in arterial pressure, due to (a) ganglion block and (b) histamine release from mast cells (see Ch. 17), which can also give rise to bronchospasm in sensitive individuals. This is unrelated to nAChRs but also occurs with **atracurium** and **mivacurium** (as well as with some unrelated drugs such as morphine; see Ch. 42). The other non-depolarising blocking drugs lack these side effects, and hence cause less hypotension. **Pancuronium** also blocks mAChRs, particularly in the heart, which results in tachycardia.

Pharmacokinetic aspects

Neuromuscular-blocking agents are used mainly in anaesthesia to produce muscle relaxation. They are given intravenously but differ in their rates of onset and recovery (Fig. 13.6 and Table 13.7).

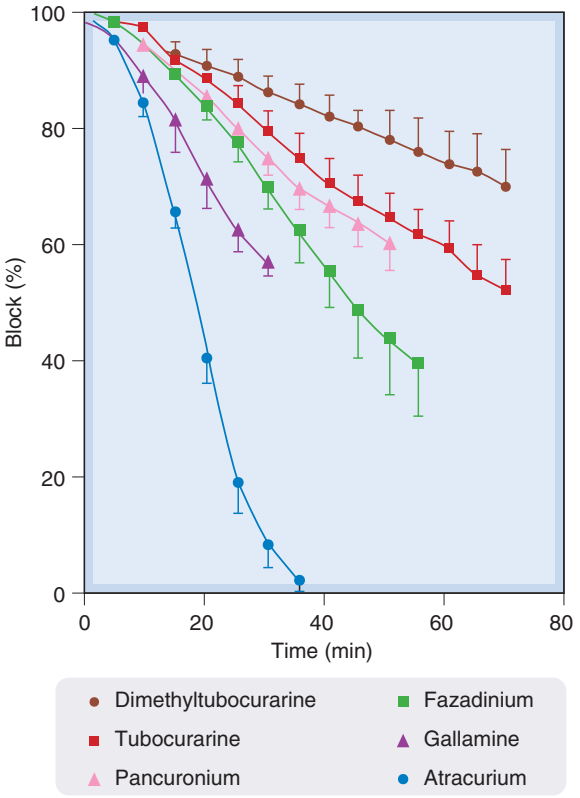


Fig. 13.6 Rate of recovery from various non-depolarising neuromuscular-blocking drugs in humans. Drugs were given intravenously to patients undergoing surgery, in doses just sufficient to cause 100% block of the tetanic tension of the indirectly stimulated adductor pollicis muscle. Recovery of tension was then followed as a function of time. *(From Payne JP, Hughes R 1981 Br J Anaesth 53, 45.)*

Most of the non-depolarising blocking agents are metabolised by the liver or excreted unchanged in the urine, exceptions being **atracurium**, which hydrolyses spontaneously in plasma, and **mivacurium**, which, like **suxamethonium** (see below), is hydrolysed by plasma cholinesterase. Their duration of action varies between about 15 min and 1–2 h (Table 13.7), by which time the patient regains enough strength to cough and breathe properly. The route of elimination is important, because many patients undergoing anaesthesia have impaired renal or hepatic function, which, depending on the drug used, can enhance or prolong the paralysis to an important degree.

Atracurium was designed to be chemically unstable at physiological pH (splitting into two inactive fragments by cleavage at one of the quaternary nitrogen atoms), although indefinitely stable when stored at an acid pH. It has a short duration of action, which is unaffected by renal or hepatic function. Because of the marked pH dependence of its degradation, however, its action becomes considerably briefer during respiratory alkalosis caused by hyperventilation.

Rapid postoperative recovery of muscle strength is needed to reduce the risk of complications. The cholinesterase inhibitor, **neostigmine** (see Table 13.8) is often used to reverse the action of non-depolarising drugs postoperatively. Co-administration of atropine is necessary to prevent unwanted parasympathomimetic effects. An alternative approach (recently licensed for reversal of neuromuscular blockade induced by **rocuronium** or **vecuronium**) is the use of a synthetic cyclodextrin, **sugammadex**, a macromolecule that selectively binds steroidal neuromuscular blocking drugs as an inactive complex in the plasma. The complex is excreted unchanged in the urine. Sugammadex is claimed to produce more rapid reversal of block with fewer unwanted effects than neostigmine.

DEPOLARISING BLOCKING AGENTS

▼ This class of neuromuscular-blocking drugs was discovered by Paton and Zaimis in their study of the effects of symmetrical bis-quaternary ammonium compounds. One of these, **decamethonium**, was found to cause paralysis without appreciable ganglion-blocking activity. Several features of its action showed it to be different from competitive blocking drugs. In particular, it was found to produce a transient twitching of skeletal muscle (fasciculation) before causing block, and when it was injected into chicks it caused a powerful extensor spasm,[5] rather than flaccid paralysis. In 1951, B.D. Burns and Paton showed that it acted as an agonist, causing a maintained depolarisation at the endplate region of the muscle fibre, which led to a loss of electrical excitability, and they coined the term 'depolarisation block'. Fasciculation occurs because the developing endplate depolarisation initially causes a discharge of action potentials in the muscle fibre. This subsides after a few seconds as the electrical excitability of the endplate region of the fibre is lost. Decamethonium itself was used clinically but has the disadvantage of too long a duration of action.

Suxamethonium (Table 13.7) – the only depolarising blocking drug currently used – is closely related in structure to both decamethonium and ACh (consisting of two ACh molecules linked by their acetyl groups) and acts similarly, but its action lasts only a few minutes, because it is quickly hydrolysed by plasma cholinesterase. When given intravenously, however, its depolarising action lasts for long enough to cause the endplate region of the muscle fibres to become inexcitable. ACh, in contrast, when released from the nerve, reaches the endplate in very brief spurts and is rapidly hydrolysed *in situ*, so it never causes sufficiently prolonged depolarisation (10 to 100 milliseconds) to result in block. If cholinesterase is inhibited, however, it is possible for the circulating ACh concentration to reach a level sufficient to cause depolarisation block.

Comparison of non-depolarising and depolarising blocking drugs

▼ There are several differences in the pattern of neuromuscular block produced by depolarising and non-depolarising mechanisms:

- Anticholinesterase drugs are very effective in overcoming the blocking action of competitive, non-depolarising agents. This is because the released ACh, protected from hydrolysis, can diffuse further within the synaptic cleft, and so gains access to a wider area of postsynaptic membrane. The chances of an ACh molecule finding an unoccupied receptor before being hydrolysed are thus increased. This diffusional effect seems to be of more importance than a truly competitive interaction, for it is unlikely that appreciable dissociation of the antagonist can occur in the

[5]Birds (and frogs) possess a special type of skeletal muscle, rare in mammals, that has many endplates scattered over the surface of each muscle fibre. Agonists cause a diffuse depolarisation in such muscles, resulting in a graded and maintained contracture. In normal skeletal muscle, with only one endplate per fibre, endplate depolarisation is too localised to cause contracture on its own.

short time for which the ACh is present. In contrast, depolarisation block is unaffected, or even increased (via potentiation of the depolarising action of ACh), by anticholinesterase drugs.
- The fasciculations seen with suxamethonium (see Table 13.7) as a prelude to paralysis do not occur with competitive drugs. There appears to be a correlation between the amount of fasciculation and the severity of the postoperative muscle pain reported after suxamethonium.
- *Tetanic fade* (a term used to describe the failure of muscle tension to be maintained during a brief period of nerve stimulation at a frequency high enough to produce a fused tetanus) is increased by non-depolarising blocking drugs, compared with normal muscle. This is due mainly to the block of presynaptic nAChRs, which normally serve to sustain transmitter release during a tetanus, and it does not occur with depolarisation block.

Unwanted effects and dangers of suxamethonium

Suxamethonium has several adverse effects (see Table 13.7), but remains in use because of the rapid recovery that follows its withdrawal – significantly more rapid than the recovery from non-depolarising agents.

Bradycardia. This is preventable by atropine and is due to a direct muscarinic action.

Potassium release. The increase in cation permeability of the motor endplates causes a net loss of K^+ from muscle, and thus a small rise in plasma K^+ concentration. In normal individuals, this is not important, but in cases of trauma, especially burns or injuries causing muscle denervation, it may be (Fig. 13.7). This is because denervation causes ACh receptors to spread to regions of the muscle fibre away from the endplates (see Ch. 12), so that a much larger area of membrane is sensitive to suxamethonium. The resulting hyperkalaemia can be enough to cause ventricular dysrhythmia or even cardiac arrest.

Increased intraocular pressure. This results from contracture of extraocular muscles applying pressure to the eyeball. It is particularly important to avoid this if the eyeball has been injured.

Prolonged paralysis. The action of suxamethonium given intravenously normally lasts for less than 5 min, because the drug is hydrolysed by plasma cholinesterase. Its action is prolonged by various factors that reduce the activity of this enzyme:

- Genetic variants of plasma cholinesterase with reduced activity (see Ch. 11). Severe deficiency, enough to increase the duration of action to 2 h or more, occurs in approximately 1 in 3500 individuals. Very rarely, the enzyme is completely absent and paralysis lasts for many hours. Biochemical testing of enzyme activity in the plasma and its sensitivity to inhibitors is used clinically to diagnose this problem; genotyping is possible but as yet not practicable for routine screening to prevent the problem.
- Anticholinesterase drugs. The use of organophosphates to treat glaucoma (see Table 13.4) can inhibit plasma cholinesterase and prolong the action of suxamethonium. Competing substrates for plasma cholinesterase (e.g. **procaine**, **propanidid**) can also have this effect.
- Neonates may have low plasma cholinesterase activity and show prolonged paralysis with suxamethonium.

Malignant hyperthermia. This is a rare inherited condition, due to a mutation of the Ca^{2+} release channel of the sarcoplasmic reticulum (the ryanodine receptor, see Ch. 4), which results in intense muscle spasm and a dramatic rise in body temperature when certain drugs are given (see Ch. 11). Suxamethonium is now the commonest culprit, although it can be precipitated by a variety of other drugs. The condition carries a very high mortality (about 65%) and is treated by administration of **dantrolene**, a drug that inhibits muscle contraction by preventing Ca^{2+} release from the sarcoplasmic reticulum.

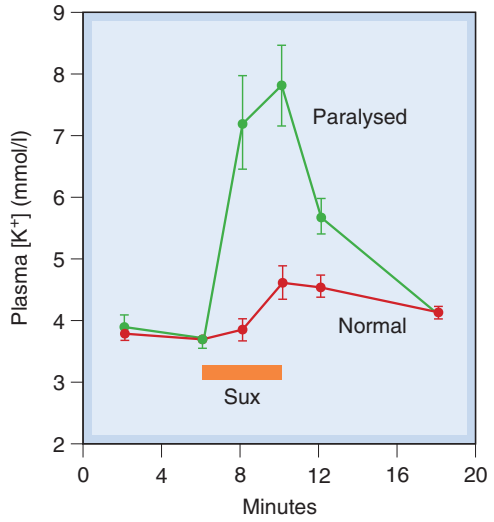


Fig. 13.7 Effect of suxamethonium (Sux) on plasma potassium concentration in humans. Blood was collected from veins draining paralysed and non-paralysed limbs of seven injured patients undergoing surgery. The injuries had resulted in motor nerve degeneration, and hence denervation supersensitivity of the affected muscles. *(From Tobey RE et al. 1972 Anaesthesiology 37, 322.)*

DRUGS THAT ACT PRESYNAPTICALLY

DRUGS THAT INHIBIT ACETYLCHOLINE SYNTHESIS

The steps in the synthesis of ACh in the presynaptic nerve terminals are shown in Figure 13.2. The rate-limiting process appears to be the transport of choline into the nerve terminal. **Hemicholinium** blocks this transport and thereby inhibits ACh synthesis. It is useful as an experimental tool but has no clinical applications. Its blocking effect on transmission develops slowly, as the existing stores of ACh become depleted. **Vesamicol**, which acts by blocking ACh transport into synaptic vesicles, has a similar effect.

DRUGS THAT INHIBIT ACETYLCHOLINE RELEASE

Acetylcholine release by a nerve impulse involves the entry of Ca^{2+} into the nerve terminal; the increase in $[Ca^{2+}]_i$ stimulates exocytosis and increases the rate of quantal release (Fig. 13.2). Agents that inhibit Ca^{2+} entry include Mg^{2+} and various aminoglycoside antibiotics (e.g. **streptomycin** and **neomycin**; see Ch. 51), which can unpredictably prolong muscle paralysis when used clinically in patients treated with neuromuscular blocking agents as an adjunct to general anaesthesia.

Neuromuscular-blocking drugs

- Substances that block choline uptake: for example **hemicholinium** (not used clinically).
- Substances that block acetylcholine release: **aminoglycoside antibiotics**, **botulinum toxin**.
- Drugs used to cause paralysis during anaesthesia are as follows:
  - Depolarising neuromuscular-blocking agents: **suxamethonium**, short acting and used during induction of anaesthesia and intubation of the airway.
  - Non-depolarising neuromuscular-blocking agents: **tubocurarine**, **pancuronium**, **atracurium**, **vecuronium**, **mivacuronium**. These act as competitive antagonists at nicotinic acetylcholine receptors and differ mainly in duration of action; used to maintain neuromuscular relaxation throughout an operation which may be of several hours duration or when unconscious in an intensive care unit.
- Important characteristics of non-depolarising and depolarising blocking drugs:
  - Non-depolarising block is reversible by anticholinesterase drugs, depolarising block is not.
  - Steroidal ('curonium') drugs (**rocuronium**, **vecuronium**) are reversed by **sugammadex**.
  - Depolarising block produces initial fasciculations and often postoperative muscle pain.
  - **Suxamethonium** is hydrolysed by plasma cholinesterase and is normally very short-acting, but may cause long-lasting paralysis in a small group of congenitally cholinesterase-deficient individuals.
- Main side effects: early curare derivatives caused ganglion block, histamine release, hence hypotension, bronchoconstriction; newer non-depolarising blocking drugs have fewer side effects; **suxamethonium** may cause bradycardia, cardiac dysrhythmias due to K^+ release (especially in burned or injured patients), increased intraocular pressure, malignant hyperthermia (rare).

Two potent neurotoxins, namely **botulinum toxin** and **β-bungarotoxin**, act specifically to inhibit ACh release. Botulinum toxin is a protein produced by the anaerobic bacillus *Clostridium botulinum*, an organism that can multiply in preserved food and can cause botulism, an extremely serious type of food poisoning.[6]

▼ The potency of botulinum toxin is extraordinary, the minimum lethal dose in a mouse being less than 10^{-12} g – only a few million molecules. It belongs to the group of potent bacterial exotoxins that includes tetanus and diphtheria toxins. They possess two subunits, one of which binds to a membrane receptor and is responsible for cellular specificity. By this means, the toxin enters the cell, where the other subunit produces the toxic effect. Botulinum toxin contains several components (A–G, see Chen et al., 2012). They are peptidases that cleave specific proteins involved in exocytosis (*synaptobrevins*, *syntaxins*, etc.; see Ch. 4), thereby producing a long-lasting block of synaptic function. Each toxin component inactivates a different functional protein – a remarkably coordinated attack by a humble bacterium on a vital component of mammalian physiology.

Botulinum poisoning causes progressive parasympathetic and motor paralysis, with dry mouth, blurred vision and difficulty in swallowing, followed by progressive respiratory paralysis. Treatment with antitoxin is effective only if given before symptoms appear, for once the toxin is bound its action cannot be reversed. Mortality is high, and recovery takes several weeks. Anticholinesterases and drugs that increase transmitter release are ineffective in restoring transmission. **Botulinum toxin**, given by local injection, has a number of clinical and cosmetic uses (a testament to Paracelsus' dictum that all drugs are poisons, the distinction lying in the dose), including:

- *blepharospasm* (persistent and disabling eyelid spasm) and other forms of unwanted movement disorder including *torsion dystonia* and *spasmodic torticollis* (twisting movements of, respectively, limbs or neck)
- *spasticity* (excessive extensor muscle tone, associated with developmental brain abnormalities or birth injury)
- *urinary incontinence* associated with bladder overactivity (given by intravesical injection)
- *squint* (given by injection into extraocular muscles)
- *hyperhidrosis* (injected intradermally into axillary skin), for excessive sweating resistant to other treatment
- *sialorrhoea* (excessive salivary secretion)
- *headache prophylaxis* (in adults with chronic migraine and frequent headaches)
- *forehead wrinkles* (injected intradermally it removes frown lines by paralysing the superficial muscles that pucker the skin).

Injections need to be repeated every few months. Botulinum toxin is antigenic, and may lose its effectiveness due to its immunogenicity. There is a risk of more general muscle paralysis if the toxin spreads beyond the injected region.

▼ β-Bungarotoxin is a protein contained in the venom of various snakes of the cobra family, and has a similar action to botulinum toxin, although its active component is a phospholipase rather than a peptidase. The same venoms also contain α-bungarotoxin (Ch. 3), which blocks postsynaptic ACh receptors. These snakes evidently cover all eventualities as far as causing paralysis of their victims is concerned.

DRUGS THAT ENHANCE CHOLINERGIC TRANSMISSION

Drugs that enhance cholinergic transmission act either by inhibiting cholinesterase (the main group) or by increasing ACh release. In this chapter, we focus on the peripheral actions of such drugs; drugs affecting cholinergic transmission in the CNS, used to treat senile dementia, are discussed in Chapter 40.

DISTRIBUTION AND FUNCTION OF CHOLINESTERASE

There are two distinct types of cholinesterase, namely *acetylcholinesterase* (AChE) and *butyrylcholinesterase* (BuChE, sometimes called pseudocholinesterase), closely related in

[6]Among the more spectacular outbreaks of botulinum poisoning was an incident on Loch Maree in Scotland in 1922, when all eight members of a fishing party died after eating duck pâté for their lunch. Their ghillies, consuming humbler fare no doubt, survived. The innkeeper committed suicide.

molecular structure but differing in their distribution, substrate specificity and functions. Both consist of globular catalytic subunits, which constitute the soluble forms found in plasma (BuChE) and cerebrospinal fluid (AChE). Elsewhere, the catalytic units are linked to accessory proteins, which tether them like a bunch of balloons to the basement membrane (at the neuromuscular junction) or to the neuronal membrane at neuronal synapses (and also, oddly, the erythrocyte membrane, where the function of the enzyme is unknown).

The bound AChE at cholinergic synapses serves to hydrolyse the released transmitter and terminate its action rapidly. Soluble AChE is also present in cholinergic nerve terminals, where it has a role in regulating the free ACh concentration, and from which it may be secreted; the function of the secreted enzyme is so far unclear. AChE is quite specific for ACh and closely related esters such as methacholine. Certain neuropeptides, such as substance P (Ch. 17) are inactivated by AChE, but it is not known whether this is of physiological significance. Overall, there is poor correspondence between the distribution of cholinergic synapses and that of AChE, both in the brain and in the periphery, and AChE most probably has synaptic functions other than disposal of ACh, although the details remain unclear (see review by Silman & Sussman, 2005; Zimmerman & Soreq, 2006).

Butyrylcholinesterase (BuChE) has a widespread distribution, being found in tissues such as liver, skin, brain and gastrointestinal smooth muscle, as well as in soluble form in the plasma. It is not particularly associated with cholinergic synapses, and its physiological function is unclear. It has a broader substrate specificity than AChE. It hydrolyses the synthetic substrate butyrylcholine more rapidly than ACh, as well as other esters, such as **procaine**, **suxamethonium** and **propanidid** (a short-acting anaesthetic agent; see Ch. 41). The plasma enzyme is important in relation to the inactivation of the drugs listed above. Genetic variants of BuChE causing significantly reduced enzymic activity occur rarely (see Ch. 11), and these partly account for the variability in the duration of action of these drugs. The very short duration of action of ACh given intravenously (see Fig. 13.1) results from its rapid hydrolysis in the plasma. Normally, AChE and BuChE between them keep the plasma ACh at an undetectably low level, so ACh is strictly a neurotransmitter and not a hormone.

▼ Both AChE and BuChE belong to the class of serine hydrolases, which includes many proteases such as trypsin. The active site of AChE comprises two distinct regions (Fig. 13.8): an *anionic site* (glutamate residue), which binds the basic (choline) moiety of ACh; and an *esteratic (catalytic) site* (histidine + serine). As with other serine hydrolases, the acidic (acetyl) group of the substrate is transferred to the serine hydroxyl group, leaving (transiently) an acetylated enzyme molecule and a molecule of free choline. Spontaneous hydrolysis of the serine acetyl group occurs rapidly, and the overall turnover number of AChE is extremely high (over 10 000 molecules of ACh hydrolysed per second by a single active site).

DRUGS THAT INHIBIT CHOLINESTERASE

Peripherally acting anticholinesterase drugs, summarised in Table 13.8, fall into three main groups according to the nature of their interaction with the active site, which determines their duration of action. Most of them inhibit AChE and BuChE about equally. Centrally acting anticholinesterases, developed for the treatment of dementia, are discussed in Chapter 40.

Short-acting anticholinesterases

The only important drug of this type is **edrophonium**, a quaternary ammonium compound that binds to the anionic site of the enzyme only. The ionic bond formed is readily reversible, and the action of the drug is very brief. It is used mainly for diagnostic purposes, because improvement of muscle strength by an anticholinesterase is characteristic of myasthenia gravis (see p. 175-176) but does not occur when muscle weakness is due to other causes.

Medium-duration anticholinesterases

These include **neostigmine** and **pyridostigmine**, which are quaternary ammonium compounds of clinical importance, and **physostigmine** (eserine), a tertiary amine, which occurs naturally in the Calabar bean.[7]

These drugs are all carbamyl, as opposed to acetyl, esters and all possess basic groups that bind to the anionic site. Transfer of the carbamyl group to the serine hydroxyl group of the esteratic site occurs as with ACh, but the carbamylated enzyme is very much slower to hydrolyse (Fig. 13.8), taking minutes rather than microseconds. The anticholinesterase drug is therefore hydrolysed, but at a negligible rate compared with ACh, and the slow recovery of the carbamylated enzyme means that the action of these drugs is quite long-lasting.

Irreversible anticholinesterases

Irreversible anticholinesterases (Table 13.8) are pentavalent phosphorus compounds containing a labile group such as fluoride (in **dyflos**) or an organic group (in **parathion** and **ecothiophate**). This group is released, leaving the serine hydroxyl group of the enzyme phosphorylated (Fig. 13.8). Most of these organophosphate compounds, of which there are many, were developed as war gases, such as **sarin**, and pesticides as well as for clinical use; they interact only with the esteratic site of the enzyme and have no cationic group. **Ecothiophate** is an exception in having a quaternary nitrogen group designed to bind also to the anionic site.

The inactive phosphorylated enzyme is usually very stable. With drugs such as dyflos, no appreciable hydrolysis occurs, and recovery of enzymic activity depends on the synthesis of new enzyme molecules, a process that may take weeks. With other drugs, such as ecothiophate, slow hydrolysis occurs over the course of a few days, so that their action is not strictly irreversible. Dyflos and parathion are volatile non-polar substances of very high lipid solubility, and are rapidly absorbed through mucous membranes and even through unbroken skin and insect cuticles; the use of these agents as war gases or insecticides relies on this property. The lack of a specificity-conferring quaternary group means that most of these drugs block other serine hydrolases (e.g. trypsin, thrombin), although their pharmacological effects result mainly from cholinesterase inhibition.

Effects of anticholinesterase drugs

Cholinesterase inhibitors affect peripheral as well as central cholinergic synapses.

Some organophosphate compounds can produce, in addition, a severe form of neurotoxicity.

[7]Otherwise known as the ordeal bean. In the Middle Ages, extracts of these beans were used to determine the guilt or innocence of those accused of crime or heresy. Death implied guilt.

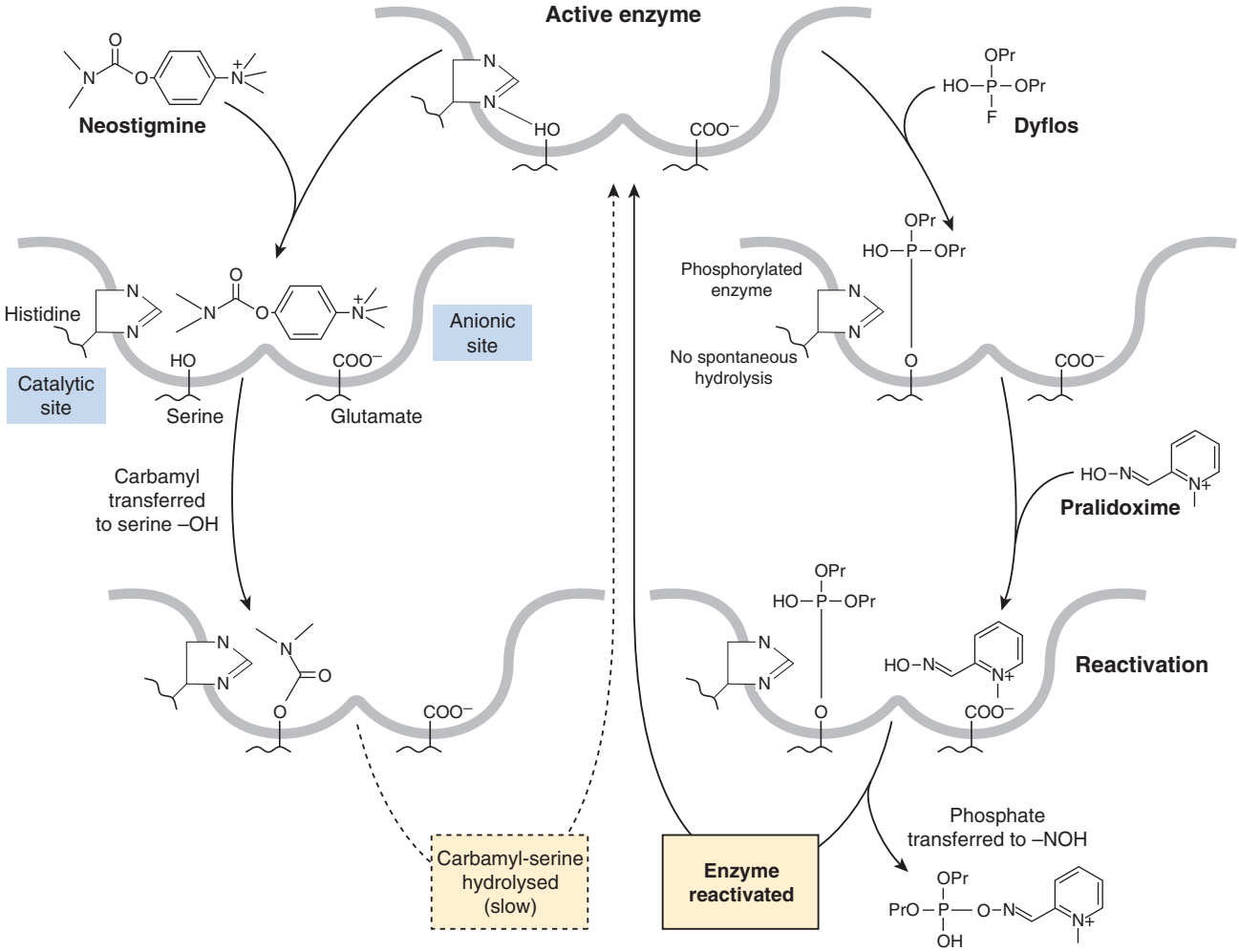


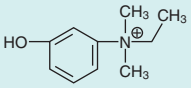
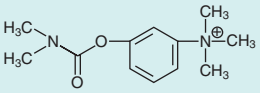
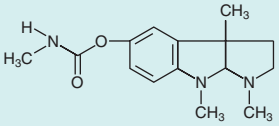
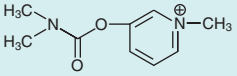
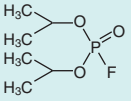
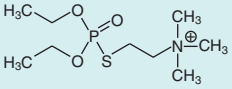
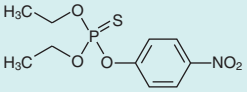
Fig. 13.8 **Action of anticholinesterase drugs.** Reversible anticholinesterase (neostigmine): recovery of activity by hydrolysis of the carbamylated enzyme takes many minutes. Irreversible anticholinesterase (dyflos): reactivation of phosphorylated enzyme by pralidoxime. The representation of the active site is purely diagrammatic and by no means representative of the actual molecular structure.

Effects on autonomic cholinergic synapses. These mainly reflect enhancement of ACh activity at parasympathetic postganglionic synapses (i.e. increased secretions from salivary, lacrimal, bronchial and gastrointestinal glands; increased peristaltic activity; bronchoconstriction; bradycardia and hypotension; pupillary constriction; fixation of accommodation for near vision; fall in intraocular pressure). Large doses can stimulate, and later block, autonomic ganglia, producing complex autonomic effects. The block, if it occurs, is a depolarisation block and is associated with a build-up of ACh in the plasma and body fluids. Neostigmine and pyridostigmine tend to affect neuromuscular transmission more than the autonomic system, whereas physostigmine and organophosphates show the reverse pattern. The reason is not clear, but therapeutic usage takes advantage of this partial selectivity.

Acute anticholinesterase poisoning (e.g. from contact with insecticides or war gases) causes severe bradycardia, hypotension and difficulty in breathing. Combined with a depolarising neuromuscular block and central effects (see p. 174), the result may be fatal.

Effects on the neuromuscular junction. The twitch tension of a muscle stimulated via its motor nerve is increased by anticholinesterases, owing to repetitive firing in the muscle fibre associated with prolongation of the epp. Normally, the ACh is hydrolysed so quickly that each stimulus initiates only one action potential in the muscle fibre, but when AChE is inhibited this is converted to a short train of action potentials in the muscle fibre, and hence greater tension. Much more important is the effect produced when transmission has been blocked by a non-depolarising blocking agent such as pancuronium. In this case, addition of an anticholinesterase can dramatically restore transmission. If a large proportion of the receptors is blocked, the majority of ACh molecules will normally encounter, and be destroyed by, an AChE molecule before reaching a vacant receptor; inhibiting AChE gives the ACh molecules a greater chance of finding a vacant receptor before being destroyed, and thus increase the epp so that it reaches threshold. In myasthenia gravis (see p. 175-176), transmission fails because there are too few ACh receptors, and cholinesterase inhibition improves transmission just as it does in curarised muscle.

Table 13.8 Anticholinesterase drugs

Drug	Structure	Duration of action	Main site of action	Notes
Edrophonium		Short	NMJ	Used mainly in diagnosis of myasthenia gravis Too short-acting for therapeutic use
Neostigmine		Medium	NMJ	Used intravenously to reverse competitive neuromuscular block Used orally in treatment of myasthenia gravis Visceral side effects
Physostigmine		Medium	P	Used as eye drops in treatment of glaucoma
Pyridostigmine		Medium	NMJ	Used orally in treatment of myasthenia gravis Better absorbed than neostigmine and has longer duration of action
Dyflos		Long	P	Highly toxic organophosphate, with very prolonged action Has been used as eye drops for glaucoma
Ecothiophate		Long	P	Used as eye drops in treatment of glaucoma Prolonged action; may cause systemic effects
Parathion		Long	–	Converted to active metabolite by replacement of sulfur by oxygen Used as insecticide but also causes poisoning in humans

Other anticholinesterase drugs developed for the treatment of dementia are described in Chapter 40.
NMJ, neuromuscular junction; P, postganglionic parasympathetic junction.

In large doses, such as can occur in poisoning, anticholinesterases initially cause twitching of muscles. This is because spontaneous ACh release can give rise to epps that reach the firing threshold. Later, paralysis may occur due to depolarisation block, which is associated with the build-up of ACh in the plasma and tissue fluids.

Effects on the CNS. Tertiary compounds, such as physostigmine, and the non-polar organophosphates penetrate the blood–brain barrier freely and affect the brain. The result is an initial excitation, which can result in convulsions, followed by depression, which can cause unconsciousness and respiratory failure. These central effects result mainly from the activation of mAChRs, and are antagonised by atropine. The use of anticholinesterases to treat senile dementia is discussed in Chapter 40.

Neurotoxicity of organophosphates. Many organophosphates can cause a severe type of delayed peripheral nerve degeneration, leading to progressive weakness and sensory loss. This is not a problem with clinically used anticholinesterases but occasionally results from poisoning with insecticides or nerve gases. In 1931, an estimated 20 000 Americans were affected, some fatally, by contamination of fruit juice with an organophosphate insecticide, and other similar outbreaks have been recorded. The mechanism of this reaction is only partly understood, but it seems to result from inhibition of a *neuropathy target esterase* distinct from cholinesterase. Chronic low level exposure of agricultural and other workers to organophosphorous pesticides has been associated with neurobehavioural disorders (Jamal et al., 2002).

The main uses of anticholinesterases are summarised in the clinical box (p. 175).

CHOLINESTERASE REACTIVATION

Spontaneous hydrolysis of phosphorylated cholinesterase is extremely slow, so poisoning with organophosphates necessitates prolonged supportive care. 3UDOLGR[LPH (Fig. 13.8) reactivates the enzyme by bringing an oxime group into close proximity with the phosphorylated esteratic site. This group is a strong nucleophile and lures the phosphate group away from the serine hydroxyl group of the enzyme. The effectiveness of pralidoxime in reactivating plasma cholinesterase activity in a poisoned subject is shown in Figure 13.9. The main limitation to its use as an antidote to organophosphate poisoning is that, within a few hours, the phosphorylated enzyme undergoes a

Cholinesterase and anticholinesterase drugs

- There are two main forms of cholinesterase: *acetylcholinesterase* (AChE), which is mainly membrane-bound, relatively specific for acetylcholine, and responsible for rapid acetylcholine hydrolysis at cholinergic synapses; and *butyrylcholinesterase* (BuChE) or pseudocholinesterase, which is relatively non-selective and occurs in plasma and many tissues. Both enzymes belong to the family of serine hydrolases.
- Anticholinesterase drugs are of three main types: short-acting (**edrophonium**); medium-acting (**neostigmine**, **physostigmine**); irreversible (organophosphates, **dyflos**, **ecothiophate**). They differ in the nature of their chemical interaction with the active site of cholinesterase.
- Effects of anticholinesterase drugs are due mainly to enhancement of cholinergic transmission at cholinergic autonomic synapses and at the neuromuscular junction. Anticholinesterases that cross the blood–brain barrier (e.g. **physostigmine**, organophosphates) also have marked central nervous system effects. Autonomic effects include bradycardia, hypotension, excessive secretions, bronchoconstriction, gastrointestinal hypermotility and decrease of intraocular pressure. Neuromuscular action causes muscle fasciculation and increased twitch tension, and can produce depolarisation block.
- Anticholinesterase poisoning may occur from exposure to insecticides or nerve gases.

Clinical uses of anticholinesterase drugs

- To reverse the action of non-depolarising neuromuscular-blocking drugs at the end of an operation (**neostigmine**). **Atropine** must be given to limit parasympathomimetic effects.
- To treat myasthenia gravis (**neostigmine** or **pyridostigmine**).
- As a test for myasthenia gravis and to distinguish weakness caused by anticholinesterase overdosage ('cholinergic crisis') from the weakness of myasthenia itself ('myasthenic crisis'): **edrophonium**, a short-acting drug given intravenously.
- Alzheimer's disease (e.g. **donepezil**; see Ch. 40).
- Glaucoma (**ecothiophate** eye drops).

chemical change ('ageing') that renders it no longer susceptible to reactivation, so that pralidoxime must be given early in order to work. Pralidoxime does not enter the brain, but related compounds have been developed to treat the central effects of organophosphate poisoning.

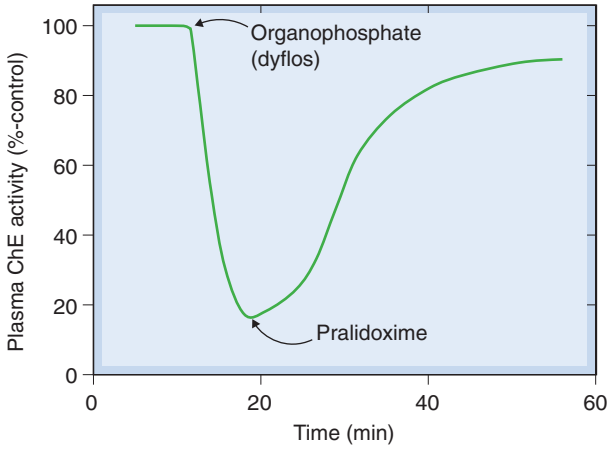


Fig. 13.9 Reactivation of plasma cholinesterase (ChE) in a volunteer subject by intravenous injection of pralidoxime.

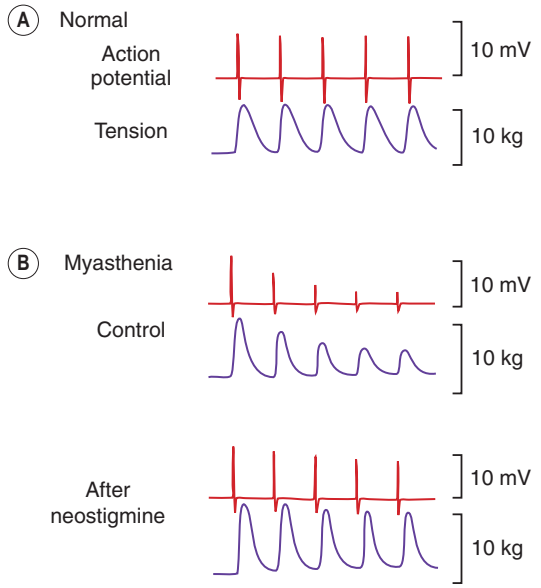


Fig. 13.10 **Neuromuscular transmission in a normal and a myasthenic human subject.** Electrical activity was recorded with a needle electrode in the adductor pollicis muscle, in response to ulnar nerve stimulation (3 Hz) at the wrist. In a normal subject, electrical and mechanical response is well sustained. In a myasthenic patient, transmission fails rapidly when the nerve is stimulated. Treatment with neostigmine improves transmission. *(From Desmedt JE 1962 Bull Acad R Med Belg VII 2, 213.)*

Myasthenia gravis

▼ The neuromuscular junction is a robust structure that very rarely fails, myasthenia gravis and the Lambert–Eaton myasthenic syndrome (see p. 176) being two of the few disorders that specifically affects it. Myasthenia gravis affects about 1 in 2000 individuals, who show muscle weakness and increased fatiguability resulting from a failure of neuromuscular transmission. The tendency for transmission to fail during repetitive activity can be seen in Figure 13.10. Functionally, it results in the inability of muscles to produce

sustained contractions, of which the characteristic drooping eyelids of myasthenic patients are a sign. The effectiveness of anticholinesterase drugs in improving muscle strength in myasthenia was discovered in 1931, long before the cause of the disease was known.

The cause of the transmission failure is an autoimmune response that causes a loss of nAChRs from the neuromuscular junction, first revealed in studies showing that the number of bungarotoxin-binding sites at the endplates of myasthenic patients was reduced by about 70% compared with normal. It had been suspected that myasthenia had an immunological basis, because removal of the thymus gland is frequently of benefit. Immunisation of rabbits with purified ACh receptor causes, after a delay, symptoms very similar to those of human myasthenia gravis. The presence of antibody directed against the ACh receptor protein can be detected in the serum of myasthenic patients, but the reason for the development of the autoimmune response in humans is unknown (Vrolix et al., 2010).

The improvement of neuromuscular function by anticholinesterase treatment (shown in Fig. 13.10) can be dramatic, but if the disease progresses too far, the number of receptors remaining may be insufficient to produce an adequate epp, and anticholinesterase drugs will then cease to be effective.

Alternative approaches to the treatment of myasthenia are to remove circulating antibody by plasma exchange, which is transiently effective, or, for a more prolonged effect, to inhibit antibody production with immunosuppressant drugs (e.g. **prednisolone**, **azathioprine**; see Ch. 26) or thymectomy.

OTHER DRUGS THAT ENHANCE CHOLINERGIC TRANSMISSION

It was observed many years ago that **tetraethylammonium**, a potassium-channel blocker and ganglion-blocking drug, could reverse the neuromuscular-blocking action of tubocurarine by prolonging the action potential in the nerve terminal and hence increasing the release of transmitter evoked by nerve stimulation. Subsequently, aminopyridines such as **amifampridine**, which also block potassium channels (see Ch. 4) were found to act similarly and to be considerably more potent and selective in their actions than tetraethylammonium. These drugs are not selective for cholinergic nerves but increase the evoked release of many different transmitters. Amifampridine (licensed in 2010) is used to treat the muscle weakness associated with Lambert–Eaton myasthenic syndrome (Maddison & Newsom-Davis, 2003), a complication of certain neoplastic diseases in which acetylcholine release is inhibited because antitumour antibodies cross react with Ca^{2+} channels on the prejunctional membrane.

▼ A related drug, **fampridine,** improves walking in patients whose walking is impaired by the demyelinating disease multiple sclerosis. It works by blocking axonal K^+ channels, thereby facilitating impulse conduction along demyelinated axons.

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14 Noradrenergic transmission

OVERVIEW

The peripheral noradrenergic neuron and the structures that it innervates are important targets for drug action, both as objects for investigation in their own right and as points of attack for many clinically useful drugs. In this chapter, we describe the physiology and function of noradrenergic neurons and the properties of adrenoceptors (the receptors on which noradrenaline and adrenaline act), and discuss the various classes of drugs that affect them. For convenience, much of the pharmacological information is summarised in tables later in the chapter.

CATECHOLAMINES

Catecholamines are compounds containing a catechol moiety (a benzene ring with two adjacent hydroxyl groups) and an amine side chain (Fig. 14.1). Pharmacologically, the most important ones are:

- **noradrenaline** (**norepinephrine**), a transmitter released by sympathetic nerve terminals
- **adrenaline** (**epinephrine**), a hormone secreted by the adrenal medulla
- **dopamine**, the metabolic precursor of noradrenaline and adrenaline, also a transmitter/neuromodulator in the central nervous system
- **isoprenaline** (**isoproterenol**), a synthetic derivative of noradrenaline, not present in the body.

CLASSIFICATION OF ADRENOCEPTORS

In 1896, Oliver and Schafer discovered that injection of extracts of adrenal gland caused a rise in arterial pressure. Following the subsequent isolation of adrenaline as the active principle, it was shown by Dale in 1913 that adrenaline causes two distinct kinds of effect, namely vasoconstriction in certain vascular beds (which normally predominates and, together with its actions on the heart – see p. 184 – causes the rise in arterial pressure) and vasodilatation in others. Dale showed that the vasoconstrictor component disappeared if the animal was first injected with an ergot derivative[1] (see Ch. 15), and noticed that adrenaline then caused a fall, instead of a rise, in arterial pressure. This result paralleled Dale's demonstration of the separate muscarinic and nicotinic components of the action of acetylcholine (see Ch. 13). He avoided interpreting it in terms of different types of receptor, but later pharmacological work, beginning with that of Ahlquist, showed clearly the existence of several subclasses of adrenoceptor with distinct tissue distributions and actions (Table 14.1).

Ahlquist found in 1948 that the rank order of the potencies of various catecholamines, including adrenaline, noradrenaline and isoprenaline, fell into two distinct patterns, depending on what response was being measured. He postulated the existence of two kinds of receptor, α and β, defined in terms of agonist potencies as follows:

α: noradrenaline > adrenaline > isoprenaline

β: isoprenaline > adrenaline > noradrenaline

It was then recognised that certain ergot alkaloids, which Dale had studied, act as selective α-receptor antagonists, and that Dale's adrenaline reversal experiment reflected the unmasking of the β effects of adrenaline by α-receptor blockade. Selective β-receptor antagonists were not developed until 1955, when their effects fully confirmed Ahlquist's original classification and also suggested the existence of further subdivisions of both α and β receptors. Subsequently it has emerged that there are two α-receptor subtypes (α_1 and α_2), each comprising three further subclasses (α_{1A}, α_{1B}, α_{1D} and α_{2A}, α_{2B}, α_{2C}) and three β-receptor subtypes (β_1, β_2 and β_3) – altogether nine distinct subtypes – all of which are typical G protein-coupled receptors (Table 14.2). Evidence from specific agonists and antagonists, as well as studies on receptor knockout mice (Philipp & Hein, 2004), has shown that α_1 receptors are particularly important in the cardiovascular system and lower urinary tract, while α_2 receptors are predominantly neuronal, acting to inhibit transmitter release both in the brain and at autonomic nerve terminals in the periphery. The distinct functions of the different subclasses of α_1 and α_2 adrenoceptors remain for the most part unclear; they are frequently co-expressed in the same tissues, and may form heterodimers, making pharmacological analysis difficult.

Each of the three main receptor subtypes is associated with a specific second messenger system (Table 14.2). Thus α_1 receptors are coupled to phospholipase C and produce their effects mainly by the release of intracellular Ca^{2+}; α_2 receptors are negatively coupled to adenylyl cyclase, and reduce cAMP formation as well as inhibiting Ca^{2+} channels and activating K^+ channels; and all three types of β receptor act by stimulation of adenylyl cyclase. The major effects that are produced by these receptors, and the main drugs that act on them, are shown in Tables 14.1 and 14.2; more detailed summaries of adrenoceptor agonists and antagonists are given later in Tables 14.4 and 14.5 respectively.

The distinction between β_1 and β_2 receptors is an important one, for β_1 receptors are found mainly in the heart, where they are responsible for the positive inotropic and chronotropic effects of catecholamines (see Ch. 21). β_2 receptors, on the other hand, are responsible for causing

[1]Dale was a new recruit in the laboratories of the Wellcome pharmaceutical company, given the job of checking the potency of batches of adrenaline coming from the factory. He tested one batch at the end of a day's experimentation on a cat that he had earlier injected with an ergot preparation. Because it produced a fall in blood pressure rather than the expected rise, he advised that the whole expensive consignment should be rejected. Unknown to him, he was given the same sample to test a few days later, and reported it to be normal. How he explained this to Wellcome's management is not recorded.

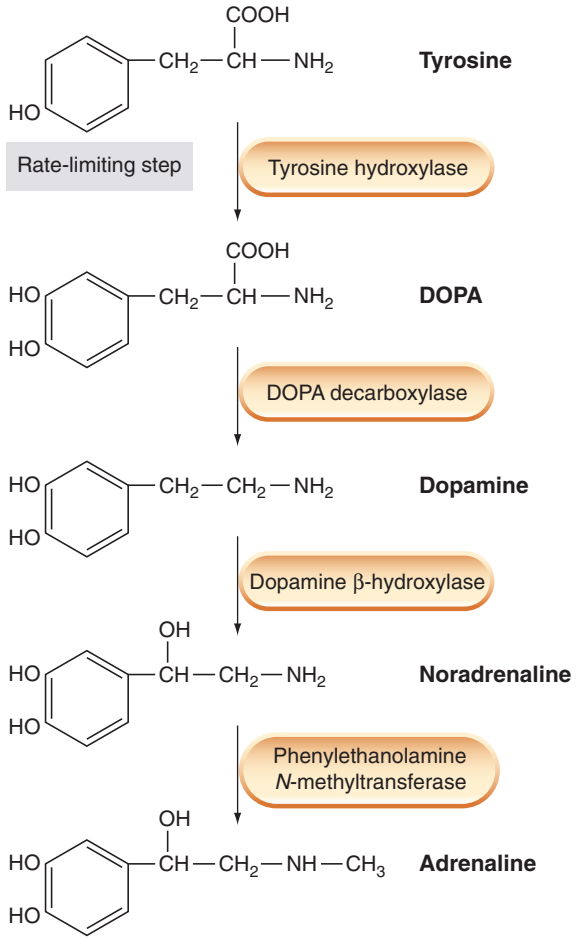

Fig. 14.1 Structures of the major catecholamines.

Classification of adrenoceptors

- Main pharmacological classification into α and β subtypes, based originally on order of potency among agonists, later on selective antagonists.
- Adrenoceptor subtypes:
  - two main α-adrenoceptor subtypes, α_1 and α_2, each divided into three further subtypes (1-/2- A,B,C)
  - three β-adrenoceptor subtypes (β_1, β_2, β_3)
  - all belong to the superfamily of G protein-coupled receptors (see Ch. 3).
- Second messengers:
  - α_1 receptors activate phospholipase C, producing inositol trisphosphate and diacylglycerol as second messengers
  - α_2 receptors inhibit adenylyl cyclase, decreasing cAMP formation
  - all types of β receptor stimulate adenylyl cyclase.
- The main effects of receptor activation are as follows:
  - α_1 receptors: vasoconstriction, relaxation of gastrointestinal smooth muscle, salivary secretion and hepatic glycogenolysis
  - α_2 receptors: *inhibition* of: transmitter release (including noradrenaline and acetylcholine release from autonomic nerves); platelet aggregation; vascular smooth muscle contraction; insulin release
  - β_1 receptors: increased cardiac rate and force
  - β_2 receptors: bronchodilatation; vasodilatation; relaxation of visceral smooth muscle; hepatic glycogenolysis; muscle tremor
  - β_3 receptors: lipolysis and thermogenesis; bladder detrusor muscle relaxation.

smooth muscle relaxation in many organs. The latter is often a useful therapeutic effect, while the former is more often harmful; consequently, considerable efforts have been made to find selective β_2 agonists, which would relax smooth muscle without affecting the heart, and selective β_1 antagonists, which would exert a useful blocking effect on the heart without at the same time blocking β_2 receptors in, for example, bronchial smooth muscle (see Table 14.1). It is important to realise that the available drugs are not completely selective, and that compounds used as selective β_1 antagonists invariably have some action on β_2 receptors as well, which can cause unwanted effects such as bronchoconstriction.

In relation to vascular control, it is important to note that both α and β receptor subtypes are expressed in smooth muscle cells, nerve terminals and endothelial cells, and their role in physiological regulation and pharmacological responses of the cardiovascular system is only partly understood (see Guimaraes & Moura, 2001).

PHYSIOLOGY OF NORADRENERGIC TRANSMISSION

THE NORADRENERGIC NEURON

Noradrenergic neurons in the periphery are postganglionic sympathetic neurons whose cell bodies are situated in sympathetic ganglia. They generally have long[2] axons that end in a series of varicosities strung along the branching terminal network. These varicosities contain numerous synaptic vesicles, which are the sites of synthesis and release of noradrenaline and of co-released mediators such as ATP and neuropeptide Y (see Ch. 12), which are stored in vesicles and released by exocytosis (Ch. 4). In most peripheral tissues, the tissue content of noradrenaline closely parallels the density of the sympathetic innervation. With the exception of the adrenal medulla, sympathetic nerve terminals account for all the noradrenaline content of peripheral tissues. Organs such as the heart, spleen, vas deferens and some blood vessels are particularly rich in noradrenaline (5–50 nmol/g of tissue) and have been widely used for studies of noradrenergic transmission. For detailed information on noradrenergic neurons, see Robertson (2004) and Cooper et al. (2002).

NORADRENALINE SYNTHESIS

The biosynthetic pathway for noradrenaline synthesis is shown in Fig. 14.1 and drugs that affect noradrenaline

[2]Just how long may be appreciated by scaling up the diameter of a neuronal cell body (4–100 μm) to that of a golf ball (≥42 670 μm diameter, a scaling factor of say 400–10 000); proportionately the axon (length from sympathetic chain ganglion to, say, a blood vessel in the calf, approximately 1 metre) will now reach some 0.4–10 km – some challenge in terms of command and control!

Table 14.1 Distribution and actions of adrenoceptors

Tissues and effects	α_1	α_2	β_1	β_2	β_3
SMOOTH MUSCLE					
Blood vessels	Constrict	Constrict/dilate	–	Dilate	–
Bronchi	Constrict	–	–	Dilate	–
Gastrointestinal tract	Relax	Relax (presynaptic effect)	–	Relax	–
Gastrointestinal sphincters	Contract	–	–	–	–
Uterus	Contract	–	–	Relax	–
Bladder detrusor	–	–	–	Relax	Relax
Bladder sphincter	Contract	–	–	–	–
Seminal tract	Contract	–	–	Relax	–
Iris (radial muscle)	Contract	–	–	–	–
Ciliary muscle	–	–	–	Relax	–
HEART					
Rate	–	–	Increase	Increase[a]	–
Force of contraction	–	–	Increase	Increase[a]	–
OTHER TISSUES/CELLS					
Skeletal muscle	–	–	–	Tremor Increased muscle mass and speed of contraction Glycogenolysis	Thermogenesis
Liver (hepatocytes)	Glycogenolysis	–	–	Glycogenolysis	–
Fat (adipocytes)	–	–	–	–	Lipolysis Thermogenesis
Pancreatic islets (B cells)	–	Decrease insulin secretion	–	–	–
Salivary gland	K^+ release	–	Amylase secretion	–	–
Platelets	–	Aggregation	–	–	–
Mast cells	–	–	–	Inhibition of histamine release	–
Brain stem	–	Inhibits sympathetic outflow	–	–	–
NERVE TERMINALS					
Adrenergic	–	Decrease release	–	Increase release	–
Cholinergic	–	Decrease release	–	–	–

[a]Minor component normally but may become significant in heart failure.

Table 14.2 Characteristics of adrenoceptors

	α_1	α_2	β_1	β_2	β_3
Second messengers and effectors	Phospholipase C activation ↑ Inositol trisphosphate ↑ Diacylglycerol ↑ Ca^{2+}	↓ cAMP ↓ Calcium channels ↑ Potassium channels	↑ cAMP	↑ cAMP	↑ cAMP
Agonist potency order	NA > A >> ISO	A > NA >> ISO	ISO > NA > A	ISO > A > NA	ISO > NA = A
Selective agonists	Phenylephrine Methoxamine	Clonidine	Dobutamine Xamoterol	Salbutamol Terbutaline Salmeterol Formoterol Clenbuterol	Mirabegron
Selective antagonists	Prazosin Doxazocin	Yohimbine Idazoxan	Atenolol Metoprolol	Butoxamine	–

A, adrenaline; ISO, isoprenaline; NA, noradrenaline.

synthesis are summarised in Table 14.6 (p. 193). The metabolic precursor for noradrenaline is *L-tyrosine*, an aromatic amino acid that is present in the body fluids and is taken up by adrenergic neurons. *Tyrosine hydroxylase*, a cytosolic enzyme that catalyses the conversion of tyrosine to *dihydroxyphenylalanine* (dopa), is found only in catecholamine-containing cells. It is a rather selective enzyme; unlike other enzymes involved in catecholamine metabolism, it does not accept indole derivatives as substrates, and so is not involved in 5-hydroxytryptamine (5-HT) metabolism. This first hydroxylation step is the main control point for noradrenaline synthesis. Tyrosine hydroxylase is inhibited by the end product of the biosynthetic pathway, noradrenaline, and this provides the mechanism for the moment-to-moment regulation of the rate of synthesis; much slower regulation, taking hours or days, occurs by changes in the rate of production of the enzyme.

The tyrosine analogue **α-methyltyrosine** strongly inhibits tyrosine hydroxylase and has been used experimentally to block noradrenaline synthesis.

The next step, conversion of dopa to dopamine, is catalysed by *dopa decarboxylase*, a cytosolic enzyme that is by no means confined to catecholamine-synthesising cells. It is a relatively non-specific enzyme, and catalyses the decarboxylation of various other L-aromatic amino acids, such as *L-histidine* and *L-tryptophan*, which are precursors in the synthesis of histamine (Ch. 17) and 5-HT (Ch. 15), respectively. Dopa decarboxylase activity is not rate-limiting for noradrenaline synthesis. Although various factors, including certain drugs, affect the enzyme, it is not an effective means of regulating noradrenaline synthesis.

Dopamine-β-hydroxylase (DBH) is also a relatively non-specific enzyme, but is restricted to catecholamine-synthesising cells. It is located in synaptic vesicles, mainly in membrane-bound form. A small amount of the enzyme is released from adrenergic nerve terminals in company with noradrenaline, representing the small proportion in a soluble form within the vesicle. Unlike noradrenaline, the released DBH is not subject to rapid degradation or uptake, so its concentration in plasma and body fluids can be used as an index of overall sympathetic nerve activity.

Many drugs inhibit DBH, including copper-chelating agents and **disulfiram** (a drug used mainly for its effect on ethanol metabolism; see Ch. 49). Such drugs can cause a partial depletion of noradrenaline stores and interference with sympathetic transmission. A rare genetic disorder, DBH deficiency, causes failure of noradrenaline synthesis resulting in severe orthostatic hypotension (see Ch. 22).

Phenylethanolamine N-*methyl transferase* (PNMT) catalyses the *N*-methylation of noradrenaline to adrenaline. The main location of this enzyme is in the adrenal medulla, which contains a population of adrenaline-releasing (A) cells separate from the smaller proportion of noradrenaline-releasing (N) cells. The A cells, which appear only after birth, lie adjacent to the adrenal cortex, and the production of PNMT is induced by an action of the steroid hormones secreted by the adrenal cortex (see Ch. 33). PNMT is also found in certain parts of the brain, where adrenaline may function as a transmitter, but little is known about its role in the central nervous system (CNS).

Noradrenaline turnover can be measured under steady-state conditions by measuring the rate at which labelled noradrenaline accumulates when a labelled precursor, such as tyrosine or dopa, is administered. The turnover time is defined as the time taken for an amount of noradrenaline equal to the total tissue content to be degraded and resynthesised. In peripheral tissues, the turnover time is generally about 5–15 h, but it becomes much shorter if sympathetic nerve activity is increased. Under normal circumstances, the rate of synthesis closely matches the rate of release, so that the noradrenaline content of tissues is constant regardless of how fast it is being released.

NORADRENALINE STORAGE

Most of the noradrenaline in nerve terminals or chromaffin cells is contained in vesicles; only a little is free in the cytoplasm under normal circumstances. The concentration in the vesicles is very high (0.3–1.0 mol/l) and is maintained by the *vesicular monoamine transporter* (VMAT), which is similar to the amine transporter responsible for noradrenaline uptake into the nerve terminal (see Ch. 12), but uses the transvesicular proton gradient as its driving force. Certain drugs, such as **reserpine** (see p. 181; Table 14.3) block this transport and cause nerve terminals to become depleted of their vesicular noradrenaline stores. The vesicles contain two major constituents besides noradrenaline, namely ATP (about four molecules per molecule of noradrenaline) and a protein called *chromogranin A*. These substances are released along with noradrenaline, and it is generally assumed that a reversible complex, depending partly on the opposite charges on the molecules of noradrenaline and ATP, is formed within the vesicle. This would serve both to reduce the osmolarity of the vesicle contents and also to reduce the tendency of noradrenaline to leak out of the vesicles within the nerve terminal.

ATP itself has a transmitter function at noradrenergic synapses (see Fig. 12.5; Ch. 16), being responsible for the fast excitatory synaptic potential and the rapid phase of contraction produced by sympathetic nerve activity in many smooth muscle tissues.

NORADRENALINE RELEASE

The processes linking the arrival of a nerve impulse at a nerve terminal to Ca^{2+} entry and the release of transmitter are described in Chapter 4. Drugs that affect noradrenaline release are summarised in Table 14.6 (p. 193).

An unusual feature of the release mechanism at the varicosities of noradrenergic nerves is that the probability of release, even of a single vesicle, when a nerve impulse arrives at a varicosity is very low (less than 1 in 50). A single neuron possesses many thousand varicosities, so one impulse leads to the discharge of a few hundred vesicles, scattered over a wide area. This contrasts sharply with the neuromuscular junction (Ch. 13), where the release probability at a single terminal is high, and release of acetylcholine is sharply localised.

Regulation of noradrenaline release

Noradrenaline release is affected by a variety of substances that act on presynaptic receptors (see Ch. 12). Many different types of nerve terminal (cholinergic, noradrenergic, dopaminergic, 5-HT-ergic, etc.) are subject to this type of control, and many different mediators (acetylcholine acting through muscarinic receptors, catecholamines acting through α and β receptors, angiotensin II, prostaglandins, purine nucleotides, neuropeptides, etc.) can act on presynaptic terminals. Presynaptic modulation represents an important physiological control mechanism throughout the nervous system.

Table 14.3 Characteristics of noradrenaline (norepinephrine) transport systems

	Neuronal (NET)	Extraneuronal (EMT)	Vesicular (VMAT)
Transport of NA (rat heart) V_{max} (nmol g^{-1} min^{-1})	1.2	100	–
K_m (μmol/l)	0.3	250	~0.2
Specificity	NA > A > ISO	A > NA > ISO	NA = A = ISO
Location	Neuronal membrane	Non-neuronal cell membrane (smooth muscle, cardiac muscle, endothelium)	Synaptic vesicle membrane
Other substrates	Tyramine Methylnoradrenaline Adrenergic neuron-blocking drugs (e.g. guanethidine) Amphetamine[a]	(+)-Noradrenaline Dopamine 5-Hydroxytryptamine Histamine	Dopamine 5-Hydroxytryptamine Guanethidine MPP^+ (see Ch. 40)
Inhibitors	Cocaine Tricyclic antidepressants (e.g. desipramine) Phenoxybenzamine Amphetamine[a]	Normetanephrine Steroid hormones (e.g. corticosterone) Phenoxybenzamine	Reserpine Tetrabenazine

[a]Amphetamine is transported slowly, so acts both as a substrate and as an inhibitor of noradrenaline uptake. For details, see Gainetdinov & Caron, 2003.
A, adrenaline; ISO, isoprenaline; NA, noradrenaline.

Furthermore, noradrenaline, by acting on presynaptic β_2 receptors, can regulate its own release, and also that of co-released ATP (see Ch. 12). This is believed to occur physiologically, so that released noradrenaline exerts a local inhibitory effect on the terminals from which it came – the so-called *autoinhibitory feedback* mechanism (Fig. 14.2; see Gilsbach & Hein, 2012). Agonists or antagonists affecting these presynaptic receptors can have large effects on sympathetic transmission. However, the physiological significance of presynaptic autoinhibition in the sympathetic nervous system is still somewhat contentious, and there is evidence that, in most tissues, it is less influential than biochemical measurements of transmitter overflow would seem to imply. Thus, although blocking autoreceptors causes large changes in noradrenaline *overflow* – the amount of noradrenaline released into the bathing solution or the bloodstream when sympathetic nerves are stimulated – the associated changes in the tissue response are often rather small. This suggests that what is measured in overflow experiments may not be the physiologically important component of transmitter release.

The inhibitory feedback mechanism operates through α_2 receptors, which inhibit adenylyl cyclase and prevent the opening of calcium channels. Sympathetic nerve terminals also possess β_2 receptors, coupled to activation of adenylyl cyclase, which *increase* noradrenaline release. Whether they have any physiological function is not yet clear.

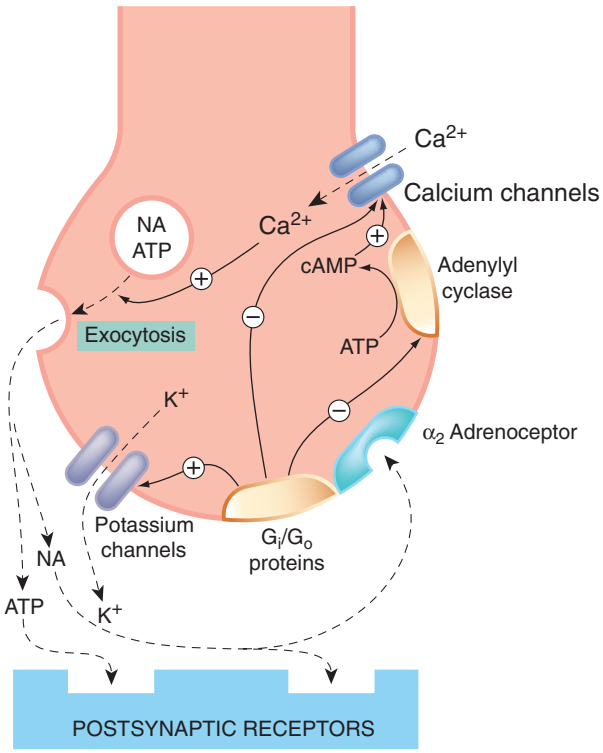

Fig. 14.2 Feedback control of noradrenaline (NA) release. The presynaptic α_2 receptor inhibits Ca^{2+} influx in response to membrane depolarisation via an action of the βγ subunits of the associated G protein on the voltage-dependent Ca^{2+} channels (Ch. 3).

UPTAKE AND DEGRADATION OF CATECHOLAMINES

The action of released noradrenaline is terminated mainly by reuptake of the transmitter into noradrenergic nerve terminals. Some is also sequestered by other cells in the vicinity. Circulating adrenaline and noradrenaline are degraded enzymically, but much more slowly than acetylcholine (see Ch. 13), where synaptically located

acetylcholinesterase inactivates the transmitter in milliseconds. The two main catecholamine-metabolising enzymes are located intracellularly, so uptake into cells necessarily precedes metabolic degradation.

UPTAKE OF CATECHOLAMINES

About 75% of the noradrenaline released by sympathetic neurons is recaptured and repackaged into vesicles. This serves to cut short the action of the released noradrenaline, as well as recycling it. The remaining 25% is captured by non-neuronal cells in the vicinity, limiting its local spread. These two uptake mechanisms depend on distinct transporter molecules. Neuronal uptake is performed by the plasma membrane noradrenaline transporter (generally known as NET, the *norepinephrine transporter*), which belongs to the family of neurotransmitter transporter proteins (NET, DAT, SERT, etc.) specific for different amine transmitters, described in Chapter 12; these act as co-transporters of Na^+, Cl^- and the amine in question, using the electrochemical gradient for Na^+ as a driving force. Packaging into vesicles occurs through the *vesicular monoamine transporter* (VMAT), driven by the proton gradient between the cytosol and the vesicle contents. Extraneuronal uptake is performed by the *extraneuronal monoamine transporter* (EMT), which belongs to a large and widely distributed family of organic cation transporters (OCTs, see Ch. 8). NET is relatively selective for noradrenaline, with high affinity and a low maximum rate of uptake, and it is important in maintaining releasable stores of noradrenaline. EMT has lower affinity and higher transport capacity than NET, and transports adrenaline and isoprenaline as well as noradrenaline. The effects of several important drugs that act on noradrenergic neurons depend on their ability either to inhibit NET or to enter the nerve terminal with its help. Table 14.3 summarises the properties of neuronal and extraneuronal uptake.

METABOLIC DEGRADATION OF CATECHOLAMINES

Endogenous and exogenous catecholamines are metabolised mainly by two intracellular enzymes: *monoamine oxidase* (MAO) and *catechol-O-methyl transferase* (COMT). MAO (of which there are two distinct isoforms, MAO-A and MAO-B; see Chs 39 and 47) is bound to the surface membrane of mitochondria. It is abundant in noradrenergic nerve terminals but is also present in liver, intestinal epithelium and other tissues. MAO converts catecholamines to their corresponding aldehydes,[3] which, in the periphery, are rapidly metabolised by *aldehyde dehydrogenase* to the corresponding carboxylic acid (3,4-dihydroxyphenylglycol being formed from noradrenaline; Fig. 14.3). MAO can also oxidise other monoamines, including dopamine and 5-HT. It is inhibited by various drugs which are used mainly for their effects on the central nervous system, where these three amines all have transmitter functions (see Ch. 39). These drugs have important side effects that are related to disturbances of peripheral noradrenergic transmission. Within sympathetic neurons, MAO controls the content of dopamine and noradrenaline, and the releasable store of noradrenaline increases if the enzyme is inhibited. MAO and its inhibitors are discussed in more detail in Chapter 47.

The second major pathway for catecholamine metabolism involves methylation of one of the catechol hydroxyl groups by COMT to give a methoxy derivative. COMT is absent from noradrenergic neurons but present in the adrenal medulla and many other cells and tissues. The final product formed by the sequential action of MAO and COMT is *3-methoxy-4-hydroxyphenylglycol* (MHPG; see Fig. 14.3). This is partly conjugated to sulfate or glucuronide derivatives, which are excreted in the urine, but most of it is converted to *vanillylmandelic acid* (VMA; Fig. 14.3) and excreted in the urine in this form. In patients with tumours of chromaffin tissue that secrete these amines (a rare cause of high blood pressure), the urinary excretion of VMA is markedly increased, this being used as a diagnostic test for this condition.

In the periphery, neither MAO nor COMT is primarily responsible for the termination of transmitter action, most of the released noradrenaline being quickly recaptured by NET. Circulating catecholamines are sequestered and inactivated by a combination of NET, EMT and COMT, the relative importance of these processes varying according to the agent concerned. Thus circulating noradrenaline is removed mainly by NET, whereas adrenaline is more dependent on EMT. Isoprenaline, on the other hand, is not a substrate for NET, and is removed by a combination of EMT and COMT.

In the central nervous system (see Ch. 39), MAO is more important as a means of terminating transmitter action than it is in the periphery, and MAO knockout mice show a greater enhancement of noradrenergic transmission in the brain than do NET knockouts, in which neuronal stores of noradrenaline are much depleted (see Gainetdinov & Caron, 2003). The main excretory product of noradrenaline released in the brain is MHPG.

DRUGS ACTING ON NORADRENERGIC TRANSMISSION

Many clinically important drugs, particularly those used to treat cardiovascular, respiratory and psychiatric disorders (see Chs 21, 22, 28 and 47), act by affecting noradrenergic neuron function, acting on adrenoceptors, transporters or catecholamine-metabolising enzymes. The properties of the most important drugs in this category are summarised in Tables 14.4–4.6.

DRUGS ACTING ON ADRENOCEPTORS

The overall activity of these drugs is governed by their affinity, efficacy and selectivity with respect to different types of adrenoceptor, and intensive research has been devoted to developing drugs with the right properties for specific clinical indications. As a result, the pharmacopoeia is awash with adrenoceptor ligands. Many clinical needs are met, it turns out, by drugs that relax smooth muscle in different organs of the body[4] and those that block the cardiac stimulant effects of the sympathetic nervous system; on the other hand, cardiac stimulation is generally undesirable in chronic disease.

[3] Aldehyde metabolites are potentially neurotoxic, and are thought to play a role in certain degenerative CNS disorders (see Ch. 40).

[4] And conversely, contracting smooth muscle is often bad news. This bald statement must not be pressed too far, but the exceptions (such as nasal decongestants and drugs acting on the eye) are surprisingly few. Even adrenaline (potentially life-saving in cardiac arrest) dilates some vessels while constricting others to less immediately essential tissues such as skin).

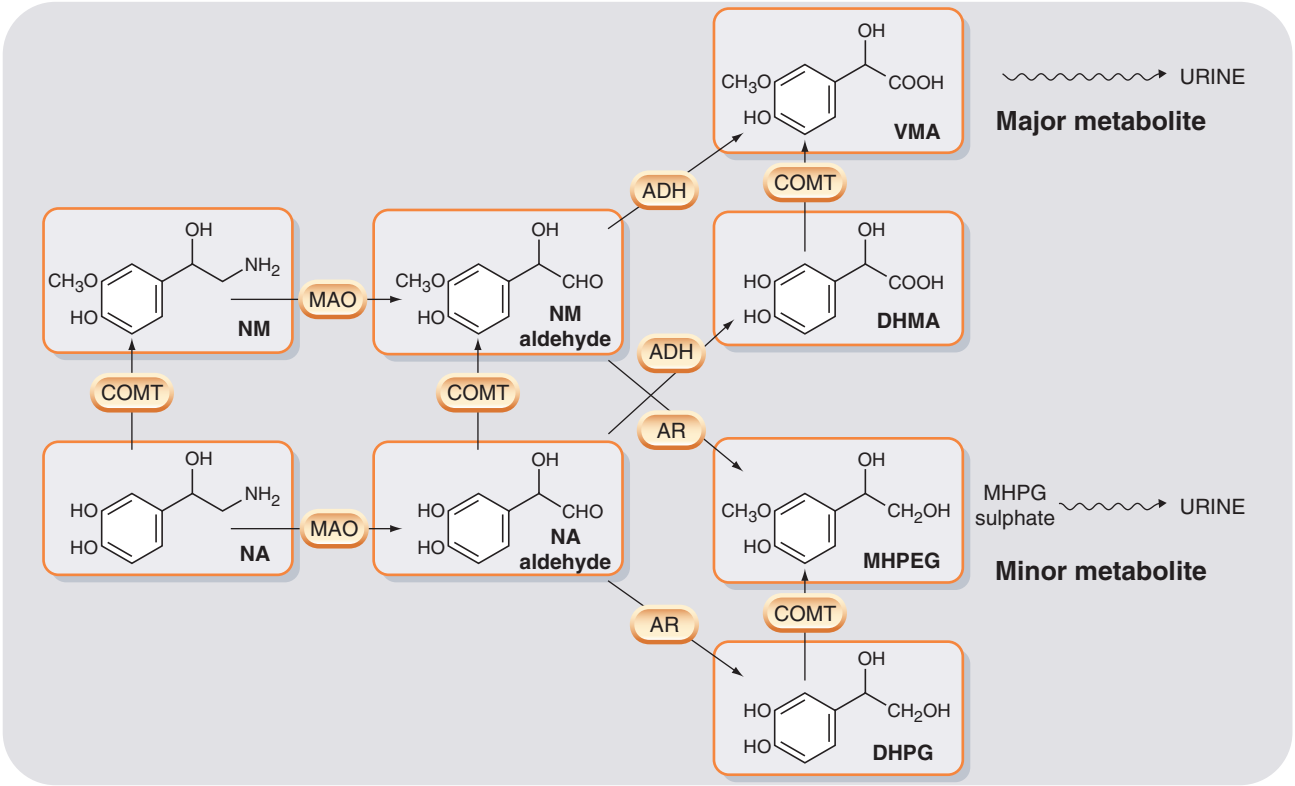

Fig. 14.3 The main pathways of noradrenaline metabolism. The oxidative branch (catalysed by ADH) predominates, giving VMA as the main urinary metabolite. The reductive branch (catalysed by AR) produces the less abundant metabolite, MHPG, which is conjugated to MHPG sulfate before being excreted. ADH, aldehyde dehydrogenase; AR, aldehyde reductase; COMT, catechol-*O*-methyl transferase; DHMA, 3,4-dihydroxymandelic acid; DHPG, 3,4-dihydroxyphenylglycol; MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylglycol; NA, noradrenaline; NM, normetanephrine; VMA, vanillylmandelic acid.

Noradrenergic transmission

- Transmitter synthesis involves the following:
  - L-tyrosine is converted to dihydroxyphenylalanine (dopa) by tyrosine hydroxylase (rate-limiting step). Tyrosine hydroxylase occurs only in catecholaminergic neurons.
  - Dopa is converted to dopamine by dopa decarboxylase.
  - Dopamine is converted to noradrenaline by dopamine β-hydroxylase (DBH), located in synaptic vesicles.
  - In the adrenal medulla, noradrenaline is converted to adrenaline by phenylethanolamine *N*-methyltransferase.
- Transmitter storage: noradrenaline is stored at high concentration in synaptic vesicles, together with ATP, chromogranin and DBH, all of which are released by exocytosis. Transport of noradrenaline into vesicles occurs by a reserpine-sensitive transporter (VMAT). Noradrenaline content of cytosol is normally low due to monoamine oxidase in nerve terminals.
- Transmitter release occurs normally by Ca^{2+}-mediated exocytosis from varicosities on the terminal network. Non-exocytotic release occurs in response to indirectly acting sympathomimetic drugs (e.g. **amphetamine**), which displace noradrenaline from vesicles. Noradrenaline escapes via the NET transporter (reverse transport).
- Transmitter action is terminated mainly by reuptake of noradrenaline into nerve terminals via the NET transporter. NET is blocked by tricyclic antidepressant drugs and **cocaine**.
- Noradrenaline release is controlled by autoinhibitory feedback mediated by α_2 receptors.
- Co-transmission occurs at many noradrenergic nerve terminals, ATP and neuropeptide Y being frequently co-released with NA. ATP mediates the early phase of smooth muscle contraction in response to sympathetic nerve activity.

Broadly speaking, β-adrenoceptor agonists are useful as smooth muscle relaxants (especially in the airways), while β-adrenoceptor antagonists (often called β blockers) are used mainly for their cardiodepressant effects. α-Adrenoceptor antagonists are used mainly for their vasodilator effects in cardiovascular indications and also for the treatment of prostatic hyperplasia. Adrenaline, with its mixture of cardiac stimulant, vasodilator and vasoconstrictor actions is uniquely important in cardiac arrest (Ch. 21). Selective α-adrenoceptor agonists have relatively few clinical uses.

ADRENOCEPTOR AGONISTS

Examples of adrenoceptor agonists (also known as *directly-acting sympathomimetic* drugs) are given in Table 14.2, and the characteristics of individual drugs are summarised in Table 14.4.

Actions

The major physiological effects mediated by different types of adrenoceptor are summarised in Table 14.1.

Smooth muscle

All types of smooth muscle, except that of the gastrointestinal tract, contract in response to stimulation of α_1-adrenoceptors, through activation of the signal transduction mechanism, leading to intracellular Ca^{2+} release described in Chapter 4.

When α agonists are given systemically to experimental animals or humans, the most important action is on vascular smooth muscle, particularly in the skin and splanchnic vascular beds, which are strongly constricted. Large arteries and veins, as well as arterioles, are also constricted, resulting in decreased vascular compliance, increased central venous pressure and increased peripheral resistance, all of which contribute to an increase in systolic and diastolic arterial pressure and increased cardiac work. Some vascular beds (e.g. cerebral, coronary and pulmonary) are relatively little affected.

In the whole animal, baroreceptor reflexes are activated by the rise in arterial pressure produced by α agonists, causing reflex bradycardia and inhibition of respiration.

Smooth muscle in the vas deferens, spleen capsule and eyelid retractor muscles (or nictitating membrane, in some species) is also stimulated by α agonists, and these organs were once widely used for pharmacological studies.

The α receptors involved in smooth muscle contraction are mainly α_1 in type, although vascular smooth muscle possesses both α_1 and α_2 receptors. It appears that α_1 receptors lie close to the sites of release (and are mainly responsible for neurally mediated vasoconstriction), while α_2 receptors lie elsewhere on the muscle fibre surface and are activated by circulating catecholamines.

Stimulation of β receptors causes relaxation of most kinds of smooth muscle by increasing cAMP formation (see Ch. 4). Additionally, β-receptor activation enhances Ca^{2+} extrusion and intracellular Ca^{2+} binding, both effects acting to reduce intracellular Ca^{2+} concentration.

Relaxation is usually produced by β_2 receptors, although the receptor that is responsible for this effect in gastrointestinal smooth muscle is not clearly β_1 or β_2. In the vascular system, β_2-mediated vasodilatation is (particularly in humans) mainly endothelium dependent and mediated by nitric oxide release (see Ch. 20). It occurs in many vascular beds and is especially marked in skeletal muscle.

The powerful inhibitory effect of the sympathetic system on gastrointestinal smooth muscle is produced by both α and β receptors, this tissue being unusual in that α receptors cause relaxation in most regions. Part of the effect is due to stimulation of presynaptic α_2 receptors (see below), which inhibit the release of excitatory transmitters (e.g. acetylcholine) from intramural nerves, but there are also α receptors on the muscle cells, stimulation of which hyperpolarises the cell (by increasing the membrane permeability to K^+) and inhibits action potential discharge. The sphincters of the gastrointestinal tract are contracted by α-receptor activation.

Bronchial smooth muscle is relaxed by activation of β_2 adrenoceptors, and selective β_2 agonists are important in the treatment of asthma (see Ch. 28). Uterine smooth muscle responds similarly, and these drugs are also used to delay premature labour (Ch. 35). Bladder detrusor muscle is relaxed by activation of β_3 adrenoceptors, and selective β_3 agonists have recently been introduced to treat symptoms of overactive bladder (see Sacco & Bientinesi, 2012).

Also, α_1 adrenoceptors mediate a long-lasting trophic response, stimulating smooth muscle proliferation in various tissues, for example in blood vessels and in the prostate gland, which is of pathological importance. *Benign prostatic hyperplasia* (see Ch. 35) is commonly treated with α-adrenoceptor antagonists. 'Cross-talk' between the α_1 adrenoceptor and the growth factor signalling pathways (see Ch. 3) probably contributes to the clinical effect, in addition to immediate symptomatic improvement which is probably mediated by smooth muscle relaxation.

Nerve terminals

Presynaptic adrenoceptors are present on both cholinergic and noradrenergic nerve terminals (see Chs 4 and 12). The main effect (α_2-mediated) is inhibitory, but a weaker facilitatory action of β receptors on noradrenergic nerve terminals has also been described.

Heart

Catecholamines, acting on β_1 receptors, exert a powerful stimulant effect on the heart (see Ch. 21). Both the heart rate (*chronotropic effect*) and the force of contraction (*inotropic effect*) are increased, resulting in a markedly increased cardiac output and cardiac oxygen consumption. The cardiac efficiency (see Ch. 21) is reduced. Catecholamines can also cause disturbance of the cardiac rhythm, culminating in ventricular fibrillation. (Paradoxically, but importantly, adrenaline is also used to treat ventricular fibrillation arrest as well as other forms of cardiac arrest; Ch. 21). Figure 14.4 shows the overall pattern of cardiovascular responses to catecholamine infusions in humans, reflecting their actions on both the heart and vascular system.

Cardiac hypertrophy occurs in response to activation of both β_1 and α_1 receptors, probably by a mechanism similar to the hypertrophy of vascular and prostatic smooth muscle. This may be important in the pathophysiology of hypertension and of cardiac failure (which is associated with sympathetic overactivity); see Chapter 21.

Metabolism

Catecholamines encourage the conversion of energy stores (glycogen and fat) to freely available fuels (glucose and free fatty acids), and cause an increase in the plasma concentration of the latter substances. The detailed biochemical mechanisms (see review by Nonogaki, 2000) vary from species to species, but in most cases the effects on carbohydrate metabolism of liver and muscle (Fig. 14.5) are mediated through β_1 receptors and the stimulation of lipolysis and thermogenesis is produced by β_3 receptors (see Table 14.1). Activation of α_2 receptors inhibits insulin secretion, an effect that further contributes to the hyperglycaemia. The production of *leptin* by adipose tissue (see Ch. 32) is also inhibited. Adrenaline-induced hyperglycaemia in humans is blocked completely by a combination of α and β antagonists but not by either on its own.

Other effects

Skeletal muscle is affected by adrenaline, acting on β_2 receptors, although the effect is far less dramatic than that

Table 14.4 Mixed (α- and β-) adrenoceptor agonists

Drug	Main action	Uses/function	Unwanted effects	Pharmacokinetic aspects	Notes
Noradrenaline (Norepinephrine)	α/β agonist	Sometimes used for hypotension in intensive care Transmitter at postganglionic sympathetic neurons, and in CNS	Hypertension, vasoconstriction, tachycardia (or reflex bradycardia), ventricular dysrhythmias	Poorly absorbed by mouth Rapid removal by tissues Metabolised by MAO and COMT Plasma $t_{1/2}$ ~2 min	–
Adrenaline (Epinephrine)	α/β agonist	Asthma (emergency treatment), anaphylactic shock, cardiac arrest Added to local anaesthetic solutions Main hormone of adrenal medulla	As norepinephrine	As norepinephrine Given i.m. or s.c. (i.v. infusion in intensive care settings)	See Ch. 28
Isoprenaline	β agonist (non-selective)	Asthma (obsolete)	Tachycardia, dysrhythmias	Some tissue uptake, followed by inactivation (COMT) Plasma $t_{1/2}$ ~2 h	Now replaced by salbutamol in treatment of asthma (see Ch. 28)
Dobutamine	β_1 agonist (non-selective)	Cardiogenic shock	Dysrhythmias	Plasma $t_{1/2}$ ~2 min Given i.v.	See Ch. 21
Salbutamol	β_2 agonist	Asthma, premature labour	Tachycardia, dysrhythmias, tremor, peripheral vasodilatation	Given orally or by aerosol Mainly excreted unchanged Plasma $t_{1/2}$ ~4 h	See Ch. 28
Salmeterol	β_2 agonist	Asthma	As salbutamol	Given by aerosol Long acting	Formoterol is similar
Terbutaline	β_2 agonist	Asthma Delay of parturition	As salbutamol	Poorly absorbed orally Given by aerosol Mainly excreted unchanged Plasma $t_{1/2}$ ~4 h	See Ch. 28
Clenbuterol	β_2 agonist	'Anabolic' action to increase muscle strength	As salbutamol	Active orally Long acting	Illicit use in sport
Mirabegron	β_3 agonist	Symptoms of overactive bladder	Tachycardia	Active orally, given once daily	See Ch. 29
Phenylephrine	α_1 agonist	Nasal decongestion	Hypertension, reflex bradycardia	Given intranasally Metabolised by MAO Short plasma $t_{1/2}$	–
Methoxamine	α agonist (non-selective)	Nasal decongestion	As phenylephrine	Given intranasally Plasma $t_{1/2}$ ~1 h	–
Clonidine	α_2 partial agonist	Hypertension, migraine	Drowsiness, orthostatic hypotension, oedema and weight gain, rebound hypertension	Well absorbed orally Excreted unchanged and as conjugate Plasma $t_{1/2}$ ~12 h	See Ch. 21

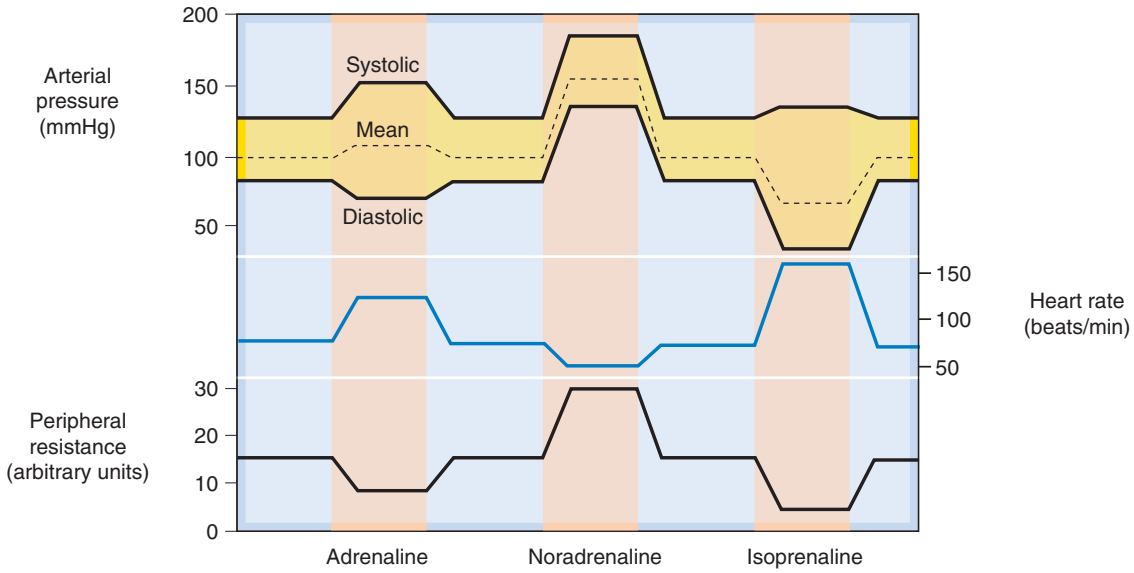

Fig. 14.4 Schematic representation of the cardiovascular effects of intravenous infusions of adrenaline, noradrenaline and isoprenaline in humans. Noradrenaline (predominantly α agonist) causes vasoconstriction and increased systolic and diastolic pressure, with a reflex bradycardia. Isoprenaline (β agonist) is a vasodilator, but strongly increases cardiac force and rate. Mean arterial pressure falls. Adrenaline combines both actions.

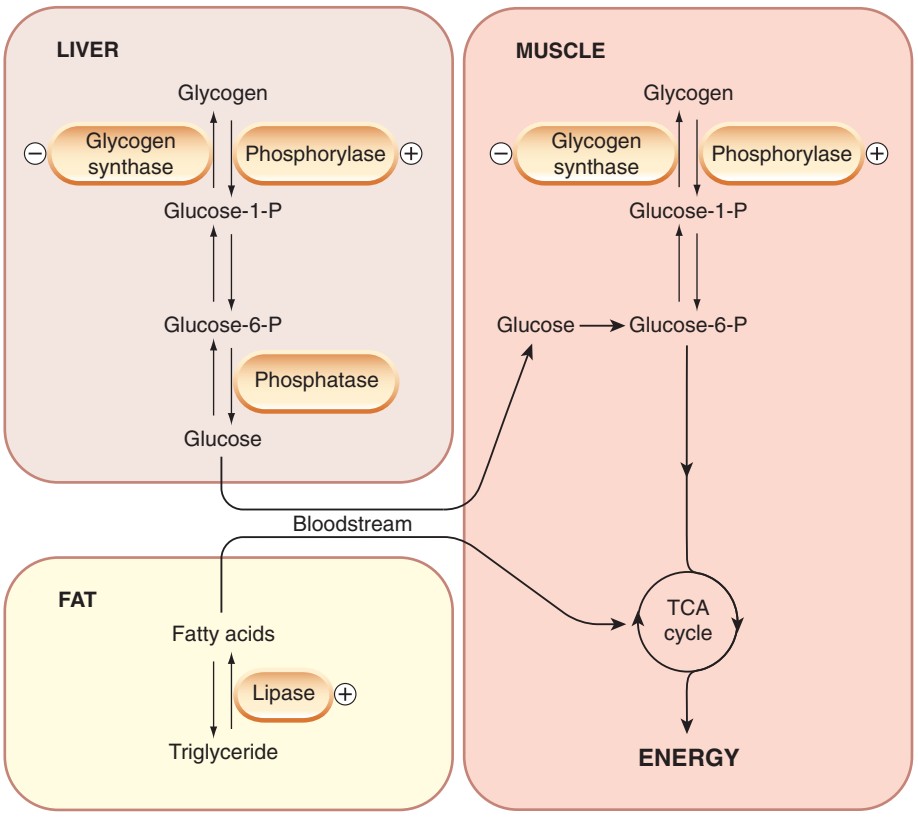

Fig. 14.5 Regulation of energy metabolism by catecholamines. The main enzymic steps that are affected by β-adrenoceptor activation are indicated by + and – signs, denoting stimulation and inhibition, respectively. The overall effect is to mobilise glycogen and fat stores to meet energy demands.

on the heart. The twitch tension of fast-contracting fibres (white muscle) is increased by adrenaline, particularly if the muscle is fatigued, whereas the twitch of slow (red) muscle is reduced. These effects depend on an action on the contractile proteins, rather than on the membrane, and the mechanism is poorly understood. In humans, adrenaline and other β_2 agonists cause a marked tremor, the shakiness that accompanies fear, excitement or the excessive use of β_2 agonists (e.g. **salbutamol**) in the treatment of asthma being examples of this. It probably results from an increase in muscle spindle discharge, coupled with an effect on the contraction kinetics of the fibres, these effects combining to produce an instability in the reflex control of muscle length. β-receptor antagonists are sometimes used to control pathological tremor. The tendency to cardiac dysrhythmias associated with β_2 agonists is thought to be partly due to hypokalaemia, caused by an increase in K^+ uptake by skeletal muscle. β_2 agonists also cause long-term changes in the expression of sarcoplasmic reticulum proteins that control contraction kinetics, and thereby increase the rate and force of contraction of skeletal muscle. **Clenbuterol**, an 'anabolic' drug used illicitly by athletes to improve performance (see Ch. 58), is a β_2 agonist that acts in this way.

Histamine release by human and guinea pig lung tissue in response to anaphylactic challenge (see Ch. 17) is inhibited by catecholamines, acting on β_2 receptors.

Lymphocytes and other cells of the immune system also express adrenoceptors (mainly β adrenoceptors). Lymphocyte proliferation, lymphocyte-mediated cell killing, and production of many cytokines are inhibited by β-adrenoceptor agonists. The physiological and clinical importance of these effects has not yet been established. For a review of the effects of the sympathetic nervous system on immune function, see Elenkov et al., 2000.

Adrenoceptor agonists

- **Noradrenaline** and **adrenaline** show relatively little receptor selectivity.
- Selective α_1 agonists include **phenylephrine** and **oxymetazoline**.
- Selective α_2 agonists include **clonidine** and **α-methylnoradrenaline**. They cause a fall in blood pressure, partly by inhibition of noradrenaline release and partly by a central action. Methylnoradrenaline is formed as a false transmitter from **methyldopa**, developed as a hypotensive drug (now largely obsolete, except during pregnancy).
- Selective β_1 agonists include **dobutamine**. Increased cardiac contractility may be useful clinically, but all β_1 agonists can cause cardiac dysrhythmias.
- Selective β_2 agonists include **salbutamol**, **terbutaline** and **salmeterol**; used mainly for their bronchodilator action in asthma.
- A selective β_3 agonist, **mirabegron**, is used to treat overactive bladder; β_3 agonists promote lipolysis and have potential in the treatment of obesity.

Clinical use

The main clinical uses of adrenoceptor agonists are summarised in the clinical box (below) and Table 14.4, the most important being the use of β-adrenoceptor agonists for the treatment of asthma (Ch. 28).

Clinical uses of adrenoceptor agonists

- Cardiovascular system:
  - cardiac arrest: **adrenaline**
  - cardiogenic shock (see Ch. 22): **dobutamine** (β_1 agonist).
- Anaphylaxis (acute hypersensitivity, see Chs 17 and 28): **adrenaline**.
- Respiratory system:
  - asthma (Ch. 28): selective β_2-receptor agonists (**salbutamol**, **terbutaline**, **salmeterol**, **formoterol**)
  - nasal decongestion: drops containing **xylometazoline** or **ephedrine** for short-term use.
- Miscellaneous indications:
  - **adrenaline**: with local anaesthetics to prolong their action (see Ch. 43)
  - premature labour (**salbutamol**; see Ch. 35)
  - α_2 agonists (e.g. **clonidine**): to lower blood pressure (Ch. 22) and intraocular pressure; as an adjunct during drug withdrawal in addicts (Ch. 49; Table 49.3, p. 181); to reduce menopausal flushing, especially when **oestrogen** is contraindicated as in patients with breast cancer; and to reduce frequency of migraine attacks (Ch. 15). Tourette syndrome, characterised by multiple tics and outbursts of foul language, is an unlicensed indication
  - A β_3 agonist, **mirabegron**: to treat urgency, increased micturition frequency and incontinence (overactive bladder symptoms).

ADRENOCEPTOR ANTAGONISTS

The main drugs are listed in Table 14.2, and further information is given in Table 14.5. Most are selective for α or β receptors, and many are also subtype-selective.

α-Adrenoceptor antagonists

The main groups of α-adrenoceptor antagonists are:

- non-selective between subtypes (e.g. **phenoxybenzamine**, **phentolamine**)
- α_1-selective (e.g. **prazosin**, **doxazosin**, **terazosin**)
- α_2-selective (e.g. **yohimbine**, **idazoxan**).

In addition, *ergot derivatives* (e.g. **ergotamine**, **dihydroergotamine**) block α receptors as well as having many other actions, notably on 5-HT receptors. They are described in Chapter 15. Their action on α adrenoceptors is of pharmacological interest but not used therapeutically.

Non-selective α-adrenoceptor antagonists

Phenoxybenzamine is not specific for α receptors, and also antagonises the actions of acetylcholine, histamine and 5-HT. It is long lasting because it binds covalently to the receptor. **Phentolamine** is more selective, but it binds reversibly and its action is short lasting. In humans, these drugs cause a fall in arterial pressure (because of block of α-receptor-mediated vasoconstriction) and postural hypotension. The cardiac output and heart rate

Table 14.5 Adrenoceptor antagonists

Drug	Main action	Uses/function	Unwanted effects	Pharmacokinetic aspects	Notes
α-Adrenoceptor antagonists					
Phenoxybenzamine	α antagonist (non-selective, irreversible) Uptake 1 inhibitor	Phaeochromocytoma	Postural hypotension, tachycardia, nasal congestion, impotence	Absorbed orally Plasma $t_{1/2}$ ~12 h	Action outlasts presence of drug in plasma, because of covalent binding to receptor
Phentolamine	α antagonist (non-selective), vasodilator	Rarely used	As phenoxybenzamine	Usually given i.v. Metabolised by liver Plasma $t_{1/2}$ ~2 h	
Prazosin	α_1 antagonist	Hypertension	As phenoxybenzamine	Absorbed orally Metabolised by liver Plasma $t_{1/2}$ ~4 h	Doxazosin, terazosin are similar but longer acting See Ch. 22
Tamsulosin	α_{1A} antagonist ('uroselective')	Prostatic hyperplasia	Failure of ejaculation	Absorbed orally Plasma $t_{1/2}$ ~5 h	Selective for α_{1A}-adrenoceptor
Yohimbine	α_2 antagonist	Not used clinically Claimed to be aphrodisiac	Excitement, hypertension	Absorbed orally Metabolised by liver Plasma $t_{1/2}$ ~4 h	
β-Adrenoceptor antagonists					
Propranolol	β antagonist (non-selective)	Angina, hypertension, cardiac dysrhythmias, anxiety, tremor, glaucoma	Bronchoconstriction, cardiac failure, cold extremities, fatigue and depression, hypoglycaemia	Absorbed orally Extensive first-pass metabolism About 90% bound to plasma protein Plasma $t_{1/2}$ ~4 h	Timolol is similar and used mainly to treat glaucoma See Ch. 21
Alprenolol	β antagonist (non-selective) (partial agonist)	As propranolol	As propranolol	Absorbed orally Metabolised by liver Plasma $t_{1/2}$ ~4 h	Oxprenolol and pindolol are similar See Ch. 21
Metoprolol	β_1 antagonist	Angina, hypertension, dysrhythmias	As propranolol, less risk of bronchoconstriction	Absorbed orally Mainly metabolised in liver Plasma $t_{1/2}$ ~3 h	Atenolol is similar, with a longer half-life See Ch. 21
Nebivolol	β_1 antagonist Enhances nitric oxide synthesis	Hypertension	Fatigue, headache	Absorbed orally $t_{1/2}$ ~10 h	–
Butoxamine	β_2-selective antagonist Weak α agonist	No clinical uses	–	–	–
Mixed (α-/β-) antagonists					
Labetalol	α/β antagonist	Hypertension in pregnancy	Postural hypotension, brochoconstriction	Absorbed orally Conjugated in liver Plasma $t_{1/2}$ ~4 h	See Chs 21 and 22
Carvedilol	β/α_1 antagonist	Heart failure	As for other β blockers Initial exacerbation of heart failure Renal failure	Absorbed orally $t_{1/2}$ ~10 h	Additional actions may contribute to clinical benefit. See Ch. 21

are increased. This is a reflex response to the fall in arterial pressure, mediated through β receptors. The concomitant block of α_2 receptors tends to increase noradrenaline release, which has the effect of enhancing the reflex tachycardia that occurs with any blood pressure-lowering agent. Phenoxybenzamine retains a niche (but vital) use in preparing patients with *phaeochromocytoma* (Ch. 22) for surgery; its irreversible antagonism and the resultant depression in the maximum of the agonist dose–response curve (see Ch. 2, Fig. 2.4) are desirable in a situation where surgical manipulation of the tumour may release a large bolus of pressor amines into the circulation.

Labetalol and **carvedilol**[5] are mixed α_1- and β-receptor-blocking drugs, although clinically they act predominantly on β receptors. Much has been made of the fact that they combine both activities in one molecule. To a pharmacologist, accustomed to putting specificity of action high on the list of pharmacological saintly virtues, this may seem like a step backwards rather than forwards. Carvedilol is used mainly to treat hypertension and heart failure (see Chs 21 and 22); labetalol is used to treat hypertension in pregnancy.

Selective α_1 antagonists

Prazosin was the first selective α_1 antagonist. Similar drugs with longer half-lives (e.g. **doxazosin**, **terazosin**), which have the advantage of allowing once-daily dosing, are now preferred. They are highly selective for α_1 adrenoceptors and cause vasodilatation and fall in arterial pressure, but less tachycardia than occurs with non-selective α-receptor antagonists, presumably because they do not increase noradrenaline release from sympathetic nerve terminals. Postural hypotension may occur, but is less problematic than with shorter-acting prazosin.

The α_1-receptor antagonists cause relaxation of the smooth muscle of the bladder neck and prostate capsule, and inhibit hypertrophy of these tissues, and are therefore useful in treating urinary retention associated with *benign prostatic hypertrophy*. **Tamsulosin**, an α_{1A}-receptor antagonist, shows some selectivity for the bladder, and causes less hypotension than drugs such as prazosin, which act on α_{1B} receptors to control vascular tone.

It is believed that α_{1A} receptors play a part in the pathological hypertrophy not only of prostatic and vascular smooth muscle, but also in the cardiac hypertrophy that occurs in hypertension and heart failure, and the use of selective α_{1A}-receptor antagonists to treat these chronic conditions is under investigation.

Selective α_2 antagonists

Yohimbine is a naturally occurring alkaloid; various synthetic analogues have been made, such as **idazoxan**. These drugs are used experimentally to analyse α-receptor subtypes, and yohimbine, possibly by virtue of its vasodilator effect, historically enjoyed notoriety as an aphrodisiac, but they are not used therapeutically.

Clinical uses and unwanted effects of α-adrenoceptor antagonists

The main uses of α-adrenoceptor antagonists are related to their cardiovascular actions, and are summarised in the clinical box (below) They have been tried for many purposes, but have only limited therapeutic applications. In hypertension, non-selective α-blocking drugs are unsatisfactory, because of their tendency to produce tachycardia and cardiac dysrhythmias, and gastrointestinal symptoms. Selective α_1-receptor antagonists (especially the longer-acting compounds **doxazosin** and **terazosin**) are, however, useful. They do not affect cardiac function appreciably, and postural hypotension is less troublesome than with prazosin or non-selective α-receptor antagonists. They have a place in treating severe hypertension, where they are added to treatment with first- and second-line drugs, but are not used as first-line agents (see Ch. 22). Unlike other antihypertensive drugs, they cause a modest decrease in low-density lipoprotein, and an increase in high-density lipoprotein cholesterol (see Ch. 23), although the clinical importance of these ostensibly beneficial effects is uncertain. They are also used to control urinary retention in patients with benign prostatic hypertrophy.

Phaeochromocytoma is a catecholamine-secreting tumour of chromaffin tissue, which causes severe and initially episodic hypertension. A combination of α- and β-receptor antagonists is the most effective way of controlling the blood pressure. The tumour may be surgically removable, and it is essential to block α and β receptors before surgery is begun, to avoid the effects of a sudden release of catecholamines when the tumour is disturbed. A combination of phenoxybenzamine and atenolol is effective for this purpose.

[5]Carvedilol is also a biased agonist, acting through the arrestin pathway (Ch. 3).

α-Adrenoceptor antagonists

- Drugs that block α_1 and α_2 adrenoceptors (e.g. **phenoxybenzamine** and **phentolamine**) were once used to produce vasodilatation in the treatment of peripheral vascular disease, but this use is now largely obsolete.
- Selective α_1 antagonists (e.g. **prazosin**, **doxazosin**, **terazosin**) are used in treating hypertension and for benign prostatic hypertrophy. Postural hypotension, stress incontinence and impotence are unwanted effects.
- **Tamsulosin** is α_{1A} selective and acts mainly on the urogenital tract. It is used to treat benign prostatic hypertrophy and may cause less postural hypotension than other α_1 agonists.
- **Yohimbine** is a selective α_2 antagonist. It is not used clinically.

Clinical uses of α-adrenoceptor antagonists

- Severe hypertension (see Ch. 22): α_1-selective antagonists (e.g. **doxazosin**) in combination with other drugs.
- Benign prostatic hypertrophy (e.g. **tamsulosin**, a selective α_{1A}-receptor antagonist).
- Phaeochromocytoma: **phenoxybenzamine** (irreversible antagonist) in preparation for surgery.

β-Adrenoceptor antagonists

The β-adrenoceptor antagonists are an important group of drugs. They were first discovered in 1958, 10 years after Ahlquist had postulated the existence of β adrenoceptors. The first compound, **dichloroisoprenaline**, had fairly low potency and was a partial agonist. Further development led to **propranolol**, which is much more potent and a pure antagonist that blocks β_1 and β_2 receptors equally. The potential clinical advantages of drugs with some partial agonist activity, and/or with selectivity for β_1 receptors, led to the development of **practolol** (selective for β_1 receptors but withdrawn because of its off-target toxicity), **oxprenolol** and **alprenolol** (non-selective with considerable partial agonist activity), and **atenolol** (β_1-selective with no agonist activity). Two newer drugs are **carvedilol** (a non-selective β-adrenoceptor antagonist with additional α_1-blocking activity) and **nebivolol** (a β_1-selective antagonist that also causes vasodilatation by inducing endothelial nitric oxide production; see Ch. 20). Both these drugs have proven more effective than conventional β-adrenoceptor antagonists in treating heart failure (see Ch. 21). The characteristics of the most important compounds are set out in Table 14.5. Most β-receptor antagonists are inactive on β_3 receptors so do not affect lipolysis.

Actions

The pharmacological actions of β-receptor antagonists can be deduced from Table 14.1. The effects produced in humans depend on the degree of sympathetic activity and are modest in subjects at rest. The most important effects are on the cardiovascular system and on bronchial smooth muscle (see Chs 21, 22 and 28).

In a healthy subject at rest, propranolol causes modest changes in heart rate, cardiac output or arterial pressure, but it markedly reduces the effect of exercise or excitement on these variables (Fig. 14.6). Drugs with partial agonist activity, such as oxprenolol, increase the heart rate at rest but reduce it during exercise. Maximum exercise tolerance is considerably reduced in normal subjects, partly because of the limitation of the cardiac response, and partly because the β-mediated vasodilatation in skeletal muscle is reduced. Coronary flow is reduced, but relatively less than the myocardial oxygen consumption, so oxygenation of the myocardium is improved, an effect of importance in the treatment of angina pectoris (see Ch. 21). In normal subjects, the reduction of the force of contraction of the heart is of no importance, but it may have important consequences for patients with heart disease (see below).

An important, and somewhat unexpected, effect of β-receptor antagonists is their antihypertensive action (see Ch. 22). Patients with hypertension (although not normotensive subjects) show a gradual fall in arterial pressure that takes several days to develop fully. The mechanism is complex and involves the following:

- reduction in cardiac output
- reduction of renin release from the juxtaglomerular cells of the kidney
- a central action, reducing sympathetic activity.

Carvedilol and nebivolol (see above) are particularly effective in lowering blood pressure, because of their additional vasodilator properties.

Blockade of the facilitatory effect of presynaptic β receptors on noradrenaline release (see Table 14.1) may also contribute to the antihypertensive effect. The antihypertensive effect of β-receptor antagonists is clinically very useful. Because reflex vasoconstriction is preserved, postural and exercise-induced hypotension are less troublesome than with many other antihypertensive drugs.

Many β-receptor antagonists have an important antidysrhythmic effect on the heart (see Ch. 21).

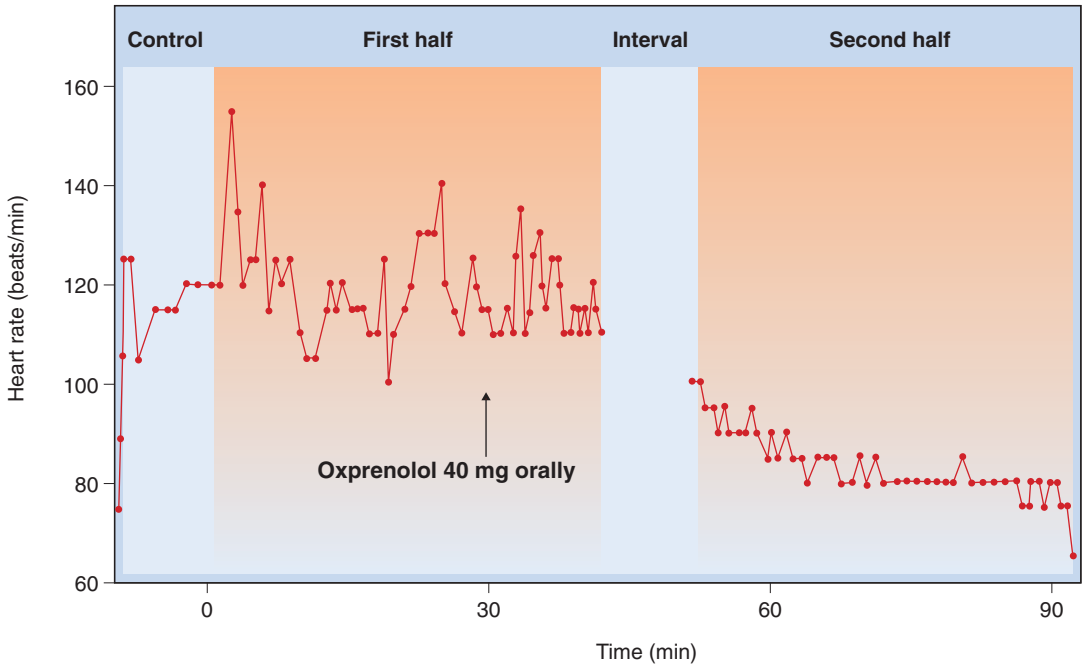

Fig. 14.6 Heart rate recorded continuously in a spectator watching a live football match, showing the effect of the β-adrenoceptor antagonist oxprenolol. *(From Taylor SH, Meeran MK 1973. In: Burley et al. (Eds) New Perspectives in Beta-Blockade. CIBA Laboratories, Horsham.)*

Airways resistance in normal subjects is only slightly increased by β-receptor antagonists, and this is of no consequence. In asthmatic subjects, however, non-selective β-receptor antagonists (such as propranolol) can cause severe bronchoconstriction, which does not, of course, respond to the usual doses of drugs such as salbutamol or adrenaline. This danger is less with β_1-selective antagonists, but none is so selective that this danger can be ignored.

Despite the involvement of β receptors in the hyperglycaemic actions of adrenaline, β-receptor antagonists cause only minor metabolic changes in normal subjects. They do not affect the onset of hypoglycaemia following an injection of insulin, but somewhat delay the recovery of blood glucose concentration. In diabetic patients, the use of β-receptor antagonists increases the likelihood of exercise-induced hypoglycaemia, because the normal adrenaline-induced release of glucose from the liver is diminished.

β-Adrenoceptor antagonists

- Non-selective between β_1 and β_2 adrenoceptors: **propranolol**, **alprenolol**, **oxprenolol**.
- β_1-selective: **atenolol**, **nebivolol**.
- **Alprenolol** and **oxprenolol** have partial agonist activity.
- Many clinical uses (see clinical box, below).
- Important hazards are bronchoconstriction, and bradycardia and cardiac failure (possibly less with partial agonists).
- Side effects include cold extremities, insomnia, depression, fatigue.
- Some show rapid first-pass metabolism, hence poor bioavailability.
- Some drugs (e.g. **labetalol**, **carvedilol**) block both α and β adrenoceptors.

Clinical use

The main uses of β-receptor antagonists are connected with their effects on the cardiovascular system, and are discussed in Chapters 21 and 22. They are as summarised in the clinical box below.

The use of β-receptor antagonists in cardiac failure deserves special mention, as clinical opinion has undergone a U-turn. Patients with heart disease may rely on a degree of sympathetic drive to the heart to maintain an adequate cardiac output, and removal of this by blocking β receptors can exacerbate cardiac failure, so using these drugs in patients with cardiac failure was considered ill-advised. In theory, drugs with partial agonist activity (e.g. oxprenolol, alprenolol) offer an advantage because they can, by their own action, maintain a degree of β_1-receptor activation, while at the same time blunting the cardiac response to increased sympathetic nerve activity or to circulating adrenaline. Clinical trials, however, have not shown a clear advantage of these drugs measurable as a reduced incidence of cardiac failure, and one such drug (**xamoterol**, since withdrawn) with particularly marked agonist activity clearly made matters worse.

Paradoxically, β-receptor antagonists are increasingly being used in low doses to treat well-compensated cardiac failure and there is strong evidence that this improves survival in carefully selected patients (Ch. 22), although at the outset there is a danger of exacerbating the problem (Bristow, 2011). **Carvedilol** is often used for this purpose. This success has led to the proposal that β-receptor antagonists might also be of value in the long-term treatment of patients with stable asthma, but this remains controversial.

Clinical uses of β-adrenoceptor antagonists

- Cardiovascular (see Chs 21 and 22):
  - angina pectoris
  - myocardial infarction, and following infarction
  - prevention of recurrent dysrhythmias (especially if triggered by sympathetic activation)
  - heart failure (in well-compensated patients)
  - hypertension (no longer first choice; Ch. 22).
- Other uses:
  - glaucoma (e.g. **timolol** eye drops)
  - thyrotoxicosis (Ch. 34), as adjunct to definitive treatment (e.g. preoperatively)
  - anxiety (Ch. 44), to control somatic symptoms (e.g. palpitations, tremor)
  - migraine prophylaxis (Ch. 15)
  - benign essential tremor (a familial disorder).

Unwanted effects

The main side effects of β-receptor antagonists result from their receptor-blocking action.

Bronchoconstriction. This is of little importance in the absence of airways disease, but in asthmatic patients the effect can be life-threatening. It is also of clinical importance in patients with other forms of obstructive lung disease (e.g. chronic bronchitis, emphysema), although the risk–benefit balance may favour cautious treatment in individual patients. As mentioned above, it has been hypothesised that β-receptor antagonists might actually be of value in treating stable asthmatic patients.

Cardiac depression. Cardiac depression can occur, leading to signs of heart failure, particularly in elderly people. Patients suffering from heart failure who are treated with β-receptor antagonists (see above) often deteriorate in the first few weeks before the beneficial effect develops.

Bradycardia. Sinus bradycardia can progress to life-threatening heart block and can occur in patients with coronary disease, particularly if they are being treated with antiarrhythmic drugs that impair cardiac conduction (see Ch. 21).

Hypoglycaemia. Glucose release in response to adrenaline is a safety device that may be important to diabetic patients and to other individuals prone to hypoglycaemic attacks. The sympathetic response to hypoglycaemia produces symptoms (especially tachycardia) that warn patients of the urgent need for carbohydrate (usually in the form of a sugary drink). β-Receptor antagonists reduce these symptoms, so incipient hypoglycaemia is more likely to go unnoticed by the patient. There is a theoretical advantage in using β_1-selective agents, because glucose release from the liver is controlled by β_2 receptors.

Fatigue. This is probably due to reduced cardiac output and reduced muscle perfusion in exercise. It is a frequent complaint of patients taking β-receptor-blocking drugs.

Cold extremities. This is common, due to a loss of β-receptor-mediated vasodilatation in cutaneous vessels. Theoretically, β_1-selective drugs are less likely to produce this effect, but it is not clear that this is so in practice.

Other adverse effects associated with β-receptor antagonists are not obviously the result of β-receptor blockade. One is the occurrence of bad dreams, which occur mainly with highly lipid-soluble drugs such as propranolol, which enter the brain easily.

▼ There are several additional factors that make β-adrenoceptor pharmacology more complicated than it appears at first sight, and may have implications for the clinical use of β-adrenoceptor antagonists:

- Several drugs that act on adrenoceptors have the characteristics of partial agonists (see Ch. 2), i.e. they block receptors and thus antagonise the actions of full agonists, but also have a weak agonist effect of their own. Some β-adrenoceptor-blocking drugs (e.g. **alprenolol**, **oxprenolol**) cause, under resting conditions, an increase in heart rate while at the same time opposing the tachycardia produced by sympathetic stimulation. This has been interpreted as a partial agonist effect, although there is evidence that mechanisms other than β-receptor activation may contribute to the tachycardia.
- The high degree of receptor specificity found for some compounds in laboratory animals is seldom found in humans.
- Though in normal hearts cardiac stimulation is mediated through β_1 receptors, in heart failure (see Ch. 21) β_2 receptors contribute significantly.
- There is evidence that β-adrenoceptor agonists and partial agonists may act not only through cAMP formation, but also through other signal transduction pathways (e.g. the mitogen-activated protein [MAP] kinase pathway; see Ch. 3), and that the relative contribution of these signals differs for different drugs. Furthermore, the pathways show different levels of constitutive activation, which is reduced by ligands that function as inverse agonists. β-Adrenoceptor antagonists in clinical use differ in respect of these properties, and drugs classified as partial agonists may actually activate one pathway while blocking another (see Baker et al., 2003).
- Genetic variants of both β_1 and β_2 receptors occur in humans, and influence the effects of agonists and antagonists (see Brodde, 2008).

DRUGS THAT AFFECT NORADRENERGIC NEURONS

Emphasis in this chapter is placed on peripheral sympathetic transmission. The same principles, however, are applicable to the central nervous system (see Ch. 37), where many of the drugs mentioned here also act. The major drugs and mechanisms are summarised in Table 14.6.

DRUGS THAT AFFECT NORADRENALINE SYNTHESIS

Only a few clinically important drugs affect noradrenaline synthesis directly. Examples are **α-methyltyrosine**, which inhibits tyrosine hydroxylase, and **carbidopa**, a hydrazine derivative of dopa, which inhibits dopa decarboxylase and is used in the treatment of parkinsonism (see Ch. 40).

Methyldopa, still used in the treatment of hypertension during pregnancy (see Ch. 22), is taken up by noradrenergic neurons, where it is converted to the false transmitter α-methylnoradrenaline. This substance is not deaminated within the neuron by MAO, so it accumulates and displaces noradrenaline from the synaptic vesicles. α-Methylnoradrenaline is released in the same way as noradrenaline, but is less active than noradrenaline on α_1 receptors and thus is less effective in causing vasoconstriction. On the other hand, it is more active on presynaptic (α_2) receptors, so the autoinhibitory feedback mechanism operates more strongly than normal, thus reducing transmitter release below the normal levels. Both of these effects (as well as a central effect, probably caused by the same cellular mechanism) contribute to the hypotensive action. It produces side effects typical of centrally acting antiadrenergic drugs (e.g. sedation), as well as carrying a risk of immune haemolytic reactions and liver toxicity, so it is now little used, except for hypertension in late pregnancy where there is considerable experience of its use and no suggestion of harm to the unborn baby.

6-Hydroxydopamine (identical with dopamine except for an extra hydroxyl group) is a neurotoxin of the Trojan horse kind. It is taken up selectively by noradrenergic nerve terminals, where it is converted to a reactive quinone, which destroys the nerve terminal, producing a 'chemical sympathectomy'. The cell bodies survive, and eventually the sympathetic innervation recovers. The drug is useful for experimental purposes but has no clinical uses. If injected directly into the brain, it selectively destroys those nerve terminals (i.e. dopaminergic, noradrenergic and adrenergic) that take it up, but it does not reach the brain if given systemically.

MPTP (**1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine**; see Ch. 40) is a similar selective neurotoxin acting on dopaminergic neurons.

Dihydroxyphenylserine (L-DOPS) is currently under investigation for treating hypotensive states associated with reduced noradrenaline synthesis. It penetrates the blood–brain barrier and can be regarded as a catecholamine prodrug being converted to noradrenaline directly by dopa decarboxylase, bypassing the DBH-catalysed hydroxylation step. It raises blood pressure by increasing noradrenaline release.

DRUGS THAT AFFECT NORADRENALINE STORAGE

Reserpine is an alkaloid from the shrub *Rauwolfia*, which has been used in India for centuries for the treatment of mental disorders. Reserpine, at very low concentration, blocks the transport of noradrenaline and other amines into synaptic vesicles, by blocking the vesicular monoamine transporter. Noradrenaline accumulates instead in the cytoplasm, where it is degraded by MAO. The noradrenaline content of tissues drops to a low level, and sympathetic transmission is blocked. Reserpine also causes depletion of 5-HT and dopamine from neurons in the brain, in which these amines are transmitters (see Ch. 39). Reserpine is now used only experimentally, but was at one time used as an antihypertensive drug. Its central effects, especially depression, which probably result from impairment of noradrenergic and 5-HT-mediated transmission in the brain (see Ch. 47), were a serious problem.

DRUGS THAT AFFECT NORADRENALINE RELEASE

Drugs can affect noradrenaline release in four main ways:

- by directly blocking release (noradrenergic neuron-blocking drugs)
- by evoking noradrenaline release in the absence of nerve terminal depolarisation (indirectly acting sympathomimetic drugs)

Table 14.6 Drugs that affect noradrenaline synthesis, release or uptake

Drug	Main action	Uses/function	Unwanted effects	Pharmacokinetic aspects	Notes
Drugs affecting NA synthesis					
α-Methyl-p-tyrosine	Inhibits tyrosine hydroxylase	Occasionally used in phaeochromocytoma	Hypotension, sedation	–	–
Carbidopa	Inhibits dopa decarboxylase	Used as adjunct to levodopa to prevent peripheral effects	–	Absorbed orally Does not enter brain	See Ch. 40
Methyldopa	False transmitter precursor	Hypertension in pregnancy	Hypotension, drowsiness, diarrhoea, impotence, hypersensitivity reactions	Absorbed slowly by mouth Excreted unchanged or as conjugate Plasma $t_{1/2}$ ~6 h	See Ch. 22
L-dihydroxyphenylserine (L-DOPS)	Converted to NA by dopa decarboxylase, thus increasing NA synthesis and release	Orthostatic hypotension	Not known	Absorbed orally Duration of action ~6 h	Currently in clinical trials
Drugs that release NA (indirectly acting sympathomimetic amines)					
Tyramine	NA release	No clinical uses Present in various foods	As norepinephrine	Normally destroyed by MAO in gut Does not enter brain	See Ch. 47
Amphetamine	NA release, MAO inhibitor, NET inhibitor, CNS stimulant	Used as CNS stimulant in narcolepsy, also (paradoxically) in hyperactive children Appetite suppressant Drug of abuse	Hypertension, tachycardia, insomnia Acute psychosis with overdose Dependence	Well absorbed orally Penetrates freely into brain Excreted unchanged in urine Plasma $t_{1/2}$ ~12 h, depending on urine flow and pH	See Ch. 48 Methylphenidate and atomoxetine are similar (used for CNS effects; see Ch. 49)
Ephedrine	NA release, β agonist, weak CNS stimulant action	Nasal decongestion	As amphetamine but less pronounced	Similar to amphetamine aspects	Contraindicated if MAO inhibitors are given
Drugs that inhibit NA release					
Reserpine	Depletes NA stores by inhibiting VMAT	Hypertension (obsolete)	As methyldopa Also depression, parkinsonism, gynaecomastia	Poorly absorbed orally Slowly metabolised Plasma $t_{1/2}$ ~100 h Excreted in milk	Antihypertensive effect develops slowly and persists when drug is stopped
Guanethidine	Inhibits NA release Also causes NA depletion and can damage NA neurons irreversibly	Hypertension (obsolete)	As methyldopa Hypertension on first administration	Poorly absorbed orally Mainly excreted unchanged in urine Plasma $t_{1/2}$ ~100 h	Action prevented by NET inhibitors
Drugs affecting NA uptake					
Imipramine	Blocks neuronal transporter (NET) Also has atropine-like action	Depression	Atropine-like side effects Cardiac dysrhythmias in overdose	Well absorbed orally 95% bound to plasma protein Converted to active metabolite (desmethylimipramine) Plasma $t_{1/2}$ ~4 h	Desipramine and amitriptyline are similar See Ch. 47
Cocaine	Local anaesthetic; blocks NET CNS stimulant	Rarely used local anaesthetic Major drug of abuse	Hypertension, excitement, convulsions, dependence	Well absorbed orally or intranasally	See Chs 43 and 49

COMT, catechol-*O*-methyltransferase; MAO, monoamine oxidase; NA, noradrenaline; VMAT, vesicular monoamine transporter.

- by acting on presynaptic receptors that indirectly inhibit or enhance depolarisation-evoked release; examples include α_2 agonists (see p. 184-187), angiotensin II, dopamine and prostaglandins
- by increasing or decreasing available stores of noradrenaline (e.g. reserpine, see p. 180; MAO inhibitors, see Ch. 47).

NORADRENERGIC NEURON-BLOCKING DRUGS

Noradrenergic neuron-blocking drugs (e.g. **guanethidine**) were first discovered in the mid-1950s when alternatives to ganglion-blocking drugs, for use in the treatment of hypertension, were being sought. The main effect of guanethidine is to inhibit the release of noradrenaline from sympathetic nerve terminals. It has little effect on the adrenal medulla, and none on nerve terminals that release transmitters other than noradrenaline. Drugs very similar to it include **bretylium**, **bethanidine** and **debrisoquin** (which is of interest mainly as a tool for studying drug metabolism; see Ch. 11).

Actions

Drugs of this class reduce or abolish the response of tissues to sympathetic nerve stimulation, but do not affect (or may potentiate) the effects of circulating noradrenaline.

The action of guanethidine on noradrenergic transmission is complex. It is selectively accumulated by noradrenergic nerve terminals, being a substrate for NET (see Table 14.6). Its initial blocking activity is due to block of impulse conduction in the nerve terminals that selectively accumulate the drug. Its action is prevented by drugs, such as *tricyclic antidepressants* (see Ch. 47), that block NET.

Guanethidine is also concentrated in synaptic vesicles by means of the vesicular transporter VMAT, possibly interfering with their ability to undergo exocytosis, and also displacing noradrenaline. In this way, it causes a gradual and long-lasting depletion of noradrenaline in sympathetic nerve endings, similar to the effect of reserpine.

Given in large doses, guanethidine causes structural damage to noradrenergic neurons, which is probably due to the fact that the terminals accumulate the drug in high concentration. It can therefore be used experimentally as a selective neurotoxin.

Guanethidine, bethanidine and debrisoquin are no longer used clinically, now that better antihypertensive drugs are available. Although extremely effective in lowering blood pressure, they produce severe side effects associated with the loss of sympathetic reflexes. The most troublesome are postural hypotension, diarrhoea, nasal congestion and failure of ejaculation.

INDIRECTLY ACTING SYMPATHOMIMETIC AMINES

Mechanism of action and structure–activity relationships

The most important drugs in the indirectly acting sympathomimetic amine category are **tyramine**, **amphetamine** and **ephedrine**, which are structurally related to noradrenaline. Drugs that act similarly and are used for their central effects (see Ch. 48) include **methylphenidate** and **atomoxetine**.

These drugs have only weak actions on adrenoceptors, but sufficiently resemble noradrenaline to be transported into nerve terminals by NET. Once inside the nerve terminals, they are taken up into the vesicles by VMAT, in exchange for noradrenaline, which escapes into the cytosol. Some of the cytosolic noradrenaline is degraded by MAO, while the rest escapes via NET, in exchange for the foreign monoamine, to act on postsynaptic receptors (Fig. 14.7). Exocytosis is not involved in the release process, so their actions do not require the presence of Ca^{2+}. They are not completely specific in their actions, and act partly by a direct effect on adrenoceptors, partly by inhibiting NET (thereby enhancing the effect of the released noradrenaline) and partly by inhibiting MAO.

As would be expected, the effects of these drugs are strongly influenced by other drugs that modify noradrenergic transmission. Thus reserpine and 6-hydroxydopamine abolish their effects by depleting the terminals of noradrenaline. MAO inhibitors, on the other hand, strongly potentiate their effects by preventing inactivation, within the terminals, of the transmitter displaced from the vesicles. MAO inhibition particularly enhances the action of tyramine, because this substance is itself a substrate for MAO. Normally, dietary tyramine is destroyed by MAO in the gut wall and liver before reaching the systemic circulation. When MAO is inhibited this is prevented, and ingestion of

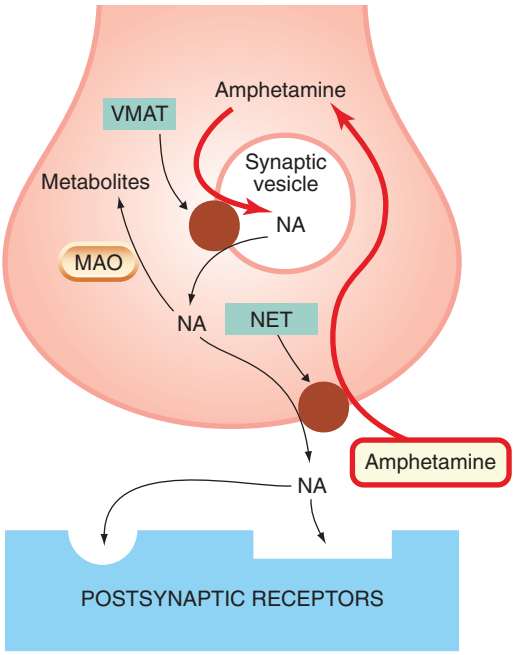

Fig. 14.7 The mode of action of amphetamine, an indirectly acting sympathomimetic amine. Amphetamine enters the nerve terminal via the noradrenaline transporter (NET) and enters synaptic vesicles via the vesicular monoamine transporter (VMAT), in exchange for NA, which accumulates in the cytosol. Some of the NA is degraded by monoamine oxidase (MAO) within the nerve terminal and some escapes, in exchange for amphetamine via the noradrenaline transporter, to act on postsynaptic receptors. Amphetamine also reduces NA reuptake via the transporter, so enhancing the action of the released NA.

tyramine-rich foods such as fermented cheese (e.g. ripe Brie) can then provoke a sudden and dangerous rise in blood pressure. Inhibitors of NET, such as **imipramine** (see Table 14.6), interfere with the effects of indirectly acting sympathomimetic amines by preventing their uptake into the nerve terminals.

These drugs, especially amphetamine, have important effects on the central nervous system (see Ch. 48) that depend on their ability to release not only noradrenaline, but also 5-HT and dopamine from nerve terminals in the brain. An important characteristic of the effects of indirectly acting sympathomimetic amines is that marked tolerance develops. Repeated doses of amphetamine or tyramine, for example, produce progressively smaller pressor responses. This is probably caused by a depletion of the releasable store of noradrenaline. A similar tolerance to the central effects also develops with repeated administration, contributing to the liability of amphetamine and related drugs to cause dependence.

Actions

The peripheral actions of the indirectly acting sympathomimetic amines include bronchodilatation, raised arterial pressure, peripheral vasoconstriction, increased heart rate and force of myocardial contraction, and inhibition of gut motility. They have important central actions, which account for their significant abuse potential and for their limited therapeutic applications (see Chs 48 and 58). Apart from ephedrine, which is still sometimes used as a nasal decongestant because it has much less central action, these drugs are no longer used for their peripheral sympathomimetic effects.

INHIBITORS OF NORADRENALINE UPTAKE

Reuptake of released noradrenaline by NET is the most important mechanism by which its action is brought to an end. Many drugs inhibit NET, and thereby enhance the effects of both sympathetic nerve activity and circulating noradrenaline. NET is not responsible for clearing circulating adrenaline, so these drugs do not affect responses to this amine.

The main class of drugs whose primary action is inhibition of NET are the *tricyclic antidepressants* (see Ch. 47), for example **imipramine**. These drugs have their major effect on the central nervous system but also cause tachycardia and cardiac dysrhythmias, reflecting their peripheral effect on sympathetic transmission. **Cocaine**, known mainly for its abuse liability (Ch. 49) and local anaesthetic activity (Ch. 43), enhances sympathetic transmission, causing tachycardia and increased arterial pressure (and with chronic use, cardiomyopathy and cardiac hypertrophy). Its central effects of euphoria and excitement (Ch. 48) are probably a manifestation of the same mechanism acting in the brain. It strongly potentiates the actions of noradrenaline in experimental animals or in isolated tissues provided the sympathetic nerve terminals are intact.

Many drugs that act mainly on other steps in sympathetic transmission also inhibit NET to some extent, presumably because the carrier molecule has structural features in common with other noradrenaline recognition sites, such as receptors and degradative enzymes.

The extraneuronal monoamine transporter EMT, which is important in clearing circulating adrenaline from the bloodstream, is not affected by most of the drugs that block NET. It is inhibited by **phenoxybenzamine**, however, and also by various *corticosteroids* (see Ch. 26). This action of corticosteroids may have some relevance to their therapeutic effect in conditions such as asthma, but is probably of minor importance.

The main sites of action of drugs that affect adrenergic transmission are summarised in Fig. 14.8.

Drugs acting on noradrenergic nerve terminals

- Drugs that inhibit noradrenaline synthesis include:
  - **α-Methyltyrosine**: blocks tyrosine hydroxylase; not used clinically
  - **carbidopa**: blocks dopa decarboxylase and is used in treatment of parkinsonism (see Ch. 40); not much effect on noradrenaline synthesis.
- **Methyldopa** gives rise to false transmitter (methylnoradrenaline), which is a potent α_2 agonist, thus causing powerful presynaptic inhibitory feedback (also central actions). Its use as an antihypertensive agent is now limited mainly to during pregnancy.
- **Reserpine** blocks noradrenaline accumulation in vesicles by VMAT, thus depleting noradrenaline stores and blocking transmission. Effective in hypertension but may cause severe depression. Clinically obsolete.
- Noradrenergic neuron-blocking drugs (e.g. **guanethidine**, **bethanidine**) are selectively concentrated in terminals and in vesicles (by NET and VMAT respectively), and block transmitter release, partly by local anaesthetic action. Effective in hypertension but cause severe side effects (postural hypotension, diarrhoea, nasal congestion, etc.), so now little used.
- **6-Hydroxydopamine** is selectively neurotoxic for noradrenergic neurons, because it is taken up and converted to a toxic metabolite. Used experimentally to eliminate noradrenergic neurons, not used clinically.
- Indirectly acting sympathomimetic amines (e.g. **amphetamine**, **ephedrine**, **tyramine**) are accumulated by NET and displace noradrenaline from vesicles, allowing it to escape. Effect is much enhanced by monoamine oxidase (MAO) inhibition, which can lead to severe hypertension following ingestion of tyramine-rich foods by patients treated with MAO inhibitors.
- Indirectly acting sympathomimetic agents are central nervous system stimulants. **Methylphenidate** and **atomoxetine** are used to treat attention deficit–hyperactivity disorder.
- Drugs that inhibit NET include **cocaine** and **tricyclic antidepressant** drugs. Sympathetic effects are enhanced by such drugs.

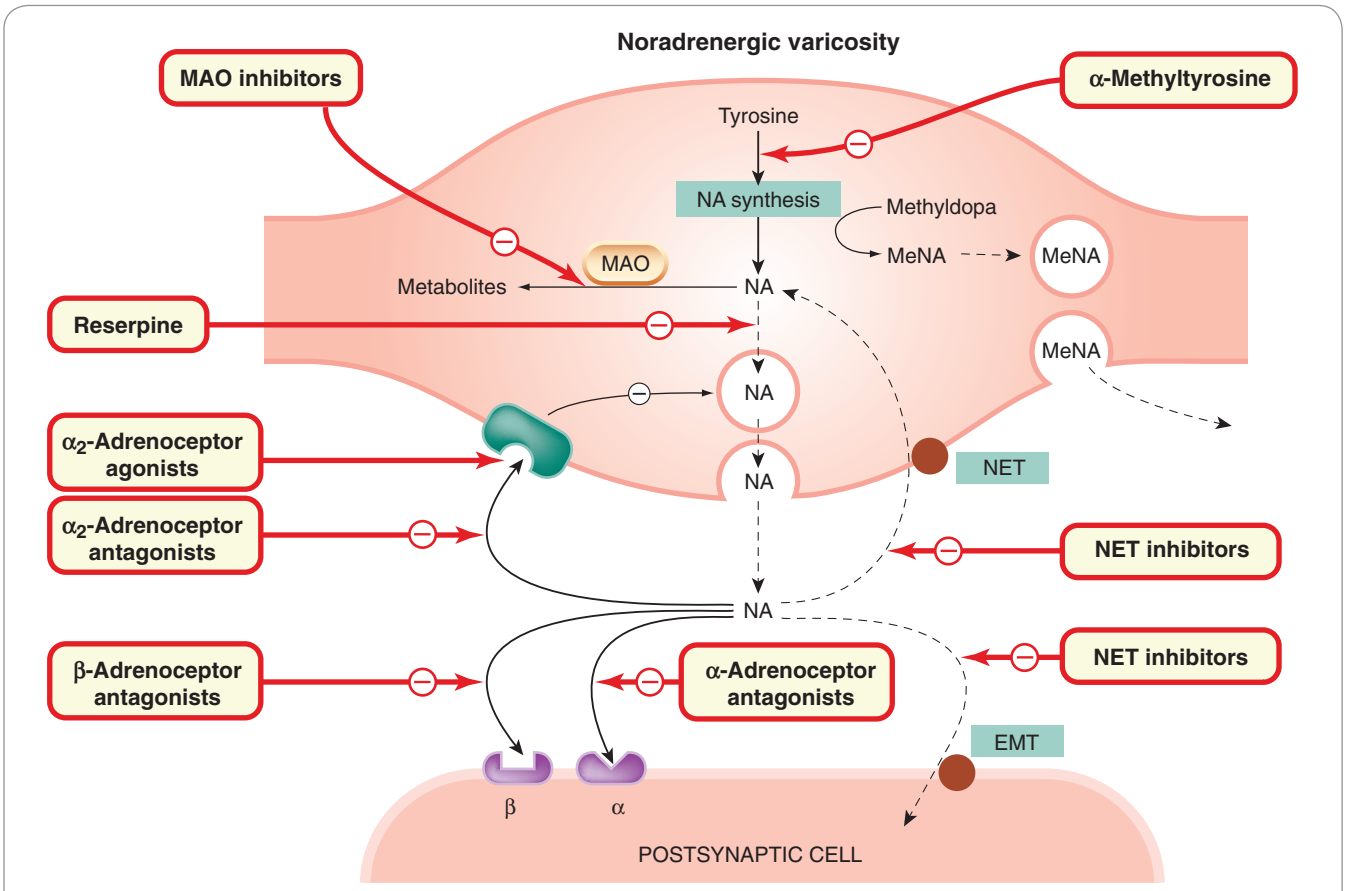

Fig. 14.8 Generalised diagram of a noradrenergic nerve terminal, showing sites of drug action. EMT, extraneuronal monoamine transporter; MAO, monoamine oxidase; MeNA, methylnoradrenaline; NA, noradrenaline; NET, neuronal noradrenaline transporter.

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5-Hydroxytryptamine and the pharmacology of migraine

15

OVERVIEW

5-Hydroxytryptamine (5-HT) is an important neurotransmitter in the brain and periphery and also a local hormone. We describe its synthesis, storage and release and its role in the pathophysiology of three disorders (migraine, carcinoid syndrome and pulmonary hypertension). We also describe the pharmacology of the numerous drugs that act at 5-HT receptors.

5-HYDROXYTRYPTAMINE

The biologically active, low-molecular-weight, factor originally detected in extracts of gut ('enteramine') and in blood serum ('serotonin') was eventually identified chemically as *5-hydroxytryptamine*. Today, the terms '5-HT' and 'serotonin' are used interchangeably. 5-HT was subsequently found in the central nervous system (CNS) and shown to function both as a neurotransmitter and as a local hormone in the peripheral vascular system. This chapter deals with the metabolism, distribution and physiological roles of 5-HT in the periphery, and with the different types of 5-HT receptor and the drugs that act on them. Further information on the role of 5-HT in the brain, and its relationship to psychiatric disorders and the actions of psychotropic drugs, is presented in Chapters 39, 46 and 47. The use of drugs that modulate 5-HT in the gut is dealt with in Chapter 30.

DISTRIBUTION, BIOSYNTHESIS AND DEGRADATION

The highest concentrations of 5-HT occur in three organs:

- *In the wall of the intestine.* Over 90% of the total amount in the body is present in the *enterochromaffin* cells (endocrine cells with distinctive staining properties) in the gut. These cells are derived from the neural crest and resemble those of the adrenal medulla. They are found mainly in the stomach and small intestine interspersed with mucosal cells. Some 5-HT also occurs in nerve cells of the myenteric plexus, where it functions as an excitatory neurotransmitter (see Chs 12 and 30).
- *In blood.* Platelets contain high concentrations of 5-HT. They accumulate it from the plasma by an active transport system and release it from cytoplasmic granules when they aggregate (hence the high concentration of 5HT in serum from clotted blood, see Ch. 24).
- *In the CNS.* 5-HT is a transmitter in the CNS and is present in high concentrations in localised regions of the midbrain. Its functional role is discussed in Chapter 39.

Although 5-HT is present in the diet, most of this is metabolised before entering the bloodstream. Endogenous 5-HT arises from a biosynthetic pathway similar to that of noradrenaline; (see Ch. 14), except that the precursor amino acid is *tryptophan* instead of tyrosine (Fig. 15.1). Tryptophan is converted to 5-hydroxytryptophan (in chromaffin cells and neurons, but not in platelets) by the action of *tryptophan hydroxylase*, an enzyme confined to 5-HT-producing cells. The 5-hydroxytryptophan is then decarboxylated to 5-HT by a ubiquitous *amino acid decarboxylase* that also participates in the synthesis of catecholamines (Ch. 14) and histamine (Ch. 17). Platelets (and neurons) possess a high-affinity 5-HT uptake mechanism. They become loaded with 5-HT as they pass through the intestinal circulation, where the local concentration is relatively high. Because the mechanisms of synthesis, storage, release and reuptake of 5-HT are very similar to those of noradrenaline, many drugs affect both processes indiscriminately (see Ch. 14). However, *selective serotonin reuptake inhibitors* (SSRIs) have been developed and are important therapeutically as anxiolytics and antidepressants (Chs 44 and 47). 5-HT is often stored in neurons and chromaffin cells as a co-transmitter together with various peptide hormones, such as *somatostatin*, *substance P* or *vasoactive intestinal polypeptide* (Ch. 18).

Degradation of 5-HT (Fig. 15.1) occurs mainly through oxidative deamination, catalysed by *monoamine oxidase A*, followed by oxidation to *5-hydroxyindoleacetic acid* (5-HIAA), the pathway again being the same as that of noradrenaline catabolism. 5-HIAA is excreted in the urine and serves as an indicator of 5-HT production in the body. This is used, for example, in the diagnosis of carcinoid syndrome.

PHARMACOLOGICAL EFFECTS

The actions of 5-HT are numerous and complex and there is considerable species variation. This complexity reflects the profusion of 5-HT receptor subtypes. The main sites of action are as follows.

Gastrointestinal tract. Most 5-HT receptor subtypes are present in the gut with the exception of those of the $5\text{-HT}_{5/6}$ family. Only about 10% of 5-HT in the intestine is located in neurons, where it acts as a neurotransmitter, while the remainder is located in the enterochromaffin cells, which act as sensors to transduce information about the state of the gut. 5-HT is released from enterochromaffin cells into the *lamina propria*. The responses observed are very complex and the reader is referred to Beattie & Smith (2008) for a recent comprehensive account. Broadly speaking, 5-HT receptors are present on most neuronal components of the enteric nervous system as well as smooth muscle, secretory and other cells. Their main function is to regulate peristalsis, intestinal motility, secretion and visceral sensitivity.

The importance of 5-HT in the gut is underlined by the widespread distribution of the *serotonin uptake transporter* (SERT), which rapidly and efficiently removes extracellular 5-HT, thus limiting its action. Inhibitors of

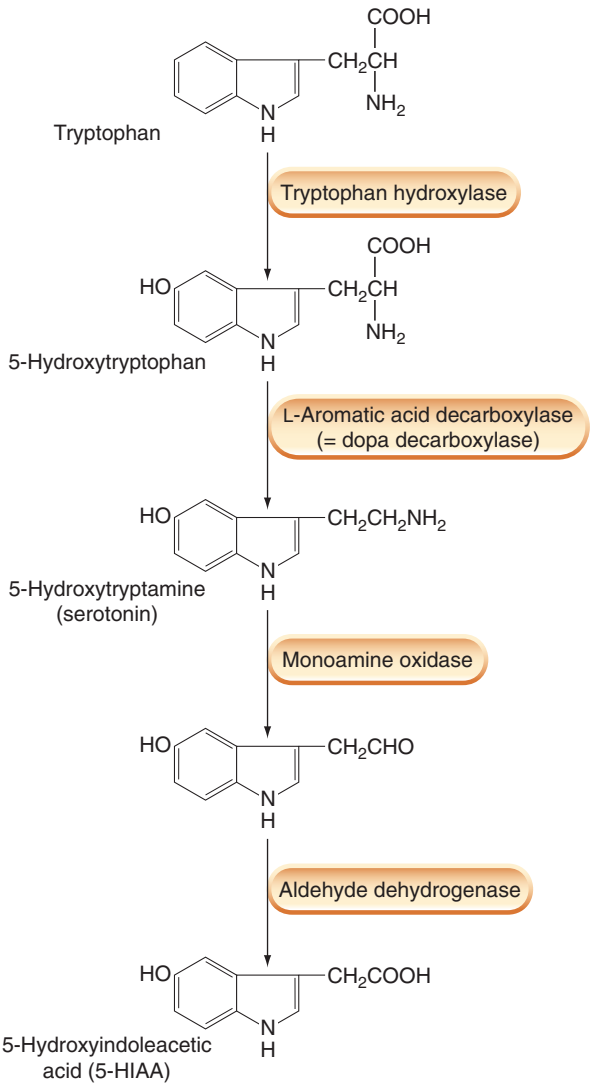

Fig. 15.1 Biosynthesis and metabolism of 5-hydroxytryptamine.

Distribution, biosynthesis and degradation of 5-hydroxytryptamine

- Tissues rich in 5-HT are:
  - gastrointestinal tract (chromaffin cells and enteric neurons)
  - platelets
  - central nervous system.
- Metabolism closely parallels that of noradrenaline.
- 5-HT is formed from dietary tryptophan, which is converted to 5-hydroxytryptophan by tryptophan hydroxylase, then to 5-HT by a non-specific decarboxylase.
- 5-HT is transported into cells by a specific serotonin uptake transporter (SERT).
- Degradation occurs mainly by monoamine oxidase, forming 5-hydroxyindoleacetic acid (5-HIAA), which is excreted in urine.

this transporter such as the SSRIs (Ch. 47) exaggerate the action of 5-HT in the gut, explaining some of the common side effects of these drugs, which include diarrhoea. Interestingly, there is evidence for genetic defects in this reuptake system in irritable bowel syndrome (Ch. 30), which might explain the rather bewildering symptoms of the disease.

Smooth muscle. In many species (although only to a minor extent in humans), smooth muscle outside of the gastrointestinal tract (e.g. uterus and bronchial tree) is also contracted by 5-HT.

Blood vessels. The effect of 5-HT on blood vessels depends on various factors, including the size of the vessel, the species and the prevailing sympathetic activity. Large vessels, both arteries and veins, are usually constricted by 5-HT, although the sensitivity varies greatly. This is the result of a direct action on vascular smooth muscle cells, mediated through 5-HT_{2A} receptors. Activation of 5-HT_1 receptors causes constriction of large intracranial vessels, dilatation of which contributes to headache. 5-HT can also cause vasodilatation, partly by acting on endothelial cells to release nitric oxide (see Ch. 20) and partly by inhibiting noradrenaline release from sympathetic nerve terminals. If 5-HT is injected intravenously, the blood pressure initially rises, owing to the constriction of large vessels, and then falls, owing to arteriolar dilatation. 5-HT may play a role in the pathology of *pulmonary hypertension* (see Ch. 22).

Platelets. 5-HT causes platelet aggregation (see Ch. 24) by acting on 5-HT_{2A} receptors, and the platelets that collect in the vessel release further 5-HT. If the endothelium is intact, 5-HT release from adherent platelets causes vasodilatation, which helps to sustain blood flow; if it is damaged (e.g. by atherosclerosis), 5-HT causes constriction and impairs blood flow further. These effects of platelet-derived 5-HT are thought to be important in vascular disease.

Nerve endings. 5-HT stimulates nociceptive (pain-mediating) sensory nerve endings, an effect mediated mainly by 5-HT_3 receptors. If injected into the skin, 5-HT causes pain; when given systemically, it elicits a variety of autonomic reflexes through stimulation of afferent fibres in the heart and lungs, which further complicate the cardiovascular response. Nettle stings contain 5-HT among other mediators. 5-HT also inhibits transmitter release from adrenergic neurons in the periphery.

Central nervous system. 5-HT excites some neurons and inhibits others; it may also act presynaptically to inhibit transmitter release from nerve terminals. Different receptor subtypes and different membrane mechanisms mediate these effects. The role of 5-HT in the CNS is discussed in Chapter 39.

CLASSIFICATION OF 5-HT RECEPTORS

▼ It was realised long ago that the actions of 5-HT are not all mediated by receptors of the same type, and various pharmacological classifications have come and gone. The current system is summarised in Table 15.1. This classification takes into account sequence data derived from cloning, signal transduction mechanisms and pharmacological specificity as well as the phenotypes of 5-HT receptor 'knockout' mice.

Their diversity is astonishing. Currently, there are some 14 known receptor subtypes (together with an extra gene in mouse). These are divided into seven classes ($5\text{-HT}_{1\text{-}7}$), one of which (5-HT_3) is a ligand-gated cation channel while the remainder are G protein-coupled receptors (GPCRs; see Ch. 3). The six GPCR families are further subdivided into some 13 receptor subtypes based on their sequence and

Table 15.1 Some significant drugs acting at the main 5-HT receptor subtypes

Receptor	Location	Main function	Signalling system	Significant drugs	
				Agonists	*Antagonists*
5-HT_{1A}	CNS	Neuronal inhibition Behavioural effects: sleep, feeding, thermoregulation, anxiety	G protein (G_i/G_o) ↓ cAMP (may also modulate Ca^{2+} channels)	8-OH-DPAT, triptans, clozapine, buspirone (PA), cabergoline	Methiothepin, yohimbine, ketanserin, pizotifen, spiperone
5-HT_{1B}	CNS, vascular smooth muscle, many other sites	Presynaptic inhibition Behavioural effects Pulmonary vasoconstriction	G protein (G_i/G_o) ↓ cAMP (may also modulate Ca^{2+} channels)	8-OH-DPAT, triptans, clozapine, cabergoline, dihydroergotamine	Methiothepin, yohimbine, ketanserin, spiperone
5-HT_{1D}	CNS, blood vessels	Cerebral vasoconstriction Behavioural effects: locomotion	G protein (G_i/G_o) ↓ cAMP (may also modulate Ca^{2+} channels)	8-OH-DPAT, triptans, clozapine, cabergoline, dihydro-ergotamine/ergotamine	Methiothepin, yohimbine, ketanserin, methysergide, spiperone
5-HT_{1E}	CNS	–	G protein (G_i/G_o) ↓ cAMP (may also modulate Ca^{2+} channels)	8-OH-DPAT, triptans; clozapine, dihydroergotamine	Methiothepin, yohimbine, methysergide
5-HT_{1F}	CNS, uterus, heart, GI tract	–	G protein (G_i/G_o) ↓ cAMP (may also modulate Ca^{2+} channels)	8-OH-DPAT, triptans; clozapine dihydro-ergotamine/ergotamine, lamistidan	Methiothepin, yohimbine, methysergide
5-HT_{2A}	CNS, PNS, smooth muscle, platelets	Neuronal excitation Behavioural effects Smooth muscle contraction (gut, bronchi, etc.) Platelet aggregation Vasoconstriction/vasodilatation	G protein (G_q/G_{11}) ↑ IP_3, Ca^{2+}	LSD, cabergoline, methysergide (PA), 8-OH-DPAT, ergotamine (PA)	Ketanserin, clozapine, methiothepin, methysergide
5-HT_{2B}	Gastric fundus	Contraction	G protein (G_q/G_{11}) ↑ IP_3, Ca^{2+}	LSD, cabergoline, methysergide (PA), 8-OH-DPAT, ergotamine (PA)	Ketanserin, clozapine, methiothepin, yohimbine
5-HT_{2C}	CNS, lymphocytes	–	G protein (G_q/G_{11}) ↑ IP_3, Ca^{2+}	LSD, cabergoline, methysergide (PA), 8-OH-DPAT, ergotamine (PA)	Ketanserin, clozapine, methiothepin, methysergide
5-HT_3	PNS, CNS	Neuronal excitation (autonomic, nociceptive neurons) Emesis Behavioural effects: anxiety	Ligand-gated cation channel	2-Me-5-HT, chloromethyl biguanide	Dolesatron, granisetron, ondansetron, palonosetron, tropisetron
5-HT_4	PNS (GI tract), CNS	Neuronal excitation GI motility	G protein (G_s) ↑ cAMP	Metoclopramide, tegaserod, cisapride	Tropisteron
5-HT_{5A}	CNS	Modulation of exploratory behaviour (rodents)?	G protein (G_s) ↑ cAMP	Triptans, 8-OH-DPAT	Methiothepin, clozapine, methysergide, yohimbine, ketanserin
5-HT_6	CNS, leukocytes	Learning and memory?	G protein (G_s) ↑ cAMP	LSD, ergotamine	Methiothepin, clozapine, spiperone, methysergide, dihydro-ergotamine
5-HT_7	CNS, GI tract, blood vessels	Thermoregulation? Circadian rhythm?	G protein (G_s) ↑ cAMP	Buspirone, cisapride, 8-OH-DPAT, LSD,	Methiothepin, clozapine, methysergide, buspirone, dihydro-ergotamine, ketanserin, yohimbine

The receptor classification system is based upon the IUPHAR database at www.iuphar-db.org.
Many drugs here are not used clinically; others have been withdrawn (e.g. fenfluramine), or are not currently available in the UK (e.g. dolesatron, tropisetron), but are included as they are often referred to in the literature.
2-Me-5-HT, 2-methyl-5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetraline; CNS, central nervous system; DAG, diacylglycerol; GI, gastrointestinal; IP_3, inositol trisphosphate; LSD, lysergic acid diethylamide; PA, partial agonist; PNS, peripheral nervous system.
The list of agonists and antagonists is not exhaustive.

Actions and functions of 5-hydroxytryptamine

- Important actions are:
  - increased gastrointestinal motility (direct excitation of smooth muscle and indirect action via enteric neurons)
  - contraction of other smooth muscle (bronchi, uterus)
  - mixture of vascular constriction (direct and via sympathetic innervation) and dilatation (endothelium dependent)
  - platelet aggregation
  - stimulation of peripheral nociceptive nerve endings
  - excitation/inhibition of central nervous system neurons.
- Postulated physiological and pathophysiological roles include:
  - in periphery: peristalsis, vomiting, platelet aggregation and haemostasis, inflammation, sensitisation of nociceptors and microvascular control
  - in CNS: many postulated functions, including control of appetite, sleep, mood, hallucinations, stereotyped behaviour, pain perception and vomiting.
- Clinical conditions associated with disturbed 5-HT include migraine, carcinoid syndrome, mood disorders and anxiety.

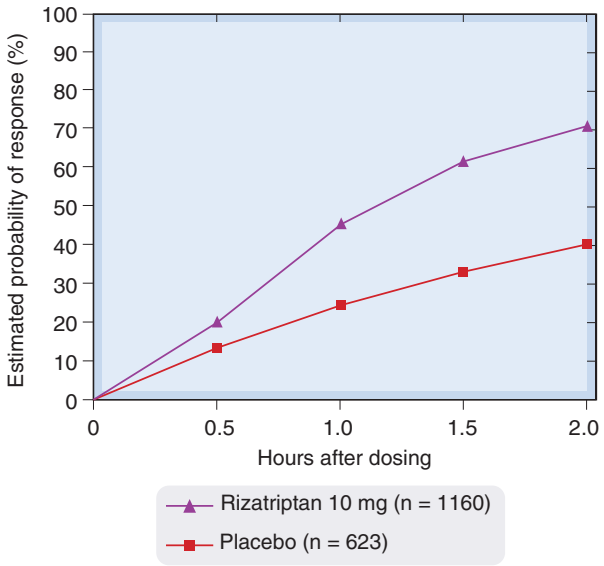

Fig. 15.2 The triptan, rizatriptan, relieves the pain associated with attacks of migraine. The graph is a Kaplan–Meier plot showing the probability of experiencing relief from the pain of the attack after treatment with placebo or with 10 mg rizatriptan. *(From Dahlof et al. 1999).*

pharmacology. Most subtypes are found in all species so far examined, but there are some exceptions (the 5-HT_{5B} gene is found in mouse but has not been found in humans). The sequences of 5-HT_1 and 5-HT_2 receptors are highly conserved among species but the 5-HT_{4-7} receptors are less conserved and are grouped together largely on pharmacological grounds. Most 5-HT GPCRs signal through adenylyl cyclase/cAMP, but some (the 5-HT_2 subtype) activate phospholipase C to generate phospholipid-derived second messengers (see Ch. 3).

In addition to these main subtypes, many genetic isoforms have been found, giving rise to four or more variants of some of these receptors. The pharmacological and pathophysiological relevance of these genetic isoforms is unclear.

With the exception of $5HT_3$- selective agents, 5-HT receptor agonists and antagonists are relatively non-selective with respect to different receptor subtypes. This makes their pharmacology difficult to interpret and summarise.

Many transgenic mice lacking functional members of this receptor family have been produced (see for example Bonasera & Tecott, 2000). The functional deficits in such animals are generally quite subtle, suggesting that these receptors may serve to tune, rather than to enable, physiological responses. Table 15.1 gives an overview of the most important receptors. Some of the more significant drug targets include the following:

5-HT_1 receptors. Those of pharmacological significance occur mainly in the brain, the subtypes being distinguished on the basis of their regional distribution and their pharmacological specificity. They function mainly as inhibitory presynaptic receptors. The 5-HT_{1A} subtype is particularly important in relation to mood and behaviour (see Chs 44, 46) and 5-HT_1 'knockout' mice exhibit defects in sleep regulation, learning ability and other CNS functions. Receptor polymorphisms may be associated with increased susceptibility to substance abuse. The 5-HT_{1B} and 5-HT_{1D} subtypes, which are expressed in cerebral blood vessels, are believed to be important in migraine and are the target for ***triptans***, such as sumatriptan an important group of drugs used to treat acute attacks (Fig. 15.2). Unfortunately, the 5-HT_{1B} receptor is also present in the vasculature of the heart and elsewhere, explaining some of the unwanted effects associated with triptan therapy. The hapless '5-HT_{1C}' receptor – actually the first to be cloned – has been officially declared non-existent, having been ignominiously reclassified as the 5-HT_{2C} receptor when it was found to be linked to inositol trisphosphate production rather than adenylyl cyclase.

5-HT_2 receptors. These are present in the CNS but are also particularly important in the periphery. The effects of 5-HT on smooth muscle and platelets, which have been known for many years, are mediated by the 5-HT_{2A} receptor, as are some of the behavioural effects of agents such as **lysergic acid diethylamide** (LSD; see Table 15.1 and Ch. 48). 5-HT_2 receptors are linked to phospholipase C and thus stimulate inositol trisphosphate formation. The 5-HT_{2A} subtype is functionally the most important, the others having a much more limited distribution and functional role. The role of 5-HT_2 receptors in normal physiology is probably a minor one, but it becomes more prominent in pathological conditions such as asthma and vascular thrombosis (see Chs 28 and 24). Mice lacking 5-HT_2 receptors exhibit defects in colonic motility (5-HT_{2A}), heart defects (5-HT_{2B}) and CNS disorders (5-HT_{2C}).

5-HT_3 receptors. 5-HT_3 receptors are exceptional in being membrane ion channels (Ch. 3) and cause excitation directly, without involvement of any second messenger. The receptor itself consists of a pentameric assembly of distinct subunits which are designated by further subscript letters (e.g. 5-HT_{3A-E} in humans). 5-HT_3 receptors occur mainly in the peripheral nervous system, particularly on nociceptive sensory neurons (see Ch. 42) and on autonomic and enteric neurons, where 5-HT exerts a strong excitatory effect. 5-HT evokes pain when injected locally; when given intravenously, it elicits a fine display of autonomic reflexes, which result from excitation of many types of vascular, pulmonary and cardiac sensory nerve fibres. 5-HT_3 receptors also occur in the brain, particularly in the *area postrema*, a region of the medulla involved in the vomiting reflex, and selective 5-HT_3 antagonists are used as antiemetic drugs (see Ch. 30). Polymorphisms in the subunits are associated with increased susceptibility to nausea and vomiting.

5-HT_4 receptors. These occur in the brain, as well as in peripheral organs such as the gastrointestinal tract, bladder and heart. Their main physiological role appears to be in the gastrointestinal tract, where they produce neuronal excitation and mediate the effect of

5-HT in stimulating peristalsis. Mice deficient in the 5-HT_4 receptor exhibit a complex phenotype including abnormal feeding behaviour in response to stress.

5-HT$_5$, 5-HT$_6$ and 5-HT$_7$ receptors. Little is known about these receptors. All are present in the CNS as well as other tissues. There are two genes for 5-HT_5 isoforms but only one codes for a functional receptor in humans although both may be functional in rodents. A recent report of selective antagonists at the 5-HT_7 receptor may open the way for a detailed examination of the role of this receptor in CNS pathology (Agosti, 2007).

5-Hydroxytryptamine receptors

- There are seven families (5-$HT_{1–7}$), with further subtypes of 5-HT_1 (A–F) and 5-HT_2 (A–C). Many polymorphisms and splice variants have also been observed.
- All are G protein-coupled receptors, except 5-HT_3, which is a ligand-gated cation channel.
  - 5-HT_1 receptors occur mainly in the CNS (all subtypes) and some blood vessels (5-$HT_{1B/D}$ subtypes). Some effects are mediated through inhibition of adenylyl cyclase, include neural inhibition and vasoconstriction. Specific agonists include triptans (used in migraine therapy) and **buspirone** (used in anxiety). Specific antagonists include **spiperone** and **methiothepin**.
  - 5-HT_2 receptors occur in the CNS and many peripheral sites (especially blood vessels, platelets, autonomic neurons). Neuronal and smooth muscle effects are excitatory and some blood vessels are dilated as a result of nitric oxide release from endothelial cells. 5-HT_2 receptors act through the phospholipase C/inositol trisphosphate pathway. Ligands include lysergic acid diethylamide (**LSD**; agonist in CNS, antagonist in periphery). Specific antagonists include **ketanserin**.
  - 5-HT_3 receptors occur in the peripheral nervous system, especially nociceptive afferent neurons and enteric neurons, and in the CNS. Effects are excitatory, mediated through direct receptor-coupled ion channels. **2-Methyl-5-HT** is a specific agonist. Specific antagonists include **ondansetron** and **tropisetron**. Antagonists are used mainly as antiemetic drugs but may also be anxiolytic.
  - 5-HT_4 receptors occur mainly in the enteric nervous system (also in the CNS). Effects are excitatory, through stimulation of adenylyl cyclase, causing increased gastrointestinal motility. Specific agonists include **metoclopramide** (used to stimulate gastric emptying).
  - 5-HT_5 receptors (one subtype in humans) are located in the CNS. Little is known about their role in humans.
  - 5-HT_6 receptors are located in the CNS and on leukocytes. Little is known about their role in humans.
  - 5-HT_7 receptors are located in the CNS and the gastrointestinal tract. Little is known about their role in humans but emerging data shows they may also be important in migraine.

DRUGS ACTING AT 5-HT RECEPTORS

Table 15.1 lists some significant agonists and antagonists at the different receptor types. Many are only partly selective. Our increasing understanding of the location and function of the different receptor subtypes has caused an upsurge of interest in developing compounds with improved receptor selectivity, and further useful new drugs are likely to appear in the near future.

Important drugs that act on 5-HT receptors in the periphery include the following:

- Although not clinically useful, selective 5-HT_{1A} agonists, such as 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), are potent hypotensive agents, acting through a central mechanism.
- 5-$HT1_{B/D}$ receptor agonists (e.g. the triptans) are used for treating migraine.
- 5-HT_2 receptor antagonists (e.g. **methysergide**, **ketanserin**) act mainly on 5-HT_{2A} receptors but may also block other 5-HT receptors, as well as α adrenoceptors and histamine receptors (Ch. 26). **Dihydroergotamine** and methysergide belong to the ergot family and are used mainly for migraine prophylaxis. Other 5-HT_2 antagonists are used to control the symptoms of carcinoid tumours.
- 5-HT_3 receptor antagonists (e.g. **dolasetron**, **granisetron**, **ondansetron**, **palonosetron**, **tropisetron**) are used as antiemetic drugs (see Chs 30 and 56), particularly for controlling the severe nausea and vomiting that occurs with many forms of cancer chemotherapy.
- 5-HT_4 receptor agonists that stimulate coordinated peristaltic activity (known as a 'prokinetic action') could be used for treating gastrointestinal disorders (see Ch. 30). **Metoclopramide** acts in this way, as well as by blocking dopamine receptors. Similar but more selective drugs such as **cisapride** and **tegaserod** were introduced to treat irritable bowel syndrome, but were withdrawn on account of cardiovascular side effects.

5-HT is also important as a neurotransmitter in the CNS, and several important antipsychotic and antidepressant drugs act on these pathways (see Chs 39, 46 and 47). LSD is a relatively non-selective 5-HT receptor agonist or partial agonist, which acts centrally as a potent hallucinogen (see Ch. 48).

ERGOT ALKALOIDS

Ergot alkaloids have preoccupied pharmacologists for more than a century. As a group, they resist classification. Many act on 5-HT receptors, but not selectively so that their effects are complex and diverse.

▼ Ergot, an extract of the fungus *Claviceps purpurea* that infests cereal crops, contains many active substances, and it was the study of their pharmacological properties that led Dale to many important discoveries concerning acetylcholine, histamine and catecholamines. Epidemics of ergot poisoning have occurred, and still occur, when contaminated grain is used for food. The symptoms include mental disturbances and intensely painful peripheral vasoconstriction leading to gangrene. This came to be known in the Middle Ages as *St Anthony's fire*, because it was believed that it could be cured by a visit to the Shrine of St Anthony (which happened to be in an ergot-free region of France).

Ergot alkaloids are complex molecules based on lysergic acid (a naturally occurring tetracyclic compound). The important members of the group (Table 15.2) include various naturally occurring and

Table 15.2 Properties of ergot alkaloids and related compounds

Drug	Actions at receptors			Uterus	Main uses	Side effects etc.
	5-HT	*α Adrenoceptor*	*Dopamine*			
Ergotamine	Antagonist/ partial agonist (5-HT_1) Antagonist (other sites)	Partial agonist (blood vessels)	Inactive	Contracts ++	Migraine (largely obsolete)	Emesis, vasospasm (avoid in peripheral vascular disease and pregnancy)
Dihydroergotamine	Antagonist/ partial agonist (5-HT_1)	Antagonist	Inactive	Contracts +	Migraine (largely obsolete)	Less emesis than with ergotamine
Ergometrine	Weak antagonist/ partial agonist (5-HT_1)	Weak antagonist/ partial agonist	Weak	Contracts +++	Prevention of postpartum haemorrhage (Ch. 35)	Nausea, vomiting
Bromocriptine	Inactive	Weak antagonist	Agonist/ partial agonist	–	Parkinson's disease (Ch. 40) Endocrine disorders (Ch. 31)	Drowsiness, emesis
Methysergide	Antagonist/ partial agonist (5-HT_2)	–	–	–	Carcinoid syndrome Migraine (prophylaxis)	Retroperitoneal and mediastinal fibrosis Emesis

synthetic derivatives with different substituent groups arranged around a common nucleus. These compounds display diverse pharmacological actions and it is difficult to discern any clear relationship between chemical structure and pharmacological properties.

Ergot alkaloids

- These active substances are produced by a fungus that infects cereal crops and are responsible for occasional poisoning incidents. The most important compounds are:
  - **ergotamine** and **dihydroergotamine**, used in migraine prophylaxis
  - **ergometrine**, used in obstetrics to prevent postpartum haemorrhage
  - **methysergide**, used to treat carcinoid syndrome, and occasionally for migraine prophylaxis
  - **bromocriptine**, used in parkinsonism and endocrine disorders.
- Main sites of action are 5-HT receptors, dopamine receptors and adrenoceptors (mixed agonist, antagonist and partial agonist effects).
- Unwanted effects include nausea and vomiting, vasoconstriction (ergot alkaloids are contraindicated in patients with peripheral vascular disease).

Actions

Most of the effects of ergot alkaloids appear to be mediated through adrenoceptors, 5-HT or dopamine receptors, although some may be produced through other mechanisms. All alkaloids stimulate smooth muscle, some being relatively selective for vascular smooth muscle while others act mainly on the uterus. **Ergotamine** and **dihydroergotamine** are, respectively, a partial agonist and an antagonist at α adrenoceptors. **Bromocriptine** is an agonist of dopamine receptors, particularly in the CNS (Ch. 39), and **methysergide** is an antagonist at 5-HT_{2A} receptors.

The main pharmacological actions and uses of these drugs are summarised in Table 15.2. As one would expect of agents having so many actions, their physiological effects are complex and rather poorly understood. Ergotamine, dihydroergotamine and methysergide are discussed here; further information on **ergometrine** and bromocriptine is given in Chapters 33, 35 and 40.

Vascular effects. When injected into an anaesthetised animal, ergotamine activates α adrenoceptors, causing vasoconstriction and a sustained rise in blood pressure. At the same time, ergotamine reverses the pressor effect of adrenaline (epinephrine; see Ch. 14). The vasoconstrictor effect of ergotamine is responsible for the peripheral gangrene of St Anthony's fire, and probably also for some of the effects of ergot on the CNS. Methysergide and dihydroergotamine have much less vasoconstrictor effect. Methysergide is a potent 5-HT_{2A} receptor antagonist, whereas ergotamine and dihydroergotamine act selectively on 5-HT_1 receptors. Although generally classified as antagonists, they show partial agonist activity in some tissues, and this may account for their activity when treating migraine attacks.

Clinical use. The only use of ergotamine is in the treatment of attacks of migraine unresponsive to simple analgesics (see Chs 26 and 42). Methysergide is occasionally used for migraine prophylaxis, but its main use is in treating the symptoms of carcinoid tumours. All these drugs can be used orally or by injection.

Unwanted effects. Ergotamine often causes nausea and vomiting, and it must be avoided in patients with peripheral vascular disease because of its vasoconstrictor action. Methysergide also causes nausea and vomiting, but its most serious side effect, which considerably restricts its clinical usefulness, is retroperitoneal and mediastinal fibrosis, which impairs the functioning of the gastrointestinal tract, kidneys, heart and lungs. The mechanism of this is unknown, but it is noteworthy that similar fibrotic reactions also occur in carcinoid syndrome, in which there is a high circulating level of 5-HT.

MIGRAINE AND OTHER CLINICAL CONDITIONS IN WHICH 5-HT PLAYS A ROLE

In this section, we discuss three situations where the peripheral actions of 5-HT are believed to be important, namely *migraine*, *carcinoid syndrome* and *pulmonary hypertension*. The use of 5-HT_3 antagonists for treating drug-induced emesis is discussed in Chapter 30. Modulation of 5-HT-mediated transmission in the CNS is an important mechanism of action of antidepressant and antipsychotic drugs (see Chs 39, 44 and 47).

MIGRAINE AND ANTIMIGRAINE DRUGS

Migraine[1] is a common and debilitating condition affecting 10–15% of people. Although the causes are not well understood, both genetic and environmental factors seem to be important. The frequency of attacks varies, with about three-quarters of *migraineurs* (as they are called) having more than one episode per month. Generally, the onset of attacks begins at puberty and wanes with increasing age. Women are twice as likely as men to suffer from the disorder and the attacks are often linked to the menstrual cycle or other reproductive events. It appears that rapidly falling oestrogen levels can precipitate bouts of migraine in susceptible subjects.

In the UK, some 25 million work or school days are lost each year because of the incapacitating effects of the disease, with an economic cost of more than £2 billion. The WHO has classified migraine as amongst the 20 most disabling lifetime conditions.

Migraines are differentiated from other types of headache (e.g. cluster headaches, tension headaches) based on strict diagnostic guidelines. The onset of an attack is heralded by a *premonitory phase*, with symptoms including nausea, mood changes and sensitivity to light and sound (photophobia and phonophobia). These may occur hours before the next phase, which is generally referred to as the *aura* during which phonophobia and photophobia are more common, and may be accompanied by more specific visual symptoms such as a slowly moving blind spot with associated flashing lights ('scintillating scotoma') or geometric patterns of coloured lights ('fortification spectra') or the illusion of looking through the wrong end of a telescope. The *headache* phase proper is characterised by a moderate or severe headache, starting unilaterally, but then usually spreading to both sides of the head. It may have a pulsating or throbbing quality accompanied by nausea, vomiting and prostration. This phase may persist for hours or even days. Following resolution of the headache, is the *postdromal* phase. This may include feelings of fatigue, altered cognition or mood changes. Whilst these different phases probably represent discrete biological events, in practice they overlap and may run in parallel. A good account of these is given by Charles (2013).

PATHOPHYSIOLOGY

The causes of migraine are incompletely understood. Historically there have been three main hypotheses advanced to account for the symptoms (see Eadie, 2005).

The classic '*vascular' theory*, first proposed around 50 years ago by Wolff, implicated an initial humorally mediated intracerebral vasoconstriction as the cause of the aura, followed by an extracerebral vasodilatation causing the headache.

The '*brain' hypothesis* (see Lauritzen, 1987) linked the symptoms to the phenomenon of *cortical spreading depression*. This is a dramatic, although poorly understood, phenomenon, triggered in experimental animals by local application of K^+ to the cortex and also thought to occur in humans after (for example) concussion. An advancing wave of profound neural inhibition progresses slowly over the cortical surface at a rate of about 2 mm/min. In the affected area, the ionic balance is grossly disturbed, with an extremely high extracellular K^+ concentration, and the blood flow is reduced.

The '*inflammation' hypothesis* (see Waeber & Moskowitz, 2005) proposes that activation of trigeminal nerve terminals in the meninges and extracranial vessels is the primary event in a migraine attack. This would cause pain directly and also induce inflammatory changes through the release of neuropeptides and other inflammatory mediators from the sensory nerve terminals (neurogenic inflammation; see Chs 18 and 42). This theory is supported by experiments showing that one such peptide (*calcitonin gene-related peptide*; see Ch. 18) is released into the meningeal circulation during a migraine attack and that an antagonist of this peptide, **telcagepant** – an investigational drug (discontinued because of liver toxicity) – was extremely effective in aborting attacks (Farinelli et al., 2008).

In practice, elements of all these phenomena seem to play a role in the pathogenesis of migraine. Current thinking (summarised by Charles, 2013) suggests that the symptoms associated with the premonitory phase are largely dopaminergic in origin. Antagonists of this neurotransmitter, such as **domperidone,** mitigate migraine attacks if administered in a timely fashion. Also, imaging studies have indicated changes in hypothalamic blood flow during this phase, suggesting a role in the pathogenesis of the attack as well as offering potential new targets for drug therapy. Imaging techniques have revealed widespread changes in brain perfusion during the aura phase. There may be hypoperfusion of some brain areas as well as hyperperfusion in others, suggesting that the physiological mechanisms that normally regulate the relationship between brain activity and blood flow become disengaged. Such *neurovascular uncoupling* is a feature of cortical spreading depression.

During the headache phase there are again vascular changes in (for example) the meningeal and middle cerebral arteries, but once again, these are not consistent and in any case not directly responsible for the pain and other

[1]The word is apparently of French origin and is probably a corruption of *hemicrania*, the Latin name for the disease.

symptoms. What does seem to be important is *central sensitisation*, which increases the migraineur's sensitivity to sound, light and other normally non-painful stimuli. This is accompanied by a release of inflammatory or nocieceptive mediators such as CGRP, nitric oxide (NO) and prostaglandins. Many of the observed vascular and other changes may persist into the postdromal phase which may last for hours or days.

It is noteworthy that none of these mechanisms can explain at the biochemical level what initiates a migraine attack or define the underlying abnormality that predisposes particular individuals to suffer such attacks. In some rare types of familial migraine, inherited mutations affecting calcium channels and Na^+-K^+-ATPase have been found, suggesting that abnormal membrane function may be responsible, but in most forms of migraine there is no clear genetic cause.

Whether one inclines to the view that migraine is primarily a vascular disorder, a type of spontaneous concussion, an inflammatory disease or just a bad headache, there are two important factors that implicate 5-HT in its pathogenesis:

1. There is a sharp increase in the urinary excretion of the main 5-HT metabolite, 5-HIAA, during the attack. The blood concentration of 5-HT falls, probably because of depletion of platelet 5-HT.
2. Many of the drugs that are effective in treating migraine are 5-HT receptor agonists or antagonists. See Figure 15.3 and the clinical box below for further information.

ANTIMIGRAINE DRUGS

Drugs used for migraine

Acute attack

- Simple analgesics (e.g. **aspirin**, **paracetamol**; see Ch. 26) with or without **metoclopramide** (see Ch. 30) to hasten absorption.
- **Ergotamine** (5-HT_{1D} receptor partial agonist).
- **Sumatriptan**, **zolmitriptan** (5-HT_{1D} agonists).

Prophylaxis

- β-Adrenoceptor antagonists (e.g. **propranolol**, **metoprolol**; see Ch. 14).
- **Pizotifen** (5-HT_2 receptor antagonist).
- Other 5-HT_2 receptor antagonists:
  - **cyproheptadine**: also has antihistamine actions
  - **methysergide**: rarely used because of risk of retroperitoneal fibrosis.
- Tricyclic antidepressants (e.g. **amitriptyline**; see Ch. 47).
- **Clonidine**, an α_2 adrenoceptor agonist (see Ch. 14).
- Calcium antagonists (e.g. dihydropyridines, **verapamil**; see Ch. 21): headache is a side effect of these drugs but, paradoxically, they may reduce frequency of migraine attacks.

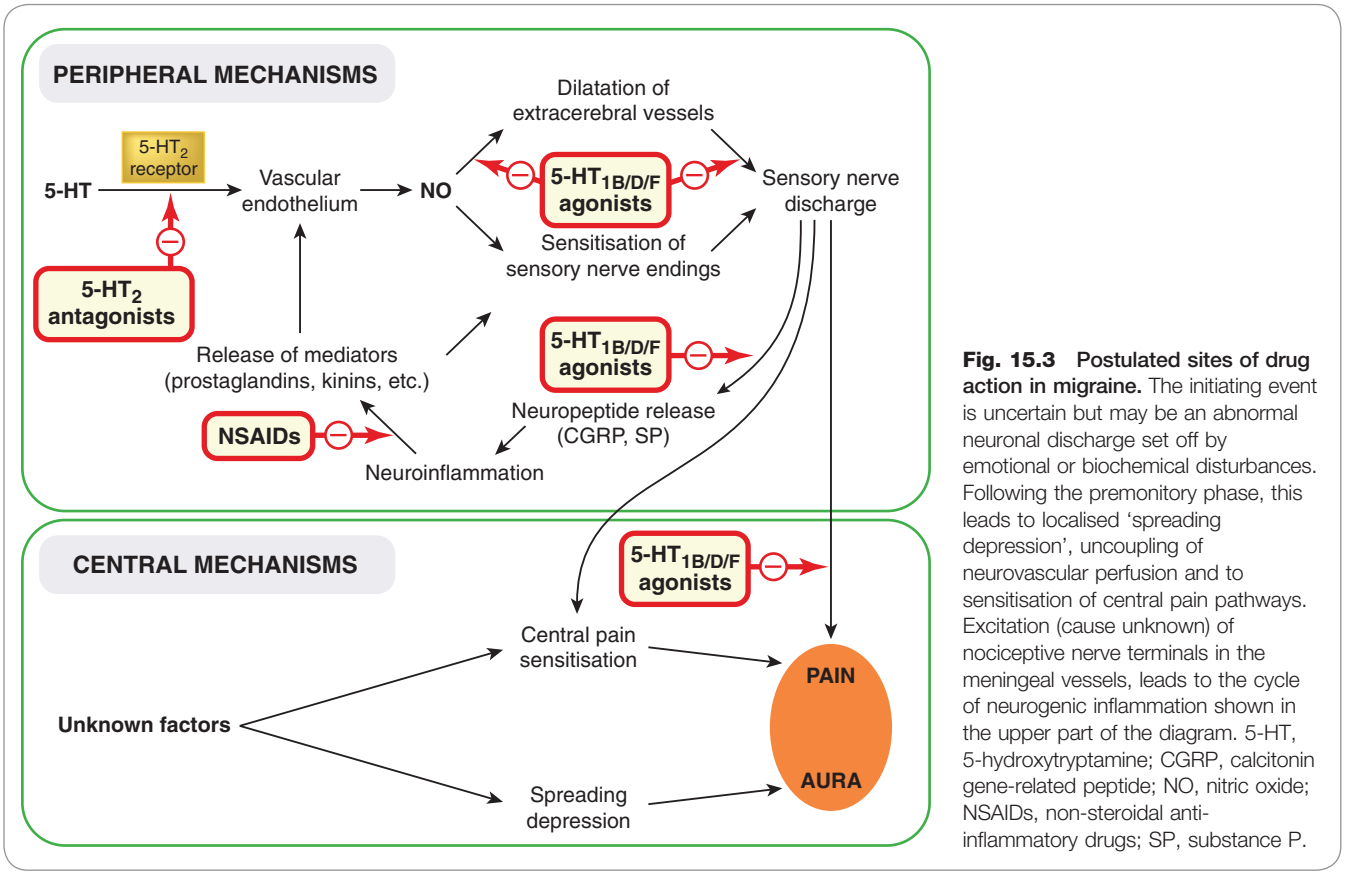

Fig. 15.3 Postulated sites of drug action in migraine. The initiating event is uncertain but may be an abnormal neuronal discharge set off by emotional or biochemical disturbances. Following the premonitory phase, this leads to localised 'spreading depression', uncoupling of neurovascular perfusion and to sensitisation of central pain pathways. Excitation (cause unknown) of nociceptive nerve terminals in the meningeal vessels, leads to the cycle of neurogenic inflammation shown in the upper part of the diagram. 5-HT, 5-hydroxytryptamine; CGRP, calcitonin gene-related peptide; NO, nitric oxide; NSAIDs, non-steroidal anti-inflammatory drugs; SP, substance P.

Table 15.3 Antimigraine drugs[a]

Use	Drug(s)	Mode of action	Side effects	Pharmacokinetic aspects	Notes
Acute	Sumatriptan	5-$HT_{1B/1D/1F}$ receptor agonist. Constricts large arteries, inhibits trigeminal nerve transmission.	Coronary vasoconstriction, dysrhythmias.	Poor oral absorption, hence delayed response. Can be given s.c. Does not cross blood–brain barrier. Plasma half-life 1.5 h.	Effective in ~70% of migraine attacks. Short duration of action is a drawback. Contraindicated in coronary disease.
Acute	Almotriptan Eletriptan Frovatriptan Naratriptan Rizatriptan Zolmitriptan	As above; additional actions on CNS.	Side effects less than with sumatriptan.	Improved bioavailability and duration of action. Able to cross blood–brain barrier.	Similar to sumatriptan; but improved pharmacokinetics and reduced cardiac side effects.
Acute	Ergotamine	5-HT_1 receptor partial agonist; also affects α adrenoceptors. Vasoconstrictor. Blocks trigeminal nerve transmission.	Peripheral vasoconstriction, including coronary vessels. Nausea and vomiting. Contracts uterus and may damage fetus.	Poorly absorbed. Can be given by suppository, inhalation, etc. Duration of action 12–24 h.	Effective, but use limited by side effects.
Prophylaxis	Methysergide	5-HT_2 receptor antagonist/partial agonist.	Nausea, vomiting, diarrhoea. Retroperitoneal or mediastinal fibrosis (rare but serious).	Used orally	Effective, but rarely used because of side effects and insidious toxicity.
Prophylaxis	Pizotifen	5-HT_2 receptor antagonist. Also histamine antagonist.	Weight gain, antimuscarinic side effects.	Used orally	–
Prophylaxis	Cyproheptadine	5-HT_2 receptor antagonist. Also blocks histamine receptors and Ca^{2+} channels.	Sedation, weight gain.	Used orally	Rarely used
Prophylaxis	Propranolol and similar drugs.	β-adrenoceptor antagonists. Mechanism of antimigraine effect not clear.	Fatigue, bronchoconstriction.	Used orally	Effective and widely used for migraine.

[a]Other drugs used for the *acute* treatment of migraine include non-steroidal anti-inflammatory drugs (NSAIDs) or opiate analgesic drugs (see Chs 42 and 47). Other drugs used for migraine *prophylaxis* include calcium channel blockers (e.g. nifedipine, see Ch. 22), antidepressants (e.g. amitriptyline; see Ch. 47), antiepileptics such as topiramate and sodium valproate (see Ch. 45) and the antihypertensive, clonidine (Ch. 14). Their efficacy is limited. Botox (botulinum toxin; type A) may be used for refractory severe cases of migraine.

The main drugs currently used to treat migraine are summarised in Table 15.3, and their postulated sites of action are shown in Figure 15.3. It is important to distinguish between drugs used *therapeutically* to treat acute attacks of migraine (appropriate when the attacks are fairly infrequent) and drugs that are used *prophylactically*. Apart from 5-HT_2 receptor antagonists, the drugs used prophylactically are a mixed bag, and their mechanisms of action are poorly understood.

The most important agents for the treatment of acute attacks are currently the triptans. These are 5-HT_1 agonists, and are usually classified as 5-$HT_{1B/1D}$ agonists, largely because it is difficult to distinguish between actions at these two receptors. However, selective high-affinity 5-HT_{1D} subtype agonists have proved disappointing in the clinic. Sumatriptan also has high affinity for the 5-HT_{1F} receptor (see Agosti, 2007), and **lasmiditan** an investigational non-triptan drug that is a selective 5HT_{1F} receptor agonist is highly effective in aborting migraine attacks (Tfelt-Hansen, 2012). Interestingly, this receptor subtype is scarce in the vasculature, casting doubt on the role of vascular changes in the disease. This is significant because a major drawback to triptan therapy is vasoconstriction in other peripheral vascular beds including the heart. Lasmiditan would be expected to be free of such effects; however, it commonly causes other adverse effects (e.g. dizziness and nausea) that can be severe.

CARCINOID SYNDROME

Carcinoid syndrome (see Creutzfeld & Stockmann, 1987) is a rare disorder associated with malignant tumours of

enterochromaffin cells, which usually arise in the small intestine and metastasise to the liver. These tumours secrete a variety of chemical mediators: 5-HT is the most important, but neuropeptides such as substance P (Ch. 18), and other agents such as prostaglandins and bradykinin (Ch. 17), are also produced. The release of these substances into the bloodstream results in several unpleasant symptoms, including flushing, diarrhoea, bronchoconstriction and hypotension, which may cause dizziness or fainting. Fibrotic stenosis of heart valves, which can result in cardiac failure, also occurs. It is reminiscent of retroperitoneal and mediastinal fibrosis, which are adverse effects of methysergide, and appears to be related to overproduction of 5-HT.

The syndrome is readily diagnosed by measuring the urinary excretion of the main metabolite of 5-HT, 5-HIAA. This may increase by as much as 20-fold when the disease is active and is raised even when the tumour is asymptomatic. 5-HT_2 antagonists, such as **cyproheptadine**, are effective in controlling some of the symptoms of carcinoid syndrome. A complementary therapeutic approach is to use **octreotide** (a long-acting agonist at somatostatin receptors), which suppresses hormone secretion from neuroendocrine, including carcinoid, cells (see Ch. 33).

PULMONARY HYPERTENSION

Pulmonary hypertension (see also Ch. 22) is an extremely serious disease characterised by the progressive remodelling of the pulmonary vascular tree. This leads to an inexorable rise in pulmonary arterial pressure which, if untreated (and treatment is difficult), inevitably leads to right heart failure and death. The role of 5-HT in this pathology was suggested by the fact that at least one form of the condition was precipitated by the use of appetite suppressants (e.g. **dexfenfluramine** and **fenfluramine**) that were at one time widely prescribed as 'weight loss' or 'slimming' aids. These drugs apparently blocked SERT and since 5-HT promotes the growth and proliferation of pulmonary arterial smooth muscle cells and also produces a net vasoconstrictor effect in this vascular bed, the hypothesis seemed reasonable.

Though this hypothesis has undergone several important changes of emphasis, pulmonary hypertension is still considered to be a disease in which 5-HT plays an important role and which therefore may become a target for novel drug development. The interested reader is referred to MacLean & Dempsie (2010) for an accessible account of the current thinking in this area, and to Chapter 22, where this topic is also covered.

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