22 Cholinergics, anticholinergics, and anticholinesterases

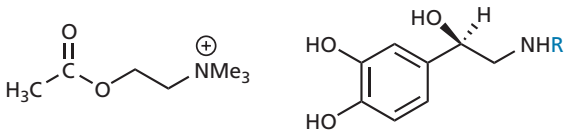
In this chapter, we shall concentrate on drugs that have an effect on the cholinergic nervous system. There are several clinically important drugs in this category which act in the peripheral and/or the central nervous system.

22.1 The peripheral nervous system

The peripheral nervous system (PNS) is so called because it is peripheral to the central nervous system (CNS; the brain and spinal column). There are many divisions and subdivisions of the peripheral system that can lead to confusion. The first distinction to make is between **sensory** and **motor nerves**:

- sensory nerves take messages from the body to the CNS;
- motor nerves carry messages from the CNS to the rest of the body.

An individual nerve cell is called a **neuron** (Appendix 4) and neurons must communicate with each other in order to relay messages. However, neurons are not physically connected. Instead, there are gaps which are called **synapses** (Fig. 22.1). If a neuron is to communicate its message to another neuron (or a target organ), it can only do so by releasing a chemical that crosses the synaptic gap and binds to receptors on the target cell. This interaction between neurotransmitter and receptor can then stimulate other processes, which, in the case of a second neuron, continues the message. As these chemicals effectively carry a message from a neuron, they are known as chemical messengers or **neurotransmitters**. There are a large number of neurotransmitters in the body, but the important ones in the peripheral nervous system are **acetylcholine** and **noradrenaline** (Fig. 22.2). The very fact that neurotransmitters are chemicals allows the medicinal chemist to design and synthesize organic compounds which can mimic (**agonists**) or block **(antagonists)** their action.



Acetylcholine

R = H Noradrenaline
R = Me Adrenaline

FIGURE 22.2 Acetylcholine, noradrenaline, and adrenaline.

22.2 Motor nerves of the PNS

In this chapter, we are concerned primarily with drugs that influence the activity of motor nerves. Motor nerves take messages from the CNS to various parts of the body, such as skeletal muscle, smooth muscle, cardiac muscle, and glands (Figs 4.1 and 22.3). The message travelling along a single neuron is often compared to an electrical pulse, but the analogy with electricity should not be taken too far as the pulse is a result of ion flow across the

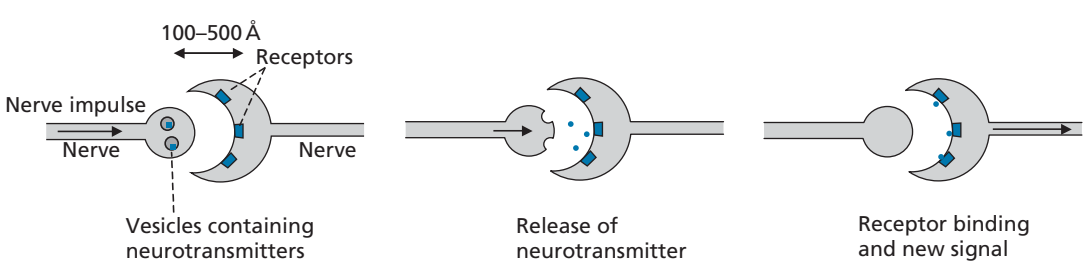


FIGURE 22.1 Signal transmission at a synapse.

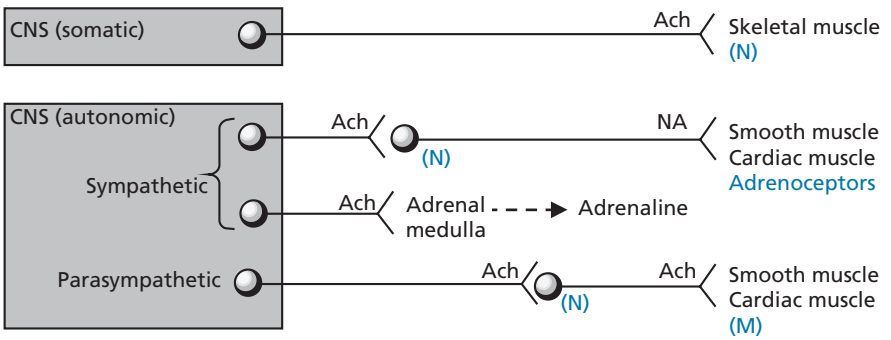


FIGURE 22.3 Motor nerves of the peripheral nervous system. N = nicotinic receptor; M = musarinic receptor; AcH = acetylcholine; NA = noradrenaline.

membranes of neurons and not a flow of electrons (see Appendix 4).

It should be evident that the workings of the human body depend crucially on an effective motor nervous system. Without it, we would not be able to operate our muscles and we would end up as flabby blobs, unable to move or breathe. We would not be able to eat, digest, or excrete our food because the smooth muscle activity of the gastrointestinal tract (GIT) and the urinary tract is controlled by motor nerves. We would not be able to control body temperature, as the smooth muscle controlling the diameter of our peripheral blood vessels would cease to function. Finally, our heart would resemble a wobbly jelly rather than a powerful pump. In short, if the motor nerves failed to function, we would be in a mess! Let us now look at the motor nerves in more detail.

The motor nerves of the PNS have been classified into three subsystems: the **somatic motor nervous system**, the **autonomic motor nervous system**, and the **enteric nervous system**. These are considered in the following sections.

22.2.1 The somatic motor nervous system

The somatic motor nerves carry messages from the CNS to the skeletal muscles. There are no synapses en route and the neurotransmitter at the neuromuscular junction is **acetylcholine**. Acetylcholine binds to cholinergic receptors within the cell membranes of muscle cells and the final result is contraction of skeletal muscle.

22.2.2 The autonomic motor nervous system

The autonomic motor nerves carry messages from the CNS to smooth muscle, cardiac muscle, and the adrenal medulla. This system can be divided into the **sympathetic** and **parasympathetic** nervous systems.

Sympathetic neurons leave the CNS and synapse almost immediately with a second neuron using acetylcholine as neurotransmitter. The second neuron then proceeds to various tissues and organs around the body. **Noradrenaline** is the neurotransmitter released from the second neuron, and this interacts with adrenergic receptors present in target cells and organs. At the heart, the action of noradrenaline leads to contraction of cardiac muscle and an increase in heart rate. Elsewhere, it relaxes smooth muscle and reduces the contractions of the gastrointestinal and urinary tracts. It also reduces salivation and the dilatation of the peripheral blood vessels. In general, the sympathetic nervous system promotes the '**fight or flight**' response by shutting down the body's housekeeping roles (digestion, defecation, urination, etc.), while stimulating the heart.

There are some neurons in the sympathetic nervous system which do not synapse with a second neuron, but go directly to a gland called the **adrenal medulla**. Acetylcholine is the neurotransmitter released by these neurons and it stimulates the adrenal medulla to release the hormone **adrenaline**, which then circulates through the blood system. **Adrenaline** reinforces the actions of noradrenaline by activating adrenergic receptors throughout the body, whether they are supplied directly with nerves or not.

Parasympathetic neurons leave the CNS, travel some distance, then synapse with a second neuron using acetylcholine as neurotransmitter. The second neuron then proceeds to synapse with the same target tissues and organs as the sympathetic neurons. However, acetylcholine acts as the neurotransmitter, rather than noradrenaline, and activates cholinergic receptors on the target cells. The resulting effects are the opposite to those caused by activation of adrenergic receptors. For

example, cardiac muscle is relaxed, whereas the smooth muscle of the digestive and urinary tracts is contracted.

As the sympathetic and parasympathetic nervous systems oppose each other in their actions, they can be looked upon as acting like a brake and an accelerator on the different tissues and organs around the body. The analogy is not quite apt because both systems are always operating and the overall result depends on which effect is the stronger.

22.2.3 The enteric system

The third constituent of the PNS is the enteric system, which is located in the walls of the GIT. It receives messages from **sympathetic** and **parasympathetic nerves**, but it also responds to local effects to provide local reflex pathways which are important in the control of GIT function. A large variety of neurotransmitters are involved including **serotonin**, **neuropeptides**, and **ATP**. **Nitric oxide** (**NO**) is also involved as a chemical messenger.

22.2.4 Defects in motor nerve transmission

Defects in motor nerve transmission would clearly lead to a large variety of ailments involving the heart, skeletal muscle, GIT, urinary tract, and many other organs. Such defects might be the result of either a deficit or an excess of neurotransmitter. Therefore, treatment involves the administration of drugs which can act as agonists or antagonists, depending on the problem. There is a difficulty with this approach, however. Usually, the problem we wish to tackle occurs at a certain location where there might, for example, be a lack of neurotransmitter. Application of an agonist to make up for low levels of neurotransmitter at the heart might solve the problem there, but would lead to problems elsewhere in the body where the levels of neurotransmitter would be normal. At those areas, the agonist would cause too much activity and cause unwanted side effects. Therefore, drugs showing selectivity for different parts of the body would, clearly, be preferred. This selectivity has been achieved to a great extent with both cholinergic and adrenergic agents. In this chapter, we concentrate on cholinergic agents (adrenergic agents are covered in Chapter 23).

22.3 The cholinergic system

22.3.1 The cholinergic signalling system

Let us look first at what happens at synapses involving acetylcholine as the neurotransmitter. Figure 22.4 shows the synapse between two neurons and the events involved when a message is transmitted from one neuron to another. The same general process takes place when a message is passed from a neuron to a muscle cell.

1. The first stage involves the biosynthesis of acetylcholine (Fig. 22.5). Acetylcholine is synthesized from **choline** and **acetyl coenzyme A** at the end of the presynaptic neuron. The reaction is catalysed by the enzyme **choline acetyltransferase**.
2. Acetylcholine is incorporated into membrane-bound vesicles by means of a specific transport protein.
3. The arrival of a nerve signal leads to an opening of calcium ion channels and an increase in intracellular calcium concentration. This induces the vesicles to fuse with the cell membrane and release the transmitter into the synaptic gap.
4. Acetylcholine crosses the synaptic gap and binds to the cholinergic receptor, resulting in stimulation of the second neuron.
5. Acetylcholine moves to an enzyme called **acetylcholinesterase**, which is situated on the postsynaptic neuron, and which catalyses the hydrolysis of acetylcholine to produce choline and acetic acid (ethanoic acid).
6. Choline is taken up into the presynaptic neuron by a transport protein to continue the cycle.

The most important thing to note is that there are several stages where it is possible to use drugs to either promote or inhibit the overall process. The greatest success so far has been with drugs targeted at stages 4 and 5 (i.e. the cholinergic receptor and the acetylcholinesterase enzyme). These are considered in more detail in subsequent sections.

22.3.2 Presynaptic control systems

Cholinergic receptors (called **autoreceptors**) are present at the terminus of the presynaptic neuron (Fig. 22.6). The purpose of these receptors is to provide a means of local control over nerve transmission. When acetylcholine is released from the neuron, some of it will find its way to these autoreceptors and switch them on. This has the effect of inhibiting further release of acetylcholine.

The presynaptic neuron also contains receptors for **noradrenaline**, which act as another control system for acetylcholine release. Branches from the sympathetic nervous system lead to the cholinergic synapses and when the sympathetic nervous system is active, noradrenaline is released and binds to these receptors. Once again, the effect is to inhibit acetylcholine release. This indirectly enhances the activity of noradrenaline at target organs by lowering cholinergic activity.

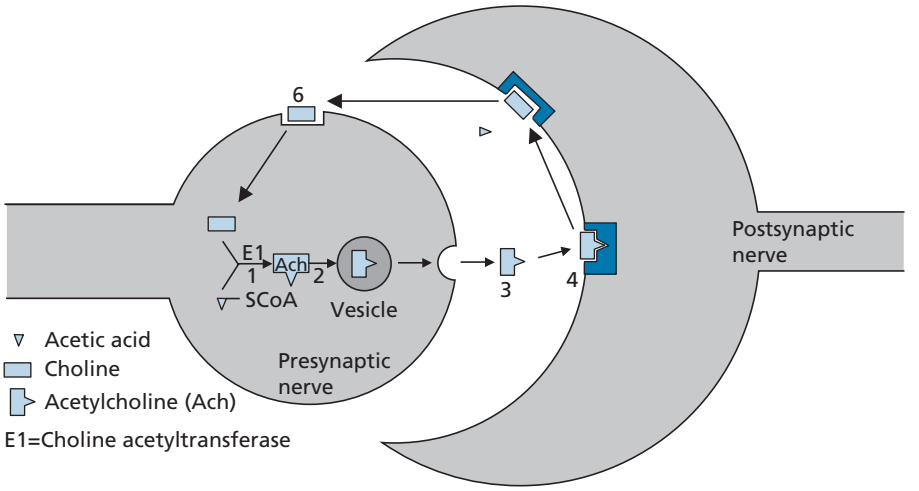


FIGURE 22.4 Synapse with acetylcholine acting as the neurotransmitter.

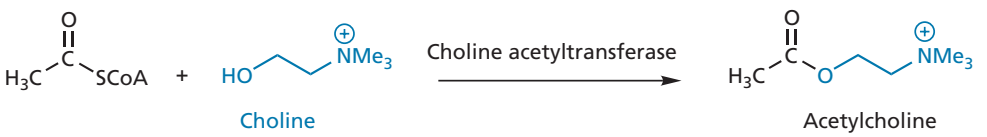


FIGURE 22.5 Biosynthesis of acetylcholine.

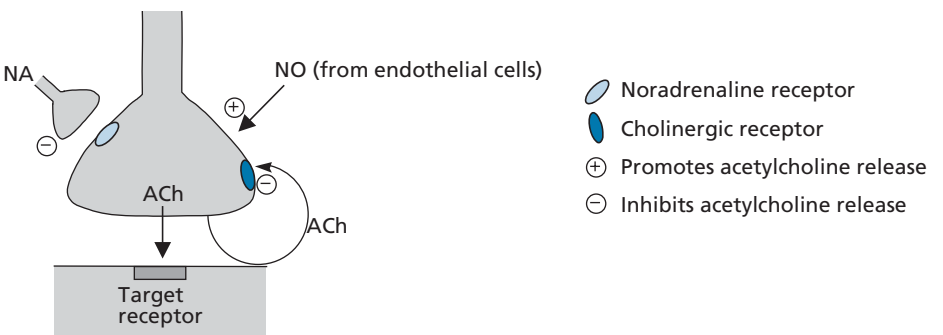


FIGURE 22.6 Presynaptic control systems.

The chemical messenger **nitric oxide** (**NO**) can also influence acetylcholine release, but, in this case, it promotes release. A large variety of other chemical messengers including **co-transmitters** (see below) are also implicated in presynaptic control. The important thing to appreciate is that presynaptic receptors offer another possible drug target to influence the cholinergic nervous system.

22.3.3 Co-transmitters

Co-transmitters are messenger molecules released along with acetylcholine. The particular co-transmitter released depends on the location and target cell of the neurons. Each co-transmitter interacts with its own receptor on the postsynaptic cell. Co-transmitters have a variety of structures and include peptides, such as **vasoactive intestinal peptide** (VIP), **gonadotrophin-releasing hormone** (GnRH), and **substance P**. The roles of these agents appear to be as follows:

- they are longer-lasting and reach more distant targets than acetylcholine, resulting in longer-lasting effects;
- the balance of co-transmitters released varies under different circumstances (e.g. presynaptic control) and so can produce different effects.

22.4 Agonists at the cholinergic receptor

One point might have occurred to you. If there is a lack of acetylcholine acting at a certain part of the body, why not just administer more acetylcholine? After all, it is easy enough to make in the laboratory (Fig. 22.7).

There are three reasons why this is not feasible.

- Acetylcholine is easily hydrolysed in the stomach by acid catalysis and cannot be given orally.
- Acetylcholine is easily hydrolysed in the blood by esterase enzymes (esterases).
- There is no selectivity of action. Additional acetylcholine will switch on all cholinergic receptors in the body.

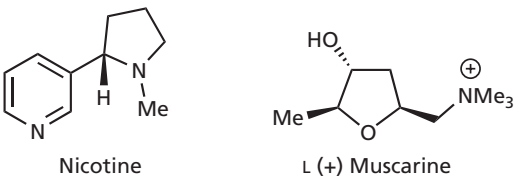
Therefore, we need analogues of acetylcholine that are more stable to hydrolysis and more selective with respect to where they act in the body. We shall look at selectivity first.

There are two ways in which selectivity can be achieved. Firstly, some drugs may be distributed more efficiently to one part of the body than another. Secondly, there are different types of cholinergic receptor, which vary in the way they are distributed in tissues. It is possible to design synthetic agents that show selectivity for these receptors and, hence, have tissue selectivity.

This is not just a peculiarity of cholinergic receptors. Differences have been observed for other types of receptors, such as those for dopamine, noradrenaline, and serotonin, and there are many types and subtypes of receptor for each chemical messenger (see Chapter 4).

The first indications that different types of cholinergic receptor existed came from the action of natural compounds. It was discovered that the compounds **nicotine** (present in tobacco) and **muscarine** (the active principle of a poisonous mushroom) (Fig. 22.8) were both cholinergic agonists, but that they had different physiological effects.

Nicotine showed selectivity for cholinergic receptors present on skeletal muscle or at the synapses between different neurons, whereas muscarine showed selectivity for cholinergic receptors present on smooth muscle and cardiac muscle. From these results, it was concluded that there was one type of cholinergic receptor on skeletal muscles and at nerve synapses (the **nicotinic receptor**), and a different type of cholinergic receptor on smooth muscle and cardiac muscle (the **muscarinic receptor**) (Fig. 22.3).



Nicotine L (+) Muscarine

FIGURE 22.8 Nicotine and muscarine.

Muscarine and nicotine were the first compounds to indicate that receptor selectivity was possible, but they are unsuitable as medicines because they have undesirable side effects resulting from their interactions with other receptors. In the search for a good drug it is important to gain selectivity for one class of receptor over another (e.g. the cholinergic receptor in preference to an adrenergic receptor) and selectivity between receptor types (e.g. the muscarinic receptor in preference to a nicotinic receptor). It is also preferable to gain selectivity for particular subtypes of a receptor. For example, not every muscarinic receptor is the same throughout the body. At present, five subtypes of the muscarinic receptor have been discovered (M1–M5) and ten subtypes of the nicotinic receptor (α1–α10).

The principle of selectivity was proven with nicotine and muscarine, and so the race was on to design novel drugs which had the selectivity of nicotine or muscarine, but not the side effects.

KEY POINTS

- The cholinergic nervous system involves nerves which use the neurotransmitter acetylcholine as a chemical messenger. These include the motor nerves which innervate skeletal muscle, nerves which synapse with other nerves in the peripheral nervous system (PNS), and the parasympathetic nerves innervating cardiac and smooth muscle.
- There are two types of cholinergic receptor. Muscarinic receptors are present in smooth and cardiac muscle. Nicotinic receptors are present in skeletal muscle and in synapses between neurons.
- Acetylcholine is hydrolysed by the enzyme acetylcholinesterase when it departs the cholinergic receptor. The hydrolytic product choline is taken up into presynaptic neurons and

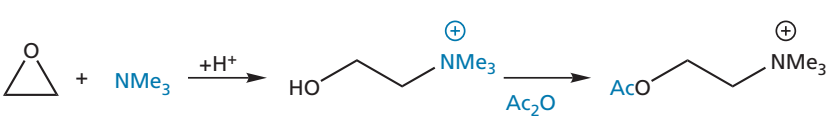


FIGURE 22.7 Synthesis of acetylcholine

acetylated back to acetylcholine. The cholinergic receptor and the enzyme acetylcholinesterase are useful drug targets.

- Acetylcholine cannot be used as a drug, because it is rapidly hydrolysed by acid and enzymes. It shows no selectivity for different types and subtypes of cholinergic receptor.

22.5 Acetylcholine: structure, structure–activity relationships, and receptor binding

The first stage in any drug development is to study the lead compound and to find out which parts of the molecule are important to activity so that they can be retained in future analogues [i.e. structure–activity relationships (SARs)]. These results also provide information about what the binding site of the cholinergic receptor looks like and help decide what changes are worth making in new analogues.

In this case, the lead compound is acetylcholine itself. The results described below are valid for both the nicotinic and muscarinic receptors, and were obtained by the synthesis of a large range of analogues.

- The positively charged nitrogen atom is essential to activity. Replacing it with a neutral carbon atom eliminates activity.
- The distance from the nitrogen to the ester group is important.
- The ester functional group is important.
- The overall size of the molecule cannot be altered much. Bigger molecules have poorer activity.
- The ethylene bridge between the ester and the nitrogen atom cannot be extended (Fig. 22.9).
- There must be two methyl groups on the nitrogen. A larger, third alkyl group is tolerated, but more than one large alkyl group leads to loss of activity.
- Bigger ester groups lead to a loss of activity.

Clearly, there is a tight fit between acetylcholine and its binding site, which leaves little scope for variation. The

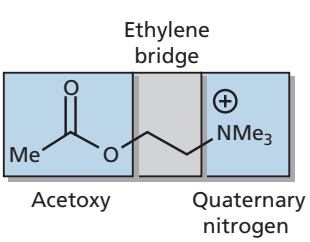


FIGURE 22.9 Acetylcholine.

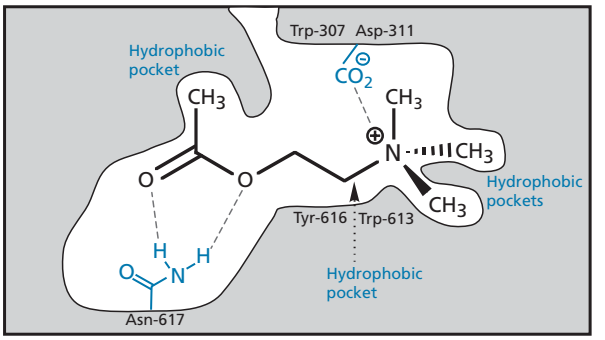


FIGURE 22.10 Muscarinic receptor binding site.

findings listed tally with a receptor binding site as shown in Fig. 22.10.

It is proposed that important hydrogen bonding interactions exist between the ester group of acetylcholine and an asparagine residue. It is also thought that a small hydrophobic pocket exists which can accommodate the methyl group of the ester, but nothing larger. This interaction is thought to be more important in the muscarinic receptor than the nicotinic receptor.

The evidence suggests that the NMe_3^+ group is placed in a hydrophobic pocket lined with three aromatic amino acids. It is also thought that the pocket contains two smaller hydrophobic pockets, which are large enough to accommodate two of the three methyl substituents on the NMe_3^+ group. The third methyl substituent on the nitrogen is positioned in an open region of the binding site and so it is possible to replace it with other groups. A strong ionic interaction has been proposed between the charged nitrogen atom and the anionic side group of an aspartate residue. The existence of this ionic interaction represents the classical view of the cholinergic receptor, but there is an alternative suggestion which states that there may be an induced dipole interaction between the NMe_3^+ group and the aromatic residues in the hydrophobic pocket.

There are several reasons for this. Firstly, the positive charge on the NMe_3^+ group is not localized on the nitrogen atom, but is spread over the three methyl groups (compare section 17.7.1). Such a diffuse charge is less likely to be involved in a localized ionic interaction and it has been shown by model studies that NMe_3^+ groups can be stabilized by binding to aromatic rings. It might seem strange that a hydrophobic aromatic ring should be capable of stabilizing a positively charged group, but it has to be remembered that aromatic rings are electron-rich, as shown by the fact they can undergo reaction with electrophiles. It is thought that the diffuse positive charge on the NMe_3^+ group is capable of distorting the π electron cloud of aromatic rings to induce a dipole moment (section 1.3.4). Induced ion–dipole interactions between the NMe_3^+ group and an aromatic residue such as tyrosine would then account for

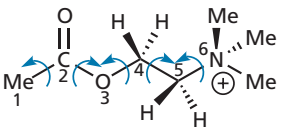


FIGURE 22.11 Bond rotations in acetylcholine leading to different conformations.

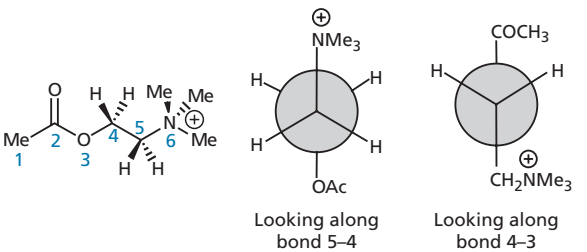


FIGURE 22.12 The sawhorse and Newman projections of acetylcholine.

the binding. The fact that three aromatic amino acids are present in the pocket adds weight to the argument.

Of course, it is possible that both types of binding interactions are taking place, which will please both parties!

A large amount of effort has been expended trying to identify the active conformation of acetylcholine, i.e. the shape adopted by the neurotransmitter when it binds to the cholinergic receptor. This has been no easy task, as acetylcholine is a highly flexible molecule (Fig. 22.11) where bond rotation along the length of its chain can lead to many possible stable conformations (or shapes).

In the past, it was assumed that a flexible neurotransmitter would adopt its most stable conformation when binding. In the case of acetylcholine, that would be the conformation represented by the sawhorse and Newman projections shown in Fig. 22.12. However, there is not a massive energy difference between alternative stable conformations such as the gauche conformation shown in Figure 22.13. The stabilization energy gained from binding interactions within the binding site could more than compensate for any energy penalties involved in adopting a slightly less stable conformation.

In order to try and establish the active conformation of acetylcholine, rigid cyclic molecules have been studied which contain the skeleton of acetylcholine within their structure; for example muscarine and the analogues shown in Fig. 22.14. In these structures, the portion of the acetylcholine skeleton which is included in a ring is locked into a particular conformation because bonds within rings cannot rotate freely. If such molecules bind to the cholinergic receptor, this indicates that this particular conformation is 'allowed' for activity.

Test your understanding and practise your molecular modelling with Exercise 22.2.

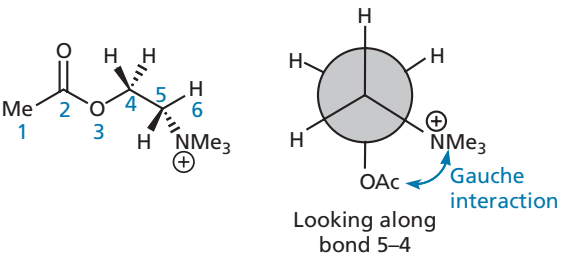


FIGURE 22.13 A gauche conformation for acetylcholine.

Many such structures have been prepared, but it has not been possible to identify one *specific* active conformation for acetylcholine. This probably indicates that the cholinergic receptor has a certain amount of latitude and can recognize the acetylcholine skeleton within the rigid analogues, even when it is not in the ideal active conformation. Nevertheless, such studies have shown that the separation between the ester group and the quaternary nitrogen is important for binding, and that this distance differs for the muscarinic and the nicotinic receptor (Fig. 22.15).

Having identified the binding interactions and pharmacophore of acetylcholine, we shall now look at how acetylcholine analogues were designed with improved stability.

Test your understanding and practise your molecular modelling with Exercises 22.1 and 22.2.

22.6 The instability of acetylcholine

As described previously, acetylcholine is prone to hydrolysis. This is explained by considering one of the conformations that the molecule can adopt (Fig. 22.16). In this conformation, the positively charged nitrogen interacts with the carbonyl oxygen and has an electron-withdrawing effect. To compensate, the oxygen atom pulls electrons from the neighbouring carbon atom and makes that carbon atom electron deficient and more prone to nucleophilic attack. Water is a poor nucleophile, but, because the carbonyl group is more electrophilic, hydrolysis takes place relatively easily. This influence of the nitrogen ion is known as **neighbouring group participation** or **anchimeric assistance**.

We shall now look at how the problem of hydrolysis was overcome, but it should be appreciated that we are doing this with the benefit of hindsight. At the time the problem was tackled the SAR studies were incomplete and the format of the cholinergic receptor binding site was unknown.

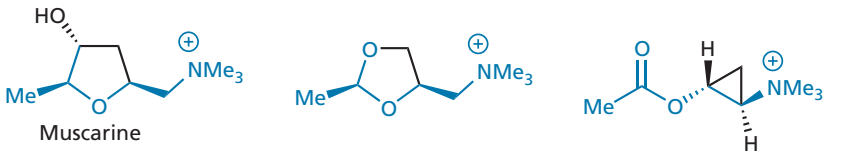


FIGURE 22.14 Rigid molecules incorporating the acetylcholine skeleton (C–C–O–C–C–N).

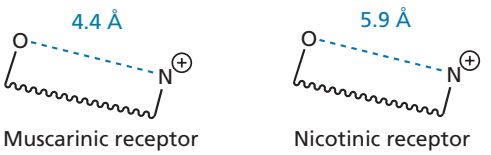


FIGURE 22.15 Pharmacophore of acetylcholine.

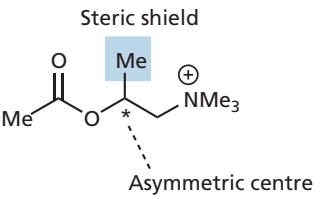


FIGURE 22.17 Methacholine (racemic mixture).

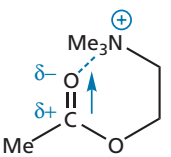


FIGURE 22.16 Neighbouring group participation. The arrow indicates the inductive pull of oxygen which increases the electrophilicity of the carbonyl carbon (see Molecular modelling exercise 22.1).

22.7 Design of acetylcholine analogues

There are two possible approaches to tackling the inherent instability of acetylcholine: steric shields and electronic stabilization.

22.7.1 Steric shields

The principle of steric shields was described in section 14.2.1 and can be demonstrated with **methacholine** (Fig. 22.17). Here, an extra methyl group has been placed on the ethylene bridge as a steric shield to protect the carbonyl group. The shield hinders the approach of any potential nucleophile and also hinders binding to esterase enzymes, thus slowing down chemical and enzymatic hydrolysis. As a result, methacholine is three times more stable to hydrolysis than acetylcholine.

The obvious question now is why not put on a bigger alkyl group like an ethyl group or a propyl group? Alternatively, why not put a bulky group on the acyl half of the molecule, as this would be closer to the carbonyl centre and have a greater shielding effect?

In fact, these approaches were tried. They certainly increased stability but they lowered cholinergic activity. We should already know why—the fit between acetylcholine and its receptor is so tight that there is little scope for enlarging the molecule. The extra methyl group is acceptable, but larger substituents hinder the molecule binding to the cholinergic receptor and decrease its activity.

Introducing a methyl steric shield has another useful effect. It was discovered that methacholine has significant muscarinic activity, but very little nicotinic activity. Therefore, methacholine shows good selectivity for the muscarinic receptor. This is perhaps more important than the gain in stability.

Selectivity for the muscarinic receptor can be explained if we compare the proposed active conformation of methacholine with muscarine (Fig. 22.18), as the methyl group of methacholine occupies the same position as a methylene group in muscarine. This is only possible for the *S*-enantiomer of methacholine and when the two enantiomers of methacholine were separated, it was found that the *S*-enantiomer was, indeed, the more active enantiomer. It is not used therapeutically, however.

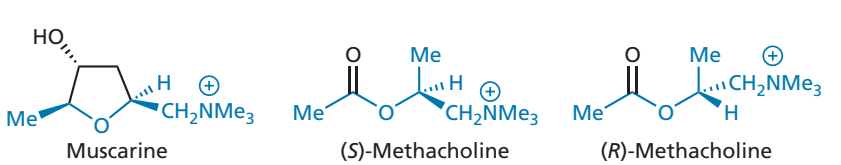


FIGURE 22.18 Comparison of muscarine and the *R*- and *S*-enantiomers of methacholine.

22.7.2 Electronic effects

The use of electronic factors to stabilize functional groups was described in sections 14.2.2 and 14.2.3, and was used in the design of **carbachol** (Fig. 22.19)—a long-acting cholinergic agent which is resistant to hydrolysis. Here, the acyl methyl group has been replaced by NH_2 which means that the ester has been replaced by a urethane or carbamate group. This functional group is more resistant to hydrolysis because the lone pair of electrons on nitrogen can interact with the neighbouring carbonyl group and lower its electrophilic character (Fig. 22.20).

The tactic worked, but it was by no means a foregone conclusion that it would. Although the NH_2 group is equivalent in size to the methyl group, the former is polar and the latter is hydrophobic, and it was by no means certain that a polar NH_2 group would be accepted into a hydrophobic pocket in the binding site. Fortunately, it is and activity is retained, which means that the amino group acts as a **bioisostere** for the methyl group. A bioisostere is a group which can replace another group without affecting the pharmacological activity of interest (sections 13.3.7 and 14.2.2). Thus, the amino group is a bioisostere for the methyl group as far as the cholinergic receptor is concerned, but not as far as the esterase enzymes are concerned.

The inclusion of the electron-donating amino group greatly increases chemical and enzymatic stability. Unfortunately, carbachol shows very little selectivity between the muscarinic and nicotinic receptors. Nevertheless, it is used clinically for the treatment of glaucoma where it can be applied locally, thus avoiding the problems of receptor selectivity. Glaucoma arises when the aqueous contents of the eye cannot be drained. This raises the pressure on the eye and can lead to blindness. Agonists cause the eye muscles to contract and allow drainage, thus relieving the pressure.

22.7.3 Combining steric and electronic effects

We have seen that the β-methyl group of methacholine increases stability and introduces receptor selectivity. Therefore, it made sense to add a β-methyl group to carbachol. The resulting compound is **bethanechol** (Fig. 22.21) which is both stable to hydrolysis and selective in its action. It is occasionally used therapeutically in stimulating the GIT and urinary bladder after surgery.

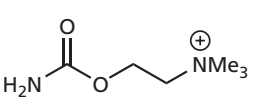


FIGURE 22.19 Carbachol.

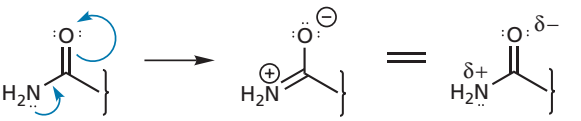
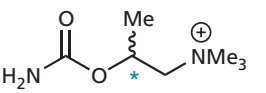


FIGURE 22.20 Resonance structures of carbachol.



* Asymmetric centre

FIGURE 22.21 Bethanechol.

Both these organs are 'shut down' with drugs during surgery (section 22.9).

22.8 Clinical uses for cholinergic agonists

22.8.1 Muscarinic agonists

A possible future use for muscarinic agonists is in the treatment of Alzheimer's disease. However, current clinical uses include:

- treatment of glaucoma;
- 'switching on' the GIT and urinary tract after surgery;
- treatment of certain heart defects by decreasing heart muscle activity and heart rate.

Pilocarpine (Fig. 22.22) is an example of a muscarinic agonist which is used in the treatment of glaucoma. It is an alkaloid obtained from the leaves of shrubs belonging to the genus *Pilocarpus*. Although there is no quaternary ammonium group present in pilocarpine, it is assumed that the drug is protonated before it interacts with the muscarinic receptor. Molecular modelling shows that pilocarpine can adopt a conformation having the correct pharmacophore for the muscarine receptor; i.e. a separation between nitrogen and oxygen of 4.4 Å.

Pilocarpine is also being considered for the treatment of Alzheimer's disease, as are other muscarinic agonists such as **oxotremorine** and various **arecoline** analogues (Fig. 22.22). At present, anticholinesterases are used clinically for the treatment of this disease (section 22.15).

22.8.2 Nicotinic agonists

Nicotinic agonists are used in the treatment of myasthenia gravis. This is an autoimmune disease where the body has produced antibodies against its own cholinergic

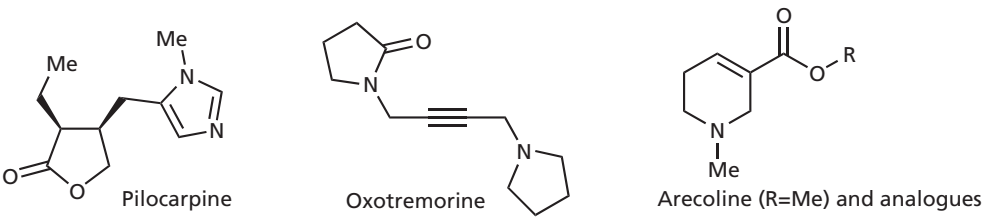


FIGURE 22.22 Examples of muscarinic agonists.

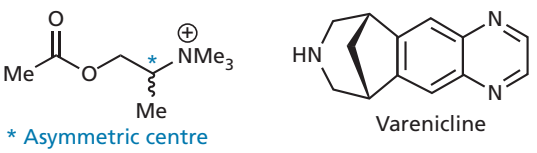


FIGURE 22.23 Examples of selective nicotinic agonists.

receptors. As a result, the number of available receptors drops and so fewer messages reach the muscle cells. In turn, this leads to severe muscle weakness and fatigue. Administering an agonist increases the chance of activating what few receptors remain. An example of a selective nicotinic agonist is the first structure shown in Fig. 22.23. This agent is very similar in structure to methacholine, and differs only in the position of the methyl substituent. This is sufficient, however, to completely alter receptor selectivity. Despite that, this particular compound is not used clinically and anticholinesterases (section 22.15.1.2) are the preferred treatment. **Varenicline** *is* used clinically, however. It is a partial agonist at nicotinic receptors and was approved in 2006 as an aid to stop smoking.

KEY POINTS

- Acetylcholine fits snugly into the binding site of cholinergic receptors and there is little scope for variation. Two of the *N*-methyl groups and the acyl methyl group fit into hydrophobic pockets. The ester is involved in hydrogen bonding, and the quaternary nitrogen is involved in ionic interactions and/or induced dipole interactions.
- Rigid analogues of acetylcholine have been used to try and identify the active conformation.
- Acetylcholine is unstable to acid because of neighbouring group participation. Stable analogues have been designed using steric shields and/or electronic effects.

22.9 Antagonists of the muscarinic cholinergic receptor

22.9.1 Actions and uses of muscarinic antagonists

Antagonists of the cholinergic receptor are drugs which bind to the receptor but do not 'switch it on'. By binding to the receptor, an antagonist acts like a plug at the receptor binding site and prevents acetylcholine from binding (Fig. 22.24). The overall effect on the body is the same as if there was a lack of acetylcholine. Therefore, antagonists have the opposite clinical effect from agonists.

The antagonists described in this section act only at the muscarinic receptor and therefore affect nerve transmissions to glands, the CNS, and the smooth muscle of the GIT and urinary tract. The clinical effects and uses of these antagonists reflect this.

The clinical effects of muscarinic antagonists are:

- reduced saliva and gastric secretions;
- reduced motility of the GIT and urinary tract by relaxation of smooth muscle;
- dilatation of eye pupils;
- CNS effects

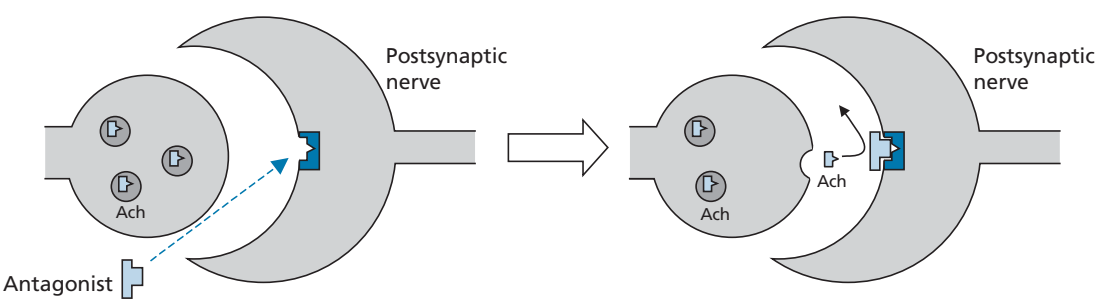


FIGURE 22.24 Action of an antagonist to block a receptor.

The clinical uses are:

- shutting down the GIT and urinary tract during surgery;
- ophthalmic examinations;
- relief of peptic ulcers;
- treatment of Parkinson's disease;
- treatment of anticholinesterase poisoning;
- treatment of motion sickness;
- a potential use for M2 antagonists is in the treatment of Alzheimer's disease.

22.9.2 Muscarinic antagonists

The first antagonists to be discovered were natural products—in particular alkaloids (nitrogen-containing compounds derived from plants).

22.9.2.1 Atropine and hyoscine

Atropine (Fig. 22.25) is present in the roots of *Atropa belladonna* (deadly nightshade) and is included in a root extract which was once used by Italian women to dilate their eye pupils. This was considered to enhance beauty, hence the name belladonna. Clinically, atropine has been used to decrease gastrointestinal motility and to counteract anticholinesterase poisoning.

Atropine has an asymmetric centre but exists as a racemate. Usually, natural products exist exclusively as one enantiomer. This is also true for atropine, which is present in the plants of the genus Solanaceae as a single enantiomer called **hyoscyamine**. As soon as the natural product is extracted into solution, however, racemization takes place. The asymmetric centre in atropine is easily racemized as it is next to a carbonyl group and an aromatic ring. This makes the proton attached to the asymmetric centre acidic and easily removed.

Hyoscine (or scopolamine) (Fig. 22.25) is obtained from the thorn apple (*Datura stramonium*) and is very similar in structure to atropine. It has been used in the treatment of motion sickness.

These two compounds bind to the cholinergic receptor, but, at first sight, they do not look anything like acetylcholine. If we look more closely though, we can see that a basic nitrogen and an ester group are present, and if we superimpose the acetylcholine skeleton on to the atropine skeleton, the distance between the ester and the nitrogen groups is similar in both molecules (Fig. 22.26). There is, of course, the problem that the nitrogen in atropine is uncharged, whereas the nitrogen in acetylcholine has a full positive charge. This implies that the nitrogen atom in atropine must be protonated and charged when it binds to the cholinergic receptor.

Therefore, atropine has two important binding features shared with acetylcholine—a charged nitrogen when protonated and an ester group. It is able to bind to the receptor, but why is it unable to switch it on? Because atropine is a larger molecule than acetylcholine, it is capable of binding to other binding regions within the binding site which are not used by acetylcholine itself. As a result, it interacts differently with the receptor and does not induce the same conformational changes (induced fit) as acetylcholine. This means that the receptor is not activated.

Test your understanding and practise your molecular modelling with Exercise 22.3.

As both atropine and hyoscine are tertiary amines rather than quaternary salts, they are able to cross the blood–brain barrier as the free base. Once they are in the brain, they can become protonated and antagonize muscarinic receptors which causes CNS effects; for example hallucinogenic activity is brought on with high doses, and both hyoscine and atropine were used by witches in past centuries to produce that very effect. Other CNS effects observed in atropine poisoning are restlessness, agitation, and hyperactivity.

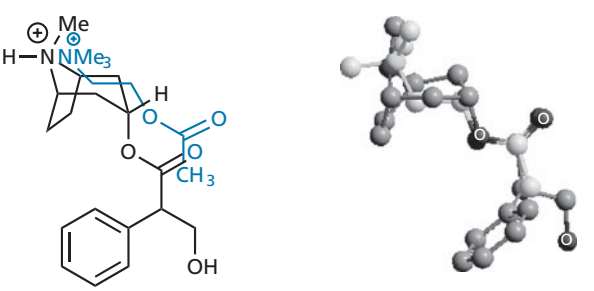


FIGURE 22.26 Acetylcholine skeleton superimposed on to the atropine skeleton.

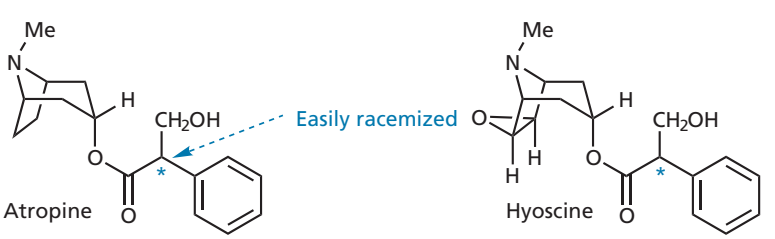


FIGURE 22.25 Atropine and hyoscine.

In recent times, the disorientating effect of scopolamine has seen it being used as a truth drug for the interrogation of spies and so it is no surprise to find it cropping up in various novels. An interesting application for scopolamine was described in Jack Higgins' novel *Day of Judgement* where it was used in association with **suxamethonium** (Fig 22.33) to torture one hapless victim. Suxamethonium was applied to the conscious victim in order to create initial convulsive muscle spasms, followed by paralysis, inability to breathe, agonizing pain, and a living impression of death. Scopolamine was then used to erase the memory of this horror, so that the impact would be just as bad when the process was repeated!

22.9.2.2 Structural analogues based on atropine

In order to reduce CNS side effects, quaternary salts of atropine and atropine analogues are used clinically (Fig. 22.27). For example, **ipratropium** is used as a bronchodilator in chronic obstructive pulmonary disease. Atropine methonitrate acts at the intestine to relieve spasm.

A large number of different analogues of atropine were synthesized to investigate the SAR of atropine, revealing the importance of the aromatic ring, the ester group, and the basic nitrogen (which is ionized).

It was further discovered that the complex ring system was not necessary for antagonist activity, so simplification could be carried out. For example, **amprotropine** (Fig. 22.28) is active and has an ester group separated from an amine by three carbon atoms.

Chain contraction to two carbon atoms can be carried out without loss of activity, and a large variety of active antagonists have been prepared having the general formula shown in Fig 22.29, for example **tridihexethyl chloride** and **propantheline bromide**.

These studies came up with the following generalizations:

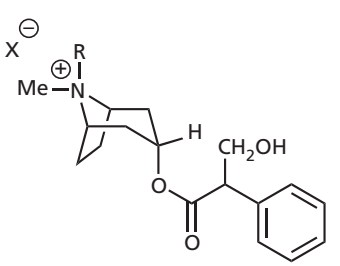
- the alkyl groups (R) on nitrogen can be larger than methyl (in contrast to agonists);
- the nitrogen can be tertiary or quaternary, whereas agonists must have a quaternary nitrogen. Note, however, that the tertiary nitrogen is probably charged when it interacts with the receptor;
- very large acyl groups are allowed (R^1 and R^2 = aromatic or heteroaromatic rings). This is in contrast to agonists where only the acetyl group is permitted.

This last point appears to be the most crucial in determining whether a compound will act as an antagonist or not. The acyl group has to be bulky, but it also has to have that bulk arranged in a certain manner; in other words, there must be some sort of branching in the acyl group.

The conclusion that can be drawn from these results is that there must be hydrophobic binding regions next to the normal acetylcholine binding site. The overall shape of the acetylcholine binding site plus the extra binding regions would have to be T- or Y-shaped in order to explain the importance of branching in antagonists (Fig. 22.30). A structure such as **propantheline**, which contains the complete acetylcholine skeleton, as well as the hydrophobic acyl side chain binds more strongly to the receptor than acetylcholine itself. The extra binding interactions mean that the conformational changes induced in the receptor will be different from those induced by acetylcholine and will fail to induce the secondary biological response. As long as the antagonist is bound, acetylcholine is unable to bind and pass on its message.

For additional material see Web article 8: photoaffinity labelling

A large variety of antagonists have proved to be useful medicines (Fig. 22.31), with many showing selectivity for specific organs. For example, **tropicamide** and **cyclopentolate** are used in eye drops to dilate pupils for ophthalmic examination, while **trihexyphenidyl and benzatropine** are used centrally to counteract movement disorders caused by Parkinson's disease. Some agents act selectively to decrease gastric secretion; others are useful in ulcer therapy. The selectivity of action for these drugs owes more to their distribution properties than to receptor selectivity. In other words, the compounds can reach some parts of the body more easily than others. Having said that, the antagonist **pirenzepine**, which is used in some countries for the treatment of peptic ulcers, is a selective M_1 antagonist with no activity against M_2 receptors.



R = Me, X = NO_3^-; Atropine methonitrate
R = ^{i}Pr, X = Br^-; Ipratropium

FIGURE 22.27 Structural analogues of atropine.

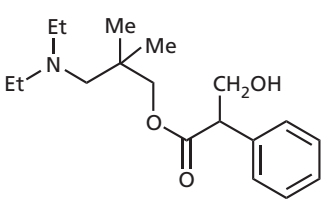
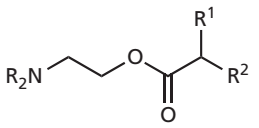
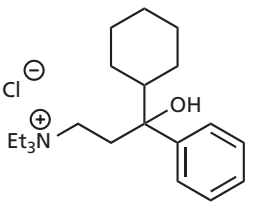


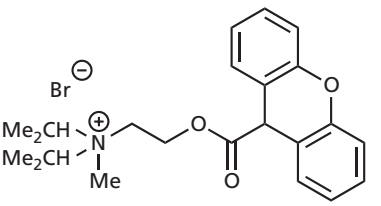
FIGURE 22.28 Amprotropine.



R^1 and R^2 = Aromatic or heteroaromatic



Tridihexethyl chloride



Propanthreline bromide

FIGURE 22.29 Simplified analogues of atropine.

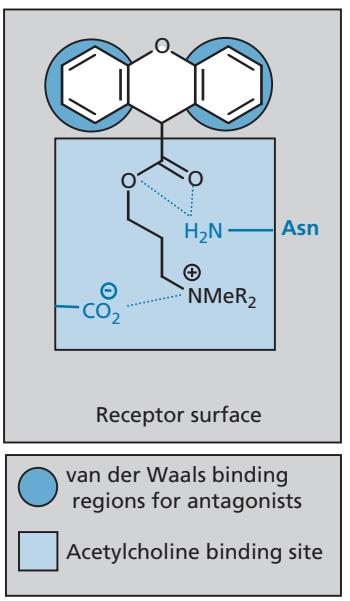
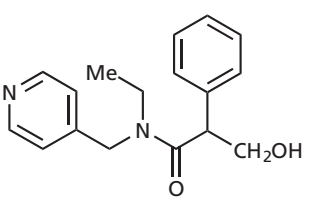


FIGURE 22.30 The binding of propantheline to the muscarinic receptor.

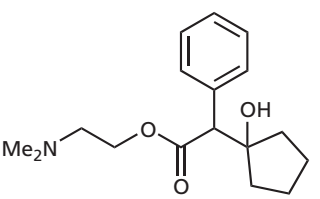
22.10 Antagonists of the nicotinic cholinergic receptor

22.10.1 Applications of nicotinic antagonists

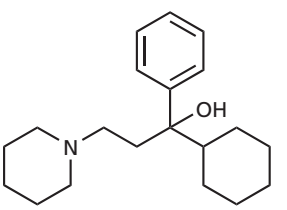
Nicotinic receptors are present in nerve synapses at ganglia, as well as at the neuromuscular synapse. However, drugs are able to show a level of selectivity between these two sites, mainly because of the distinctive routes which have to be taken to reach them. Antagonists of ganglionic nicotinic receptor sites are not therapeutically useful because they cannot distinguish between the ganglia of the sympathetic nervous system and the ganglia of the parasympathetic nervous system (both use nicotinic receptors) (Fig. 22.3). Consequently, they have many side effects. However, antagonists of the neuromuscular junction are therapeutically useful and are known as **neuromuscular blocking agents**.



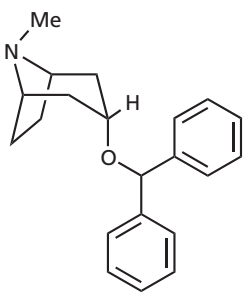
Tropicamide



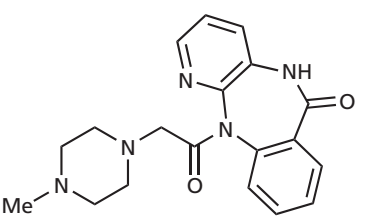
Cyclopentolate



Trihexyphenidyl



Benzatropine



Pirenzepine

FIGURE 22.31 Some examples of clinically useful cholinergic antagonists.

22.10.2 Nicotinic antagonists

22.10.2.1 Curare and tubocurarine

Curare was first identified in the sixteenth century when Spanish soldiers in South America found themselves under attack by indigenous people using poisoned arrows. It was discovered that the Indians were using a crude, dried extract from a plant called *Chondrodendron tomentosum,* which stopped the heart and also caused paralysis. Curare is a mixture of compounds, but the active principle is a cholinergic antagonist that blocks nerve transmissions from nerve to muscle.

It might seem strange to consider such a compound for medicinal use, but at the right dose levels and under proper control, there are useful applications for this sort of action. The main application is in the relaxation of abdominal muscles in preparation for surgery. This allows the surgeon to use lower levels of general anaesthetic than would otherwise be required and increase the safety margin for operations.

As mentioned previously, curare is actually a mixture of compounds, and it was not until 1935 that the active principle (**tubocurarine**) was isolated. The determination of the structure took even longer, and it was not established until 1970 (Fig. 22.32). Tubocurarine was used clinically as a neuromuscular blocker, but it had undesirable side effects as it also acted as an antagonist at the nicotinic receptors of the autonomic nervous system (Fig. 22.2). Better agents are now available.

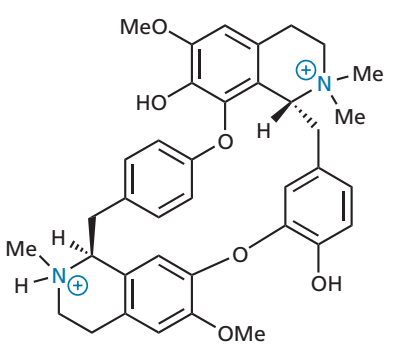


FIGURE 22.32 Tubocurarine.

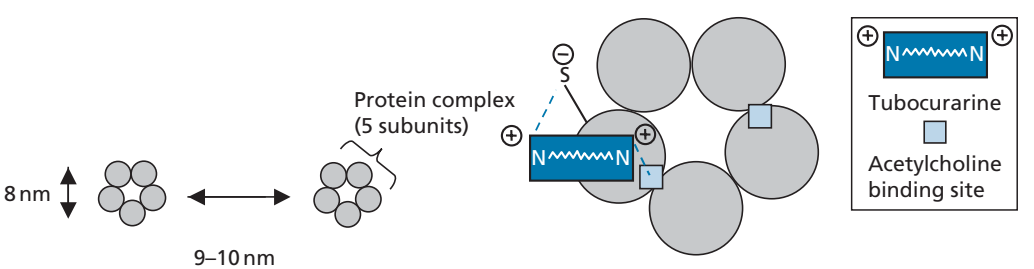
The structure of tubocurarine presents a problem to our theory of receptor binding. Although it has a couple of charged nitrogen centres, there is no ester to interact with the acetyl binding region. Studies on the compounds discussed so far show that the positively charged nitrogen on its own is not sufficient for good binding, so why should tubocurarine bind to the nicotinic receptor?

Test your understanding and practise your molecular modelling with Exercise 22.4.

The answer lies in the fact that the molecule has *two* positively charged nitrogen atoms (one tertiary, which is protonated, and one quaternary). Originally, it was believed that the distance between the two centres (1.15 nm) might be equivalent to the distance between two separate cholinergic receptors and that the tubocurarine molecule could bridge the two binding sites, and act as a steric shield for both. However pleasing that theory may be, the dimensions of the nicotinic receptor make this impossible. The nicotinic receptor is a protein dimer made up of two identical protein complexes separated by 9–10 nm—far too large to be bridged by the tubocurarine molecule (Fig. 22.33 and section 22.11).

Another possibility is that the tubocurarine molecule bridges two acetylcholine binding sites within the one protein complex. As there are two such sites within the complex, this appears to be an attractive theory. However, the two sites are more than 1.15 nm apart and so this too has to be ruled out. It has now been proposed that one of the positively charged nitrogens on tubocurarine binds to the anionic binding region of an acetylcholine binding site, while the other binds to a nearby cysteine residue 0.9–1.2 nm away (Fig. 22.33).

Despite the uncertainty surrounding the binding interactions of tubocurarine, it seems highly probable that two ionic binding regions are involved. Such an interaction is extremely strong and would more than



(a) Receptor dimer **(b)** Interaction with tubocurarine

FIGURE 22.33 Tubocurarine binding to the cholinergic receptor.

make up for the lack of the ester binding interaction. It is also clear that the distance between the two positively charged nitrogen atoms is crucial to activity. Therefore, analogues that retain this distance should also be good antagonists. Strong evidence for this comes from the fact that the simple molecule decamethonium is a good antagonist (section 22.10.2.2).

22.10.2.2 Decamethonium and suxamethonium

Decamethonium (Fig. 22.34) is as simple an analogue of tubocurarine as one could imagine. It is a flexible, straight-chain molecule and is capable of a large number of conformations. The fully extended conformation places the nitrogen atoms 1.4 nm apart, but there are other more folded conformations that position the nitrogen centres 1.14 nm apart, which compares well with the equivalent distance in tubocurarine (1.15 nm) (see also Box 17.4 and Molecular modelling exercise 22.4).

The drug binds strongly to cholinergic receptors and has proved a useful clinical agent, but it suffers from several disadvantages. For example, when it binds initially to nicotinic receptors, it acts as an agonist rather than an antagonist. In other words, it switches on receptors such that sodium ion channels open up to depolarize muscle cell membranes and cause brief contractions of the muscle. Because the drug is not rapidly hydrolysed in the same way as acetylcholine, it remains bound to the receptor leading to persistent depolarization and subsequent desensitization of the end plate. At that stage, it can be viewed as an antagonist as it no longer stimulates muscle contraction and blocks access to acetylcholine. (A theory of how such an effect might take place is described in section 8.6.) Another disadvantage is that it binds too strongly, so patients take a long time to recover from its effects.

We now face the opposite problem from the one faced when designing cholinergic agonists. Instead of stabilizing a molecule, we need to introduce some instability—a sort of timer control whereby the molecule can be inactivated more quickly. Success was first achieved with **suxamethonium** (Fig. 22.34) where two ester groups are incorporated into the chain in such a way that the distance between the charged nitrogens remains the same. The ester groups are susceptible to chemical and enzymatic hydrolysis and, once this takes place, the molecule can no longer bridge the two binding regions on the receptor and is inactivated. The ester groups are also introduced such that suxamethonium mimics two acetylcholine molecules linked end on. Suxamethonium has a fast onset and short duration of action (5–10 minutes), but suffers from various side effects. Furthermore, about one person in every 2000 lacks the plasma cholinesterase enzyme which hydrolyses suxamethonium. Nevertheless, it is still used clinically in short surgical procedures, such as the insertion of tracheal tubes.

Both decamethonium and suxamethonium are classed as depolarizing neuromuscular blockers and have effects on the autonomic ganglia, which explains some of their side effects. Decamethonium also lacks total selectivity for the neuromuscular junction and has an effect on cholinergic receptors in the heart. This leads to an increased heart rate and a fall in blood pressure.

22.10.2.3 Steroidal neuromuscular blocking agents

The design of **pancuronium**, **vecuronium**, and **rocuronium** (Fig. 22.35) was based on tubocurarine, but involved a steroid nucleus acting as a spacer between the two nitrogen groups. The distance between the quaternary nitrogens is 1.09 nm compared with 1.15 nm in tubocurarine. Acyl groups were also added to introduce one or two acetylcholine skeletons into the molecule in order to improve affinity for the receptor sites. These compounds have a faster onset of action than tubocurarine and do not affect blood pressure. They are not as rapid in onset as suxamethonium and have a longer duration of action (45 minutes). Their main advantage is that they have fewer side effects and so they are widely used clinically. Unlike decamethonium and suxamethonium, these agents have no agonist activity and act as pure antagonists, so they have no depolarizing effect on target muscle cells. The neuromuscular blocking activity of rocuronium can be reversed with a cyclodextrin called s**ugammadex** (Box 10.3).

22.10.2.4 Atracurium and mivacurium

The design of atracurium (Fig. 22.36) was based on the structures of tubocurarine and suxamethonium. It is

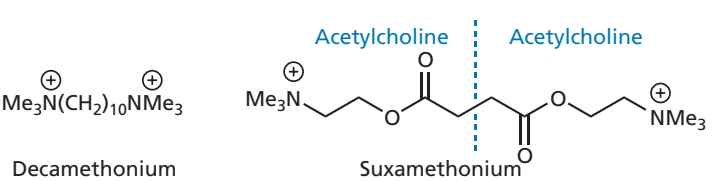


FIGURE 22.34 Decamethonium and suxamethonium.

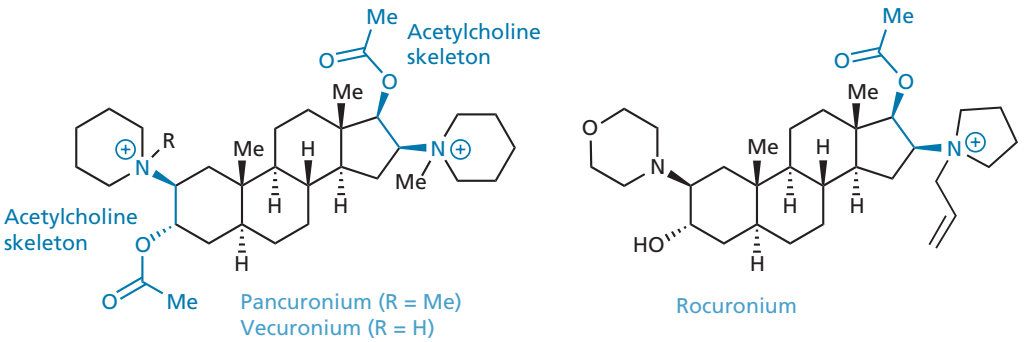


FIGURE 22.35 Steroidal neuromuscular blocking agents.

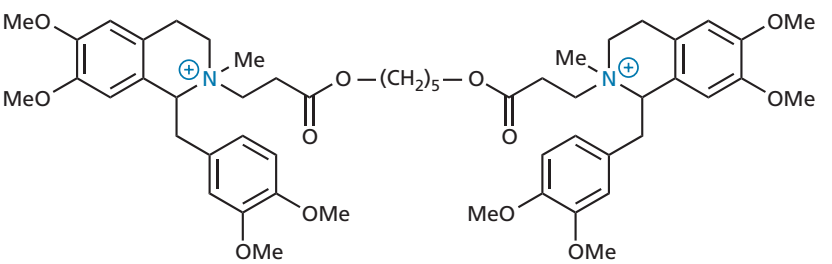


FIGURE 22.36 Atracurium.

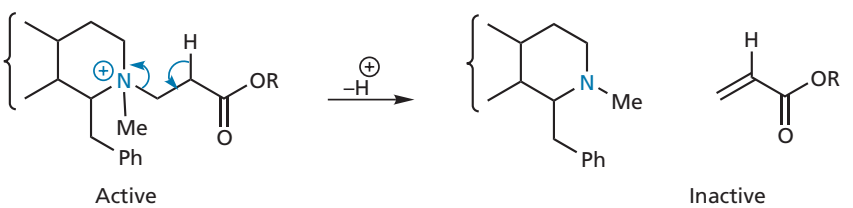


FIGURE 22.37 Hofmann elimination of atracurium.

superior to both as it lacks cardiac side effects and is rapidly broken down in blood. This rapid breakdown allows the drug to be administered as an intravenous drip.

The rapid breakdown is due to a self-destruct mechanism. At the slightly alkaline pH of blood (pH = 7.4), the molecule can undergo a **Hofmann elimination** (Fig. 22.37). Once this happens, the compound is inactivated because the positive charge on the nitrogen is lost and the molecule is split in two. It is a particularly clever example of drug design in that the very element responsible for the molecule's biological activity promotes its deactivation.

The important features of atracurium are:

- *the spacer*—a 13-atom chain connects the two quaternary centres;
- *the blocking units*—the cyclic structures at either end of the molecule which block the binding site from acetylcholine;
- *the quaternary centres*—these are essential for receptor binding. If one is lost through Hofmann elimination, the binding interaction is too weak and the antagonist leaves the binding site;
- *the Hofmann elimination*—the ester groups within the spacer chain are crucial to the rapid deactivation process. Hofmann eliminations normally require strong alkaline conditions and high temperatures—hardly normal physiological conditions. However, if a good electron-withdrawing group is present on the carbon that is *beta* to the quaternary nitrogen centre, it allows the reaction to proceed under the much milder alkaline conditions present in blood (pH 7.4). The electron-withdrawing ester group increases the acidity of the hydrogen on the *beta*-carbon such that it is easily lost. The Hofmann elimination does not occur at acid pH, and so the drug is stable in solution at a pH of 3–4 and can be stored safely in a refrigerator.

Because the drug acts very briefly (approximately 30 minutes), it is added intravenously for as long as it is needed. As soon as surgery is over, the intravenous drip is stopped and antagonism ceases almost instantaneously.

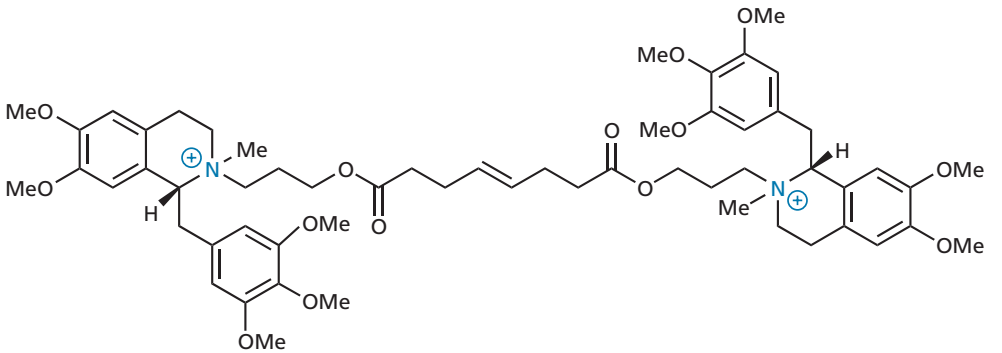


FIGURE 22.38 Mivacurium.

Another major advantage is that the drug does not require enzymes to become deactivated and so deactivation occurs at a constant rate between patients. With previous neuromuscular blockers, deactivation depended on metabolic mechanisms involving enzymic deactivation and/or excretion. The efficiency of these processes varies from patient to patient and is particularly poor for patients with kidney failure or with low levels of plasma esterases.

Mivacurium (Fig. 22.38) is a newer drug which is similar to atracurium and is inactivated rapidly by plasma enzymes, as well as by the Hofmann elimination. It has a faster onset (about 2 minutes) and shorter duration of action (about 15 minutes), although the duration is longer if the patients have liver disease or enzyme deficiencies.

22.10.2.5 Other nicotinic antagonists

Local anaesthetics and barbiturates appear to prevent the changes in ion permeability which would normally result from the interaction of acetylcholine with the nicotinic receptor. They do not, however, bind to the cholinergic binding site. It is believed that they bind instead to the part of the receptor which is on the inside of the cell membrane, perhaps binding to the ion channel itself and blocking it.

Certain snake toxins have been found to bind irreversibly to the nicotinic receptor, thus blocking cholinergic transmissions. These include toxins such as **α-bungarotoxin** from the Indian cobra. The toxin is a polypeptide containing 70 amino acids which cross-links the α- and β-subunits of the cholinergic receptor (section 22.11).

Finally, the antidepressant and antismoking drug **bupropion** (section 23.12.4) has been shown to be a nicotinic antagonist, as well as a reuptake inhibitor of noradrenaline and dopamine. It is possible that the drug's effectiveness as an antismoking aid may be related to its blockage of neuronal nicotinic receptors in the brain.

KEY POINTS

- Cholinergic antagonists bind to cholinergic receptors but fail to activate them. They block binding of acetylcholine and have a variety of clinical uses.
- Muscarinic antagonists normally contain a tertiary or quaternary nitrogen, a functional group involving oxygen, and a branch point containing two hydrophobic ring substituents.
- Nicotinic antagonists are useful as neuromuscular blockers in surgery.
- The pharmacophore for a nicotinic antagonist consists of two charged nitrogen atoms separated by a spacer molecule such that the centres are a specific distance apart.
- One of the charged nitrogens binds to the cholinergic binding site; the other interacts with a nucleophilic group neighbouring the binding site.
- Neuromuscular blockers should have a fast onset of action, minimal side effects, and a short duration of action to allow fast recovery. The lifetime of neuromuscular blockers can be decreased by introducing ester groups which are susceptible to enzymatic hydrolysis.
- Neuromuscular blockers which degrade chemically by means of the Hofmann elimination are not dependent on metabolic reactions and are more consistent from patient to patient.

22.11 Receptor structures

The nicotinic receptor has been isolated successfully from the electric ray (*Torpedo marmorata*)—a fish found in the Atlantic and the Mediterranean—allowing the receptor to be studied carefully. As a result, a great deal is known about its structure and operation. It is a protein complex made up of five subunits, two of which are the same. The five subunits (two α, one β, one γ, and one δ) form a cylindrical or barrel shape which traverses the cell membrane

(section 4.6.2). The centre of the cylinder acts as an ion channel for sodium, and a gating or lock system is controlled by the interaction of the nicotinic receptor with acetylcholine. In the absence of acetylcholine, the gate is shut. When acetylcholine binds, the gate is opened. The binding site for acetylcholine is situated mainly on the α-subunit and there are two binding sites per ion channel complex. It is usually found that nicotinic receptors occur in pairs, linked together by a disulphide bridge between the δ-subunits.

This is the make up of the nicotinic receptor at neuromuscular junctions. The nicotinic receptors at ganglia and in the CNS are more diverse in nature involving different α- and β-subunits. This allows drugs to act selectively on neuromuscular, rather than neuronal, receptors. For example, decamethonium is only a weak antagonist at autonomic ganglia, whereas **epibatidine** (extracted from a South American frog) is a selective agonist for neuronal receptors. The snake toxin **α-bungarotoxin** is specific for receptors at neuromuscular junctions.

Muscarinic receptors belong to the superfamily of G-protein-coupled receptors (section 4.7) which operate by activation of a signal transduction process (sections 5.1–5.3). Five subtypes of muscarinic receptors have been identified and are labelled M_1–M_5. These subtypes tend to be concentrated in specific tissues. For example, M_2 receptors occur mainly in the heart, whereas M_4 receptors are found mainly in the CNS. M_2 receptors are also used as the autoreceptors on presynaptic cholinergic neurons (section 22.3.2).

The M_1, M_3, and M_5 receptors are associated with a signal transduction process involving the secondary messenger **inositol triphosphate** (IP_3) (section 5.3). The M_2 and M_4 receptors involve a process which inhibits the production of the secondary messenger **cyclic-AMP** (section 5.2). Lack of M_1 activity is thought to be associated with dementia.

KEY POINTS

- The nicotinic receptor is an ion channel consisting of five protein subunits. There are two binding sites for each ion channel.
- The muscarinic receptor is a G-protein-coupled receptor. Various subtypes of muscarinic receptor predominate in different tissues.

22.12 Anticholinesterases and acetylcholinesterase

22.12.1 Effect of anticholinesterases

Anticholinesterases are inhibitors of acetylcholinesterase—the enzyme that hydrolyses acetylcholine (section 22.3.1). If acetylcholine is not destroyed, it can return to reactivate the cholinergic receptor and increase cholinergic effects (Fig. 22.39). Therefore, an acetylcholinesterase inhibitor will have the same biological effect as a cholinergic agonist.

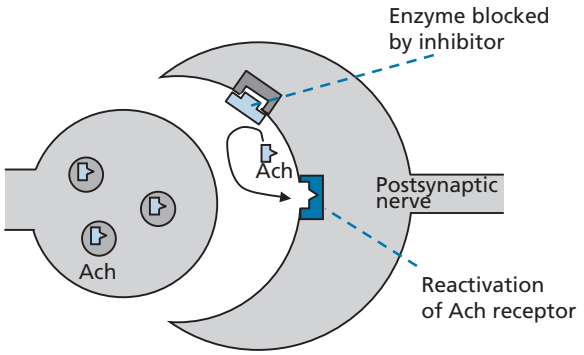


FIGURE 22.39 Effect of anticholinesterases (Ach = acetylcholine).

22.12.2 Structure of the acetylcholinesterase enzyme

The acetylcholinesterase enzyme has a fascinating tree-like structure (Fig. 22.40). The trunk of the tree is a collagen molecule which is anchored to the cell membrane. There are three branches with disulphide bridges that lead off from the trunk, each of which holds the acetylcholinesterase enzyme above the surface of the membrane. The enzyme itself is made up of four protein subunits, each of which has an active site. Therefore, each enzyme tree has 12 active sites. The trees are rooted immediately next to the cholinergic receptors such that they efficiently capture acetylcholine as it departs the receptor. In fact, the acetylcholinesterase enzyme is one of the most efficient enzymes known. A soluble cholinesterase enzyme called **butyrylcholinesterase** is also present in various tissues and plasma. This enzyme has a broader substrate specificity than acetylcholinesterase and can hydrolyse a

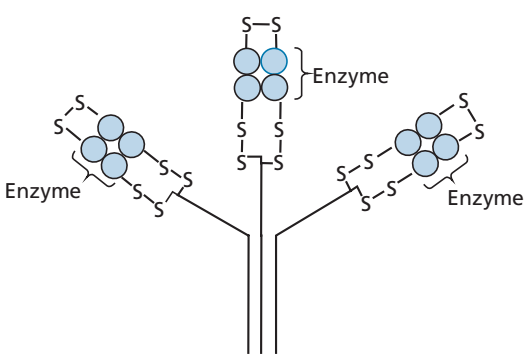


FIGURE 22.40 The acetylcholinesterase enzyme.

variety of esters. Its physiological function is not totally clear, but it has been found to catalyse the hydrolysis of toxic esters, such as cocaine, and appears to have a non-catalytic role in cell differentiation and development. It is also more effective than acetylcholinesterase at hydrolysing high levels of acetylcholine when the acetylcholinesterase enzyme itself becomes substrate inhibited.

22.12.3 The active site of acetylcholinesterase

The design of anticholinesterases depends on the shape of the enzyme's active site, the binding interactions involved with acetylcholine, and the mechanism of hydrolysis. The active site itself is at the foot of a narrow gorge (Fig. 22.41a) and, at the entrance to the gorge, there is a peripheral binding site. It is believed that this site plays a crucial role in recognizing acetylcholine as the substrate. One of the key interactions is a weak π-cation interaction between the heteroaromatic ring of a tryptophan residue and the charged quaternary nitrogen of acetylcholine (Fig. 22.41b). After acetylcholine has been 'captured' it is rapidly transferred down the gorge to the active site (Fig. 22.41c). This process is aided by the fact that the gorge is lined with 14 conserved aromatic residues, which can also form π-cation interactions with acetylcholine and thus channel the substrate down the gorge into the active site. Once acetylcholine enters the active site, another tryptophan residue forms yet another π-cation interaction (Fig. 22.41d). An electrostatic gradient running down the gorge encourages the movement of acetylcholine. The gradient is due to several negatively charged amino acid residues in the active site, which create a dipole that points down the gorge to serve as an electronic steering mechanism for the positively charged substrate. The tryptophan residues in the peripheral binding site and the active site are 12A apart and this is

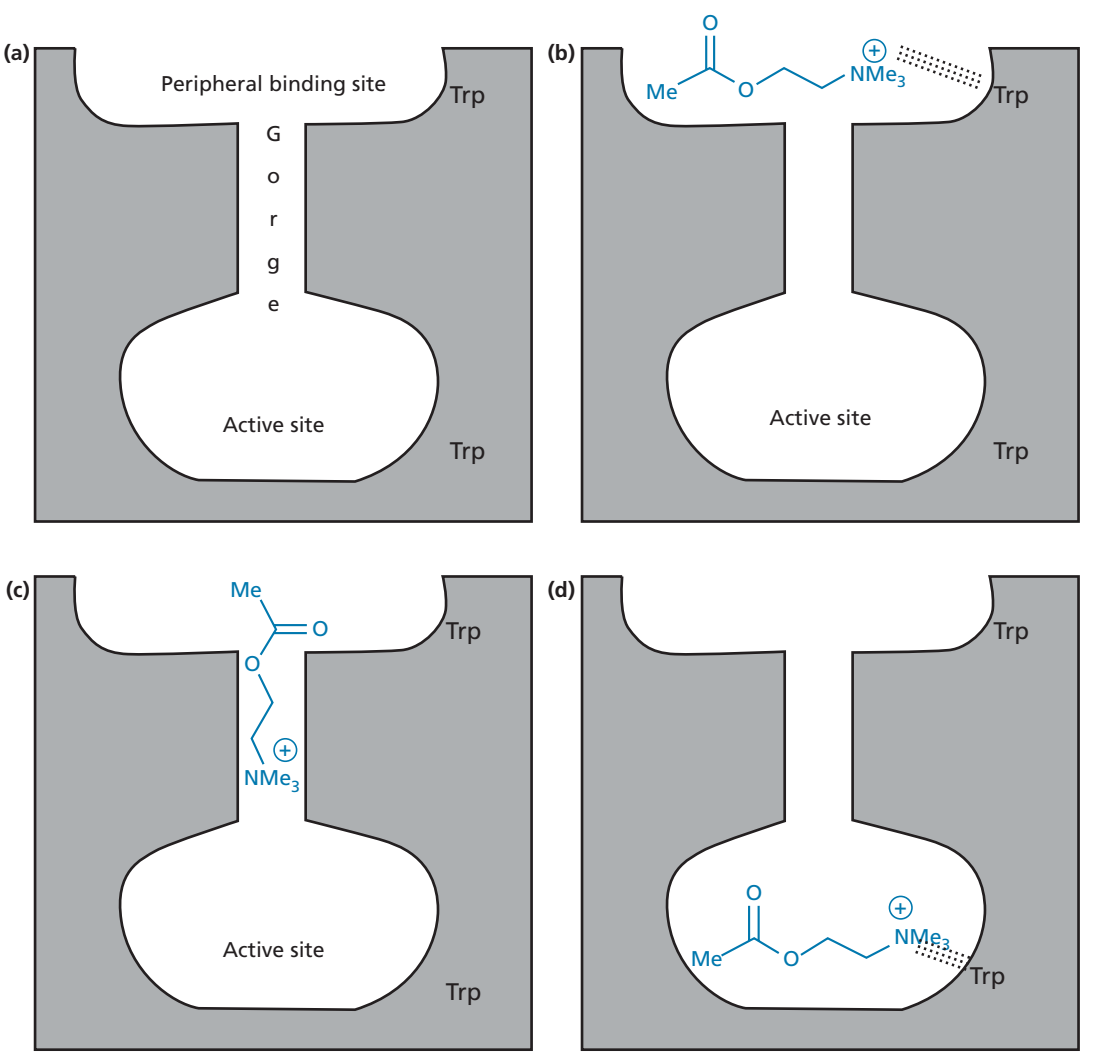


FIGURE 22.41 Process by which acetylcholine is recognized and bound.

significant when it comes to designing potential **dual-action drugs** (section 22.15.2).

22.12.3.1 Crucial amino acids within the active site

The important amino acids within the active site are those which bind acetylcholine, as well as those involved in the mechanism of hydrolysis. As far as binding is concerned, several amino acids are thought to be involved, but a key interaction is the interaction between a tryptophan residue and the quaternary nitrogen atom (Fig. 22.42). The key amino acid residues involved in the catalytic mechanism are serine, histidine, and glutamate.

22.12.3.2 Mechanism of hydrolysis

The histidine residue acts as an acid–base catalyst throughout the mechanism, while serine acts as a nucleophile. This is not a particularly good role for serine, as an aliphatic alcohol is a poor nucleophile and is unable to hydrolyse an ester, but the acid/base catalysis provided by histidine overcomes that disadvantage. The glutamate residue interacts with the histidine residue and serves to orientate and activate the ring (compare chymotrypsin—section 3.5.3). There are several stages to the mechanism (Fig. 22.43):

1. Acetylcholine approaches and binds to the active site. Serine acts as a nucleophile and uses a lone pair of electrons to form a bond to the ester of acetylcholine. Nucleophilic addition to the ester takes place and opens up the carbonyl group
2. The histidine residue catalyses this reaction by acting as a base and removing a proton, thus making serine more nucleophilic
3. Histidine now acts as an acid catalyst and protonates the alkoxy (OR) portion of the intermediate, turning it into a much better leaving group
4. The carbonyl group reforms and expels the alcohol portion of the ester (i.e. choline)
5. The acyl portion of acetylcholine is now covalently bound to the active site. Choline leaves the active site and is replaced by water
6. Water acts as a nucleophile and uses a lone pair of electrons on oxygen to attack the acyl group
7. Water is normally a poor nucleophile, but, histidine aids the process again by acting as a basic catalyst and removing a proton
8. Histidine acts as an acid catalyst by protonating the intermediate
9. The carbonyl group is reformed and the serine residue is released. Because it is now protonated, it is a much better leaving group
10. Ethanoic acid leaves the active site and the cycle can be repeated.

The enzymatic process is remarkably efficient owing to the close proximity of the glutamate residue (not shown), the serine nucleophile, and the histidine acid–base catalyst. As a result, hydrolysis by acetylcholinesterase is 10^8 (one hundred million) times faster than in its absence. The process is so efficient that acetylcholine is hydrolysed within a 100 μs of reaching the enzyme.

22.13 Anticholinesterase drugs

Anticholinesterase drugs act as inhibitors of the enzyme acetylcholinesterase. This inhibition can be either reversible or irreversible depending on how the drug interacts with

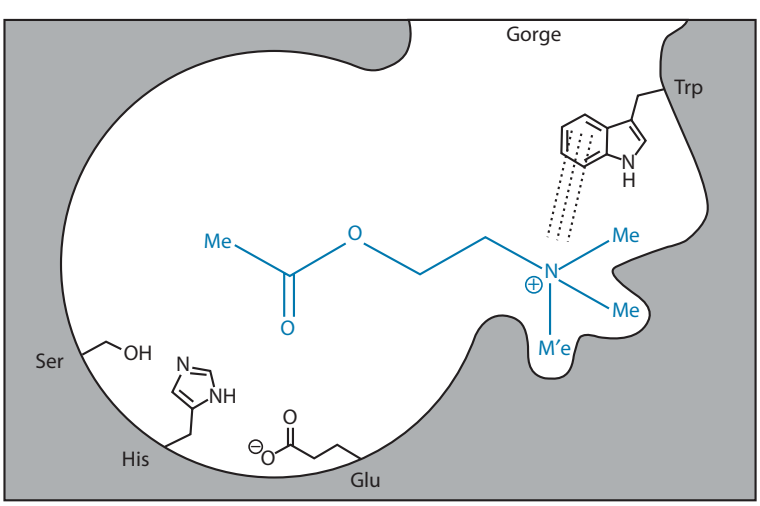


FIGURE 22.42 Key amino acid residues within the active site.

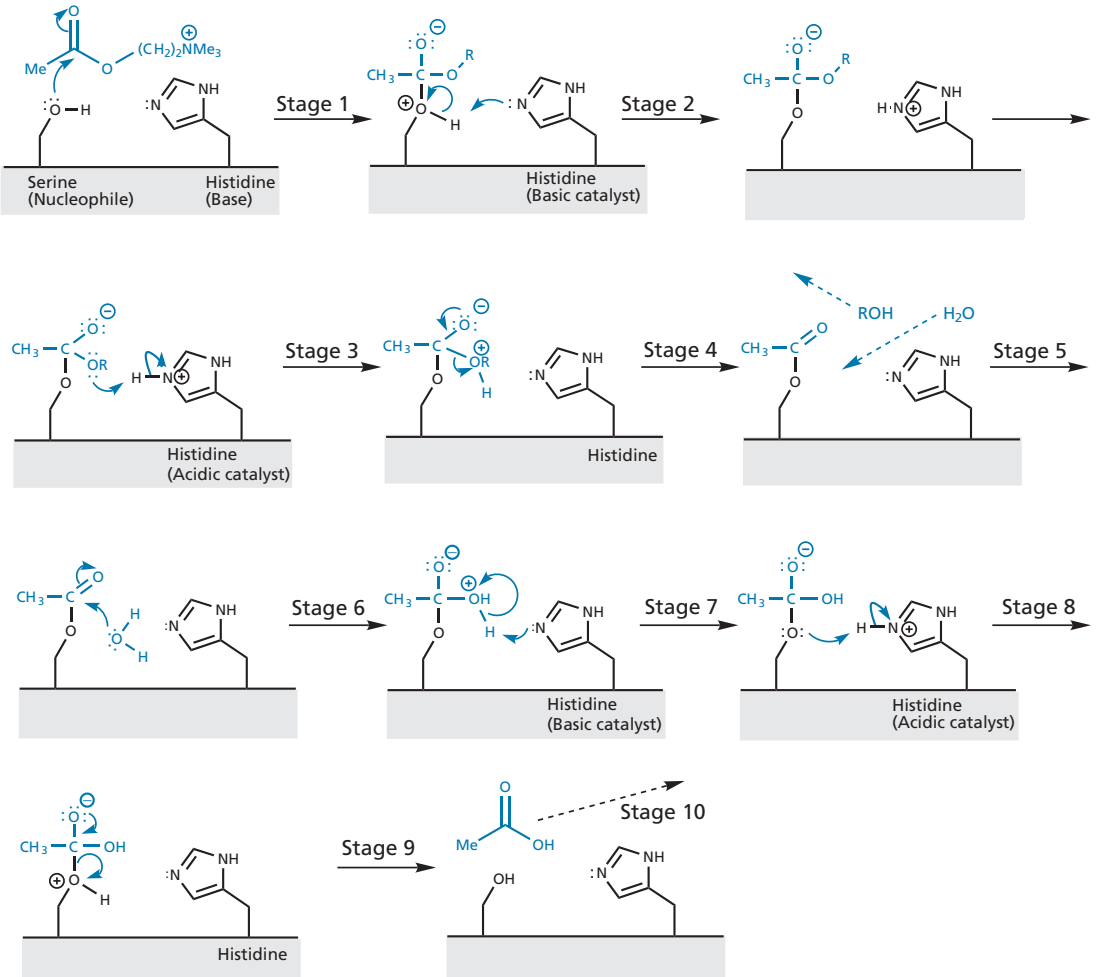


FIGURE 22.43 Mechanism of hydrolysis for the acetylcholinesterase enzyme (the glutamate component of the catalytic triad is not shown).

the active site. Two main groups of acetylcholinesterases are considered here—carbamates and organophosphorus agents.

22.13.1 Carbamates

22.13.1.1 Physostigmine

As in so many fields of medicinal chemistry, it was a natural product that provided the lead for the carbamate inhibitors. The natural product was **physostigmine** (Fig. 22.44) (also called **eserine**) which was discovered in 1864 as a product of the poisonous **calabar bean** (the ordeal bean, *Physostigma venenosum*) from West Africa. Extracts of these beans were fed to criminals to assess whether they were guilty or innocent. Death indicated a guilty verdict. The structure was established in 1925 and physostigmine is still used clinically to treat glaucoma.

SAR studies of physostigmine demonstrate that:

- the carbamate group is essential to activity;
- the benzene ring is important;
- the pyrrolidine nitrogen is important and is ionized at blood pH.

Working backwards, the positively charged pyrrolidine nitrogen is important because it binds to the anionic binding region of the enzyme. The benzene ring may be involved in some extra hydrophobic bonding

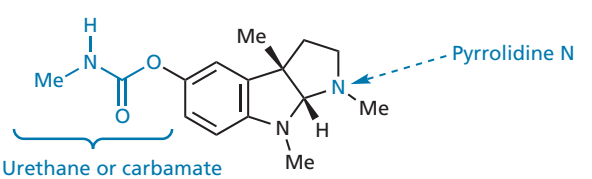


FIGURE 22.44 Physostigmine.

with the active site. Alternatively, it may be important in the mechanism of inhibition as it provides a good leaving group. The carbamate group is the crucial group responsible for physostigmine's inhibitory properties. To understand why, we must look at what happens when physostigmine acts as the substrate for acetylcholinesterase (Fig. 22.45).

The first four stages proceed as normal, with histidine catalysing the nucleophilic attack of the serine residue on physostigmine (stages 1 and 2). The leaving group (this time a phenol) is expelled with the aid of acid catalysis from histidine (stages 3 and 4) and departs the active site to be replaced by a water molecule.

The next stage turns out to be extremely slow. Despite the fact that histidine can still act as a basic catalyst, water finds it difficult to attack the carbamoyl intermediate. This step becomes the rate-determining step for the whole process and the overall rate of hydrolysis of physostigmine is 40×10^6 times slower than that of acetylcholine. As a result, the cholinesterase active site becomes blocked and is unable to react with acetylcholine.

The final stage is slow because of the stability of the carbamoyl–enzyme complex. This is because the nitrogen can feed a lone pair of electrons into the carbonyl group and drastically reduce its electrophilic character (Fig. 22.46) (cf. section 22.9.2).

22.13.1.2 Analogues of physostigmine

Physostigmine has limited medicinal use because of serious side effects, and it has only been used in the treatment of glaucoma or as an antidote for atropine poisoning. Simpler analogues, however, have been used in the treatment of myasthenia gravis and as an antidote to curare poisoning.

Miotine (Fig. 22.47) still has the necessary carbamate, aromatic, and tertiary aliphatic nitrogen groups. It is active as an antagonist but it also has disadvantages: it is susceptible to chemical hydrolysis and it can cross the blood–brain barrier (section 11.4.5) as the free base, resulting in side effects due to its action in the CNS.

Neostigmine and **pyridostigmine** (Fig. 22.47) were designed to deal with both these problems. Firstly, a quaternary nitrogen atom is present and so there is no chance of the free base being formed. As the molecule is permanently charged, it cannot cross the blood–brain barrier

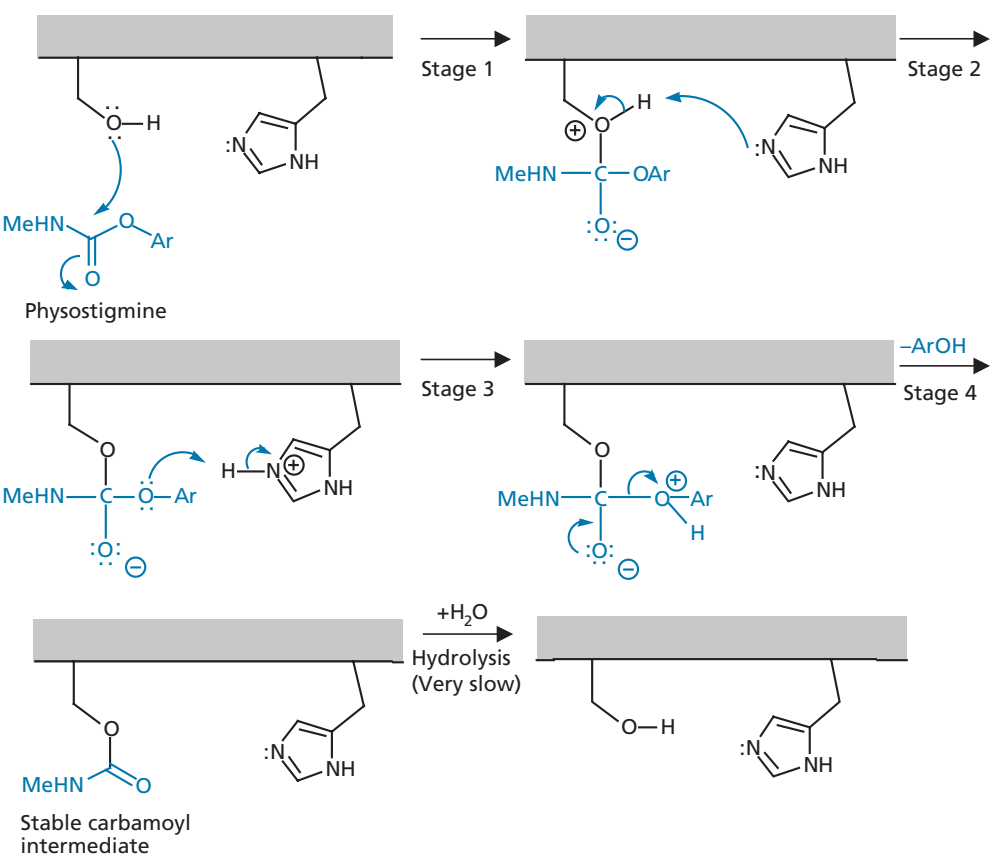


FIGURE 22.45 Mechanism of inhibition by physostigmine (Ar represents the tricyclic system of physostigmine).

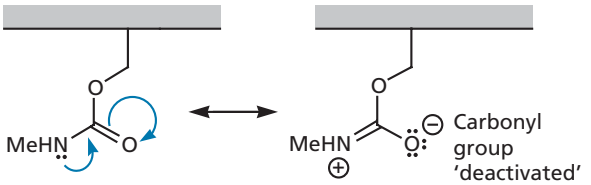


FIGURE 22.46 Stabilization of the carbamoyl–enzyme intermediate.

and so the drug is free of CNS side effects. Increased stability is achieved by using a dimethylcarbamate group rather than a methylcarbamate group. Two further points to note about neostigmine are:

- the quaternary nitrogen is 4.7 Å away from the carbamate group;
- the direct bonding of the quaternary centre to the aromatic ring reduces the number of conformations that the molecule can adopt. This is an advantage if the active conformation is retained because the molecule is more likely to be in the active conformation when it approaches the active site.

Both neostigmine and pyridostigmine are in use today. They are given intravenously to reverse the actions of neuromuscular blockers or used orally in the treatment of myasthenia gravis. Pyridostigmine was one of the drugs used in the chemical cocktail provided to allied troops in Iraq during **Operation Desert Shield**. The agent was present to help protect against possible exposure to **organophosphate nerve gases**. **Edrophonium** is a similar agent used to reverse neuromuscular blocking and is also used as a treatment of myasthenia gravis.

22.13.2 Organophosphorus compounds

The potential of organophosphorus compounds as nerve agents was first recognized by German scientists in the 1920s and 1930s, and research was carried out to investigate their potential as weapons of war. When World War II broke out, governments in the UK, USA, Sweden, and Russia recognized the danger of Germany perfecting these weapons and started their own research efforts during the 1940s. In the UK, this was carried out at the **Porton Down Defence Centre**. Fortunately, these agents were never used, but researchers in different countries continued work to find suitable antidotes that would protect troops from a possible attack. It has not been proved whether the organophosphate nerve gases have ever been used in combat, but many believe that they were part of the chemical weapons arsenal that was used against the Kurds by the Iraqi government. It has also been proposed that **sarin** (Fig. 22.48) may have been released when Iraqi chemical plants and ammunition dumps were bombed during the period 1990–91, and that this might be a possible cause of the mystery illness that afflicted many of the veterans of that war—**Gulf War syndrome**. Bosnians, Serbs, and Croats have also been accused of using nerve agents during the break-up of Yugoslavia in the 1990s. Certainly, nerve agents have been used by terrorist groups: the most notorious example was the release of sarin in the Tokyo subway during 1995.

The organophosphate nerve agents are examples of the weapons of mass destruction which several Western countries feared might be used by Iraq on its neighbours or supplied to extremist groups. The invasion of Iraq in

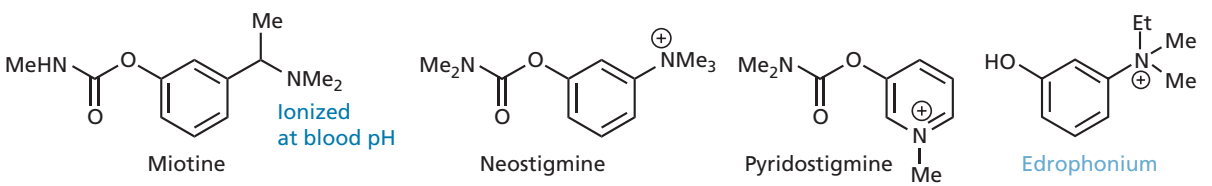


FIGURE 22.47 Analogues of physostigmine. Miotine is a chiral molecule that has been studied as a racemate.

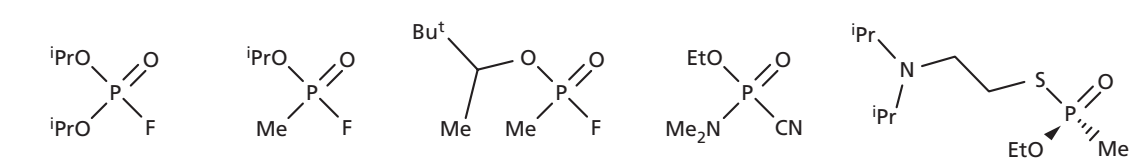


FIGURE 22.48 Examples of nerve agents.

2003 was designed to combat this threat, but subsequent searches failed to reveal any such weapons.

It would be wrong to give the impression that the only use for organophosphates is as weapons of war and terror. They are also extremely important insecticides used in agriculture and animal husbandry, and have a variety of uses in medicine. We shall consider these aspects in the following sections.

22.13.2.1 Nerve agents

The nerve gases **dyflos** and **sarin** (**GB**) (Figure 22.48) were discovered and perfected long before their mode of action was known. Both agents inhibit acetylcholinesterase by irreversibly phosphorylating the serine residue at the active site (Fig. 22.49).

The early part of the mechanism is similar to the normal mechanism, but the phosphorylated adduct which is formed is extremely resistant to hydrolysis. Consequently, the enzyme is permanently inactivated. As acetylcholine cannot be hydrolysed, the cholinergic system is continually stimulated. This results in permanent contraction of skeletal muscle, resulting in death.

Other nerve agents include **tabun** (**GA**), **soman** (**GD**), and **VX**. VX is the most toxic of the nerve agents, having an LD_{50} of 10 mg through skin contact. It was discovered at Porton Down in the UK in 1954 then traded to the USA in exchange for technological information on nuclear weapons. The USA produced several tons of the material for its chemical warfare programme, but decided to dispose of its stockpiles in the late 1960s—a process that was only completed in 2008. Much of the nerve agent now lies at the bottom of the Atlantic Ocean.

22.13.2.2 Medicines

Once the mechanism of action of nerve agents was discovered, compounds such as **ecothiopate** (Fig. 22.50) were designed to fit the active site more effectively by including a quaternary amine to bind with the anionic region. This meant that lower doses would be more effective. Ecothiopate is used medicinally in the form of eye drops for the treatment of glaucoma and has advantages over dyflos, which has also been used in this way. Unlike dyflos, ecothiopate slowly hydrolyses from the enzyme over a matter of days.

22.13.2.3 Insecticides

The insecticides **parathion**, **malathion**, and **chlorpyrifos** (Fig. 22.50) are good examples of how a detailed knowledge of biosynthetic pathways can be useful in drug design. These agents are relatively non-toxic compared with nerve gases because the P = S double bond prevents inhibition of the acetylcholinesterase enzymes. In contrast, the equivalent compounds containing a P = O double bond are highly lethal.

Fortunately, there are no metabolic pathways in mammals which can convert the P = S double bond to a P = O double bond. In insects, however, the insecticides act as prodrugs and are metabolized by oxidative desulphurization. The resulting anticholinesterases prove lethal.

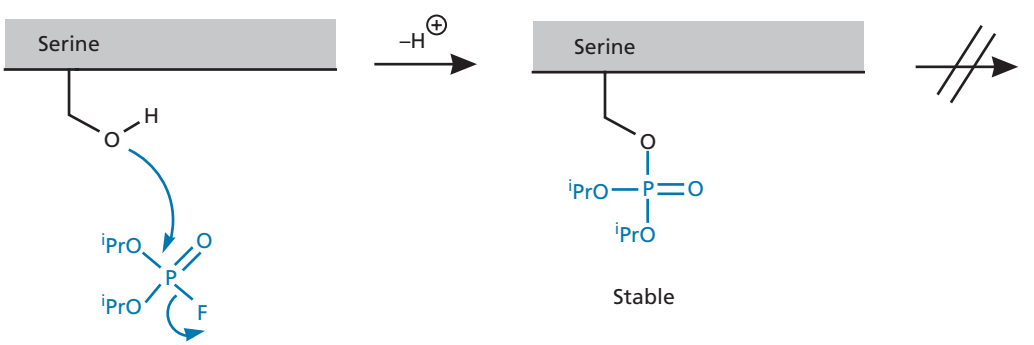


FIGURE 22.49 Simplified mechanism of action of dyflos at the active site of acetylcholinesterase.

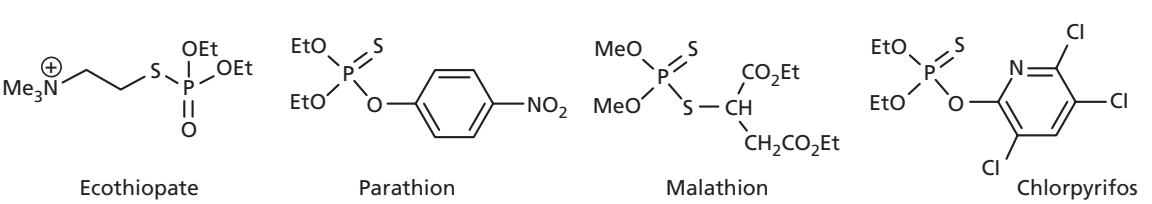


FIGURE 22.50 Organophosphates used as medicines and insecticides.

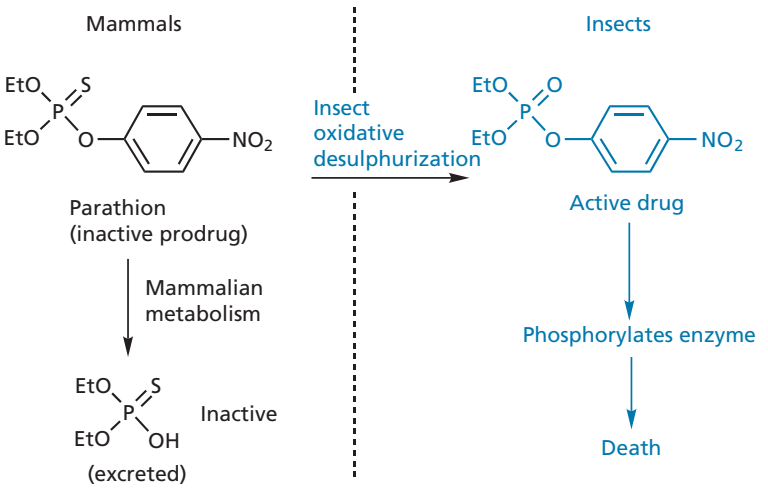


FIGURE 22.51 Metabolism of insecticides in mammals and insects.

In mammals, the same compounds are metabolized in a different way to give inactive compounds which are then excreted (Fig. 22.51). Despite this, organophosphate insecticides are not totally safe and prolonged exposure to them can cause serious side effects if they are not handled with care. Parathion has high lipid solubility and is absorbed easily through mucous membranes, and can also be absorbed through the skin. Preparations of malathion are used medicinally for the treatment of head lice, crab lice, and scabies, but should not be used too frequently or over prolonged periods.

22.14 Pralidoxime: an organophosphate antidote

Pralidoxime (Fig. 22.52) is an antidote to organophosphate poisoning and represents one of the early examples of rational drug design, Any antidote for organophosphate poisoning has to displace the organophosphate moiety from serine by hydrolysing the phosphate–serine bond. However, this is a strong bond and not easily broken. Therefore, a strong nucleophile is required.

The literature revealed that phosphates can be hydrolysed with hydroxylamine (Fig. 22.53). This proved too toxic a compound to be used on humans, so the next stage was to design an equally reactive nucleophilic group which would specifically target the acetylcholinesterase enzyme. If such a compound could be designed, then there was less chance of the antidote taking part in toxic side reactions.

The designers' job was made easier by the knowledge that the organophosphate group does not fill the active site, and the anionic binding region is vacant. The obvious thing to do was to find a suitable group to bind to this anionic centre and attach a hydroxylamine moiety to it. Once positioned in the active site, the hydroxylamine group could react with the phosphate ester (Fig. 22.52).

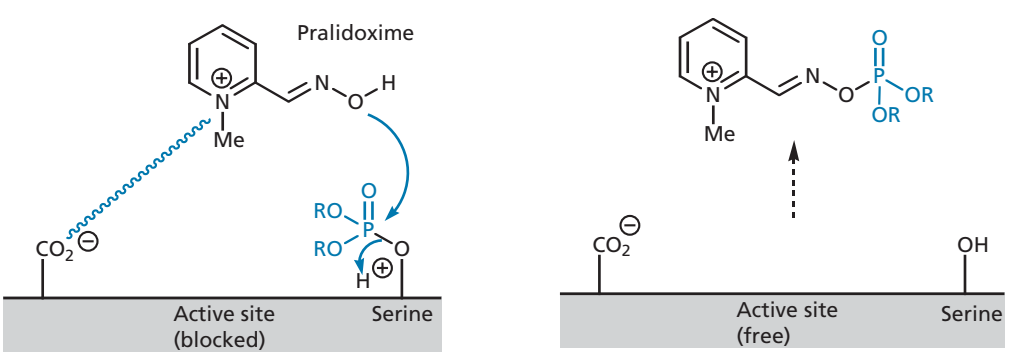


FIGURE 22.52 Pralidoxime as an antidote for organophosphate poisoning.

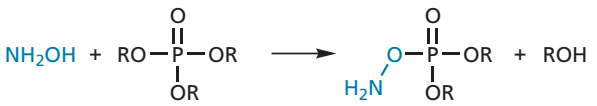


FIGURE 22.53 Hydrolysis of phosphates.

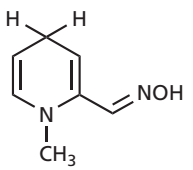


FIGURE 22.54 ProPAM.

Pralidoxime was the result. The positive charge is provided by a methylated pyridine ring and the nucleophilic side group is attached to the *ortho* position, as it was calculated that this would place the nucleophilic hydroxyl group in exactly the correct position to react with the phosphate ester. The results were spectacular, with pralidoxime showing a potency as an antidote 10^6 times greater than hydroxylamine.

Because pralidoxime has a quaternary nitrogen, it is fully charged and cannot pass through the blood–brain barrier into the CNS. This means that the antidote cannot work on any enzymes that have been inhibited in the brain. Pro-2-PAM (Fig. 22.54) is a prodrug of pralidoxime which avoids this problem. As a tertiary amine it can pass through the blood–brain barrier and is oxidized to pralidoxime once it has entered the CNS.

22.15 Anticholinesterases as 'smart drugs'

22.15.1 Acetylcholinesterase inhibitors

Acetylcholine is an important neurotransmitter in the CNS, as well as in the PNS. It has been proposed that the memory loss, intellectual deterioration, and personality changes associated with Alzheimer's disease may, in part, be due to the destruction of cholinergic nerves in the brain. Such damage is associated with the appearance of extracellular protein plaques and intracellular protein tangles in nerve fibres. These aberrant protein structures are neurotoxic and responsible for the destruction of neurons.

Although Alzheimer's disease is primarily a disease of the elderly, it can strike victims as young as 30 years of age and is the fourth leading cause of death in the developed world, affecting nearly 50% of those aged 85 years or more. It has been predicted that there will be 70 million sufferers worldwide by 2050, representing 1.2% of the total population.

The destruction of cholinergic nerves results in a drop in both cholinergic receptors and acetylcholine levels in the brain. Therefore, research has been carried out into the use of anticholinesterases for the treatment of Alzheimer's disease—the so-called **smart drugs**. There is no evidence that these compounds assist general memory improvement and so they are not a student's answer to exam cramming! The treatment does not offer a cure for Alzheimer's disease either, but it can alleviate the symptoms by increasing the duration of action of acetylcholine such that activation of the cholinergic receptors remaining is prolonged. Unlike anticholinesterases acting in the periphery, 'smart drugs' have to cross the blood–brain barrier and so structures containing quaternary nitrogen atoms are not suitable. Tests with physostigmine were carried out in 1979, but the compound was not ideal as it does not enter the brain sufficiently well and shows short-lived, non-selective inhibition. The first drug to be approved for the treatment of Alzheimer's disease was **tacrine** (Fig. 22.55) in 1993. However, this is an extremely toxic drug and is only beneficial for about a year. Other agents which have subsequently been introduced include **donepezil** in 1997, **rivastigmine** in 2000, and **galantamine** (obtained from daffodils or snowdrop bulbs) in 2001. Rivastigmine (an analogue of physostigmine) was the first drug to be approved in all countries of the European Union. It shows selectivity for the brain and has beneficial effects on cognition, memory, concentration, and functional abilities, such as day-to-day tasks or hobbies. The drug has a short half-life, reducing the risk of accumulation or drug–drug interactions. **Metrifonate** (an organophosphate) and **anabaseine** (from ants and marine worms) have also been tested for the treatment of Alzheimer's disease. Herbal medicines have been used in the past to treat the symptoms of Alzheimer's disease and may provide useful lead compounds for further research (Box 22.1).

The anticholinesterase drugs have been shown to be beneficial in the early stages of Alzheimer's disease, but are of less benefit when the disease has become advanced. One disadvantage with the long-term use of these agents is the fact that they increase acetylcholine levels all round the body and not just in the brain; this leads to gastrointestinal side effects. Another problem is that the increased acetylcholine levels result in an increased activation of presynaptic cholinergic receptors which act as a feedback control to lower the amounts of acetylcholine released. As a result, there has been research into finding selective cholinergic agonists that could be used to treat the symptoms of the disease.

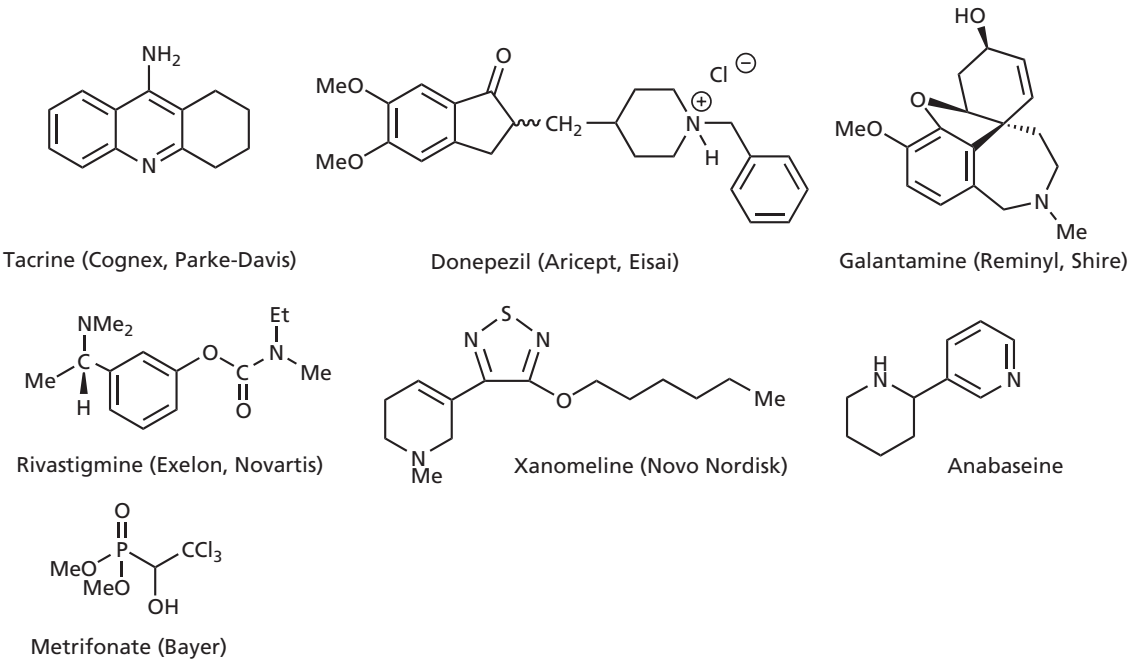
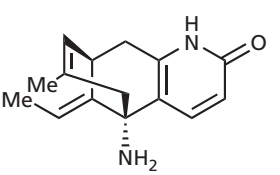


FIGURE 22.55 'Smart drugs'.

BOX 22.1 Mosses play it smart

An extract from the club moss *Huperzia serrata* has been used for centuries in Chinese herbal medicine to treat ailments varying from confusion in Alzheimer's disease to schizophrenia. The extract contains a novel alkaloid called **huperzine A**, which acts as an anticholinesterase. Binding is very specific and so the drug can be used in small doses, thus minimizing the risk of side effects. Huperzine A has been approved for clinical use in China and has been shown to have memory-enhancing effects.

A synthetic route to the natural product has been worked out which has allowed the synthesis of different analogues, but none of these is as active as the natural product. The tricyclic ring system seems to be necessary for good activity, ruling out the possibility of significant simplification. All the functional groups in the molecule are also required for good activity.



Huperzine A.

22.15.2 Dual-action agents acting on the acetylcholinesterase enzyme

In recent years, it has been discovered that the acetylcholinesterase enzyme appears to do more than just catalyse the hydrolysis of acetylcholine. Under normal conditions, the enzyme plays a non-catalytic role in neural development, cell adhesion, and differentiation. Protein–protein interactions involving the interaction of the peripheral binding site of acetylcholinesterase with other proteins promote these processes, with the tryptophan residue described previously (section 22.12.3) playing a crucial role.

Unfortunately, it has also been discovered that the enzyme can play an active role in promoting the deposits of aberrant protein that are found in the brain of Alzheimer's sufferers. Studies have shown that the peripheral binding site of the enzyme is capable of binding **β-amyloid**

protein, which is normally soluble and has an antioxidant role. However, on binding to acetylcholinesterase, the protein undergoes a conformational change which causes it to become insoluble, leading to the appearance of the protein plaques and tangles associated with Alzheimer's disease. The enzyme has been described as a **pathological chaperone** for this process and becomes associated with the protein deposits. Moreover, soluble oligomers of the protein are also formed within cells, which disrupt mitochondria function and increase oxidative stress, resulting in cell toxicity and cell death. These, indeed, may be more relevant to the disease than the visible extracellular plaques.

There is an exciting possibility that drugs might be developed which could halt the progression of the disease by preventing the binding of β-amyloid protein to the peripheral binding site of acetylcholinesterase. Research is currently in progess aimed at designing dual-action drugs that are capable of inhibiting this process, as well as acting as acetylcholinesterase inhibitors. **Donepezil** (Fig. 22.55) is one currently used inhibitor that can span the gorge to interact with both the peripheral binding site and the active site. It has also been shown to have an inhibitory effect on protein aggregation. However, much of the early work has looked at tacrine dimers. **Tacrine** (Fig. 22.55) is believed to enter the active site of the enzyme in a similar manner to acetylcholine; in other words, it is protonated and binds initially to the peripheral binding site. It is then transferred down the gorge into the active site (Fig. 22.41). A dimer was designed where two tacrine molecules were linked by a hydrocarbon chain of sufficient length to allow one tacrine moiety to bind to the active site while the other interacted simultaneously with the peripheral binding site. Different lengths of linker were tried and it was found that a seven-carbon chain was ideal—**bis(7)-tacrine** (Fig. 22.56). This compound was found to be 150–1000 times more potent as an enzyme inhibitor, depending on the source of enzyme studied. Studies have shown that the key tryptophan residues in the active site and the peripheral binding site can form π-cation interactions with each of the tacrine components. The linker can also form van der Waals interactions with the gorge and there is an entropy gain achieved by the displacement of water from the gorge. However, there is an entropy penalty resulting from the restriction in flexibility of the linker once it is constrained within the gorge. The tricyclic hydrophobic nature of the tacrine moieties is also important as there is only a small desolvation penalty involved when the structure binds. Stronger π-cation interactions would be possible if one of the tacrine ring systems was replaced with a simpler amine, but the latter would be strongly solvated and would require a higher desolvation penalty.

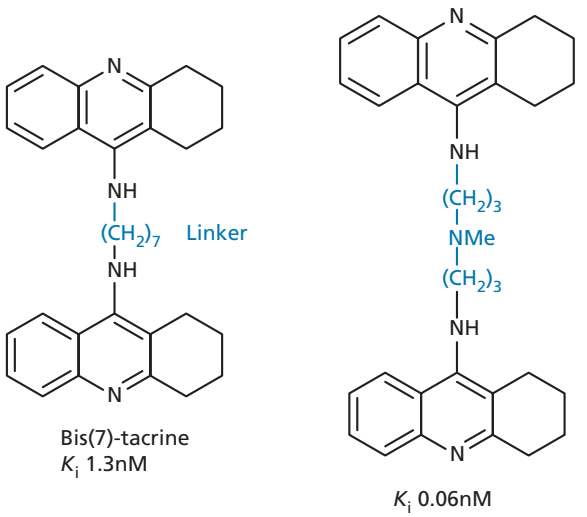


FIGURE 22.56 Tacrine dimers as dual-action agents.

Test your understanding and practise your molecular modelling with Exercises 22.5 and 22.6.

The introduction of an *N*-methyl group into the linker resulted in further binding interactions and increased potency. The *N*-methyl group is protonated when the dimer binds and so it can form π-cation interactions with the aromatic residues lining the gorge. Following on from this work, a large number of structures were synthesized including homodimers of **galantamine** and **huperzine B**, as well as heterodimers containing two different acetylcholinesterase inhibitors. Other dual-action structures have been prepared consisting of a standard acetylcholinesterase inhibitor linked to a moiety designed to bind more effectively with the peripheral binding site. Many of these have been shown to inhibit both the catalytic activity of acetylcholinesterase, as well as protein aggregation. Nevertheless, none of these compounds has entered the clinic to date.

There is a good chance that a dual-action agent will eventually reach the clinic. However, there are many factors involved in Alzheimer's disease and so drugs interacting with acetylcholinesterase alone are unlikely to provide a total cure. Attention is now turning to treatments that can address more than one of the various targets implicated in Alzheimer's disease. These treatments could involve a cocktail of different drugs acting at different targets. An alternative approach is to use an agent that can interact with different targets in a predictable way (**multiple-target directed ligands**) (see also section 13.3.14). For example, dual-action agents that inhibit the acetylcholinesterase enzyme have been designed which have one or more of the following properties;

- antioxidant activity and/or the ability to chelate metals;

- the ability to inhibit enzymes, such as butyrylcholinesterase, monoamine oxidase, or BACE1;
- antagonist activity at α_2-adrenoceptors, $5HT_3$ receptors, *N*-methyl-D-aspartate (NMDA) receptors, muscarinic (M_2) receptors, or H_3 receptors;
- inhibition of serotonin reuptake from nerve synapses;
- the blockade of calcium ion channels.

22.15.3 Multi-targeted agents acting on the acetylcholinesterase enzyme and the muscarinic M_2 receptor

As an example of one area of research into multi-targeted directed ligands, we shall consider agents that have been designed to act as dual-action agents at the acetylcholinesterase enzyme (AChE), as well as antagonists of the M_2 receptor. The M_2 receptor is an autoreceptor present on presynaptic cholinergic neurons. Activation of the autoreceptor inhibits the release of acetylcholine from the presynaptic neuron (section 22.3.2 and 22.11) and so M_2 antagonists will increase acetylcholine release and help to raise acetylcholine levels. The lead compound for this work was a polyamine structure called **benextramine** (Fig. 22.57). This is an irreversible α-adrenoceptor antagonist, but it also shows activity as an anticholinesterase and M_2 receptor antagonist. Polyamines have been identified as good lead compounds for multi-targeted directed ligands as the protonated nitrogens present have the capability of forming π-cation interactions with aromatic residues in virtually any protein target. Moreover, the flexible linear structure allows the polyamine to adopt a huge number of different conformations, making it more likely that suitable conformations are present that allow interaction with different targets. Such compounds are defined as **promiscuous ligands** (section 12.2.7).

Studies showed that the 2-methoxybenzyl group was important to activity, but not the disulphide bridge. Varying the chain length led to **methoctramine**, which had improved M_2 activity, while retaining good acetylcholinesterase (AChE) activity. It was also shown that a diamine diamide backbone retained affinity for M_2 and so the two 'internal' amines were replaced with amides in order to improve lipophilicity. This decreased affinity for M_2 receptors and butyrylcholinesterase (BuChE), but increased affinity for AChE. *N*-Methylation further increased affinity for AChE resulting in the discovery of **caproctamine**.

Compared with benextramine, caproctamine was found to be 42 times more active as an AChE inhibitor and two times less active as a BuChE inhibitor, while retaining affinity for the M_2 receptor. It was also demonstrated that the structure could bind simultaneously to the tryptophan residues in the active and peripheral binding sites, while the linker formed hydrophobic interactions with aromatic residues in the gorge. However, caproctamine showed very little ability to inhibit AChE-induced Aβ aggregation, which demonstrated that the ability to interact with the peripheral binding site does not necessarily block protein aggregation.

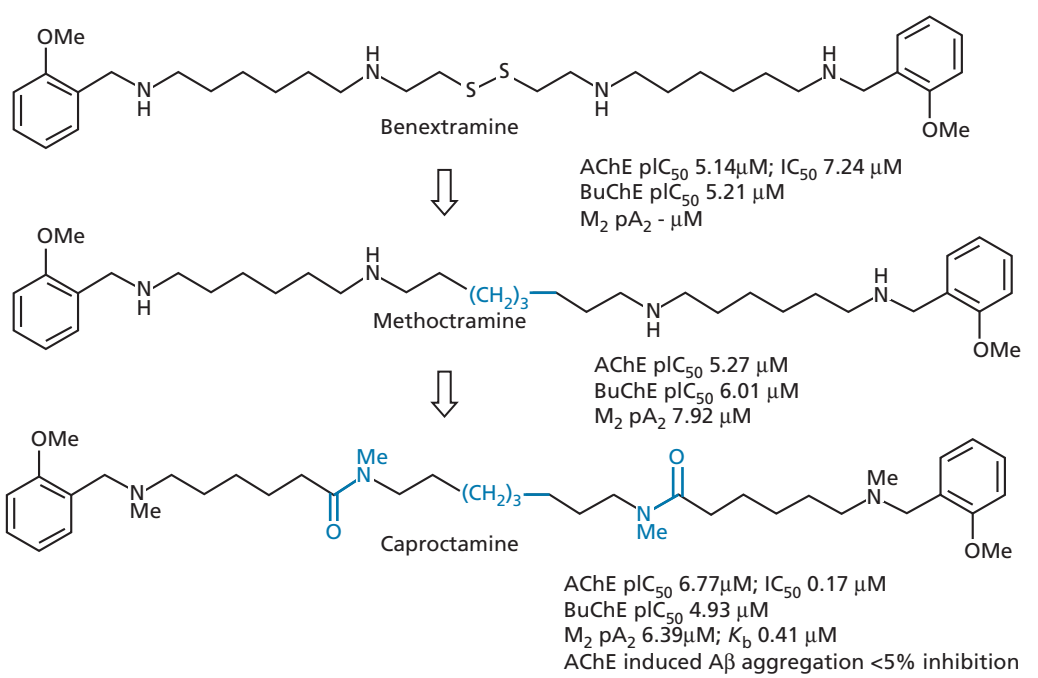


FIGURE 22.57 Development of caproctamine from benextramine.

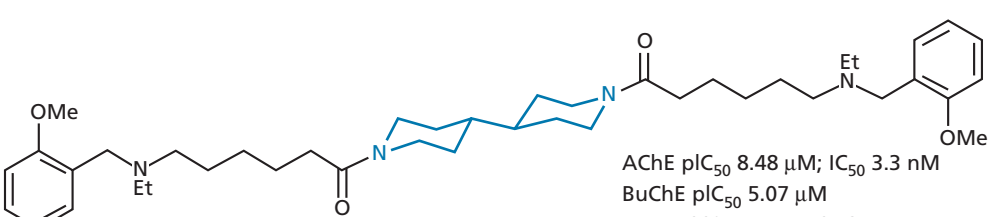
Further work was done to introduce some rigidity into the linker chain by introducing piperidine rings (Fig. 22.58). This resulted in increased anticholinesterase activity and M_2 antagonism, as well as inhibition of AChE-induced Aβ aggregation.

A more substantial aromatic system was introduced into the middle of the linker because it was believed that this would form π–π interactions with aromatic residues in the gorge of AChE and would also allow the structure to interact directly with Aβ proteins to inhibit self-induced aggregation of the protein. This led to the structure shown in Fig. 22.59, which proved to have nanomolar activity as an AChE inhibitor. The structure also proved more active in its ability to inhibit AChE-induced Aβ aggregation. Its activity as an M_2 antagonist was not reported, however.

Docking experiments indicated that the structure could bind to the key tryptophan residues in the catalytic and peripheral binding sites, while the central tetracyclic ring system interacts by π–π or van der Waals interactions with aromatic residues in the gorge. Hydrogen bonding is possible between the methoxy groups and a tyrosine residue in the active site. It remains to be seen whether further development of this compound will result in a clinically useful agent.

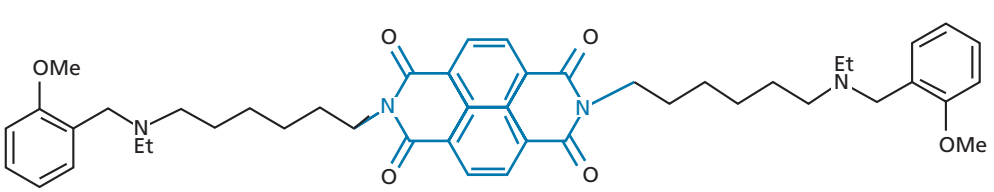
KEY POINTS

- Anticholinesterases inhibit the enzyme acetylcholinesterase and have the same clinical effects as cholinergic agonists.
- The active site for acetylcholinesterase is similar to the binding site for the cholinergic receptor, but also includes a catalytic triad of amino acids—histidine, serine, and glutamate.
- Histidine acts as an acid–base catalyst, while serine acts as a nucleophile during the hydrolytic mechanism. Glutamate orientates and activates histidine.
- The carbamate inhibitors are derived from the lead compound physostigmine. They react with acetylcholinesterase to produce a carbamoyl-bound intermediate which is stable and slow to hydrolyse.
- Organophosphorus agents have been used as nerve gases, medicines, and insecticides. They irreversibly phosphorylate serine in the active site.
- Pralidoxime was designed as an antidote for organophosphate poisoning. It can bind to the active site of phosphorylated enzymes and displace the phosphate group from serine.
- Anticholinesterases have been used as smart drugs in the treatment of Alzheimer's disease. They have to cross the blood–brain barrier and cannot be permanently charged.
- Dual-action agents have been designed as potential drugs for the treatment of Alzheimer's disease. They are designed to bind simultaneously to the active site and the peripheral binding site.
- Multi-target agents have been deigned that target acetylcholinesterase and other targets that have been implicated in Alzheimer's disease.



AChE pIC_{50} 8.48 μM; IC_{50} 3.3 nM
BuChE pIC_{50} 5.07 μM
M_2 K_b 660 nM; pA_2 6.18 μM
Aβ(AChE) 41% at 100 μM

FIGURE 22.58 Rigidification of caproctamine.

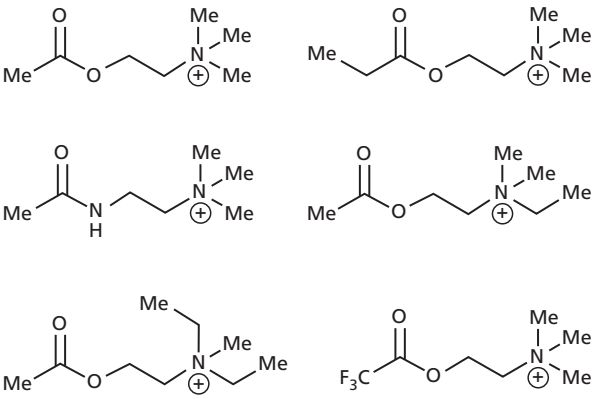


hAChE IC_{50} 0.37nM; AChE-induced Aβ aggregation >90% inhibition;
Self-induced Aβ aggregation 54.5% inhibition.

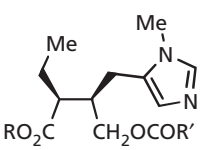
FIGURE 22.59 Further rigidified analogue of caproctamine.

QUESTIONS

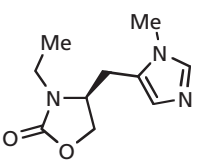
1. Based on the binding site described in Fig. 22.10, suggest whether the following structures are likely to act as agonists or not.



2. Suggest a mechanism by which atropine is racemized.
3. A fine balance of binding interactions is required of a neurotransmitter. What do you think is meant by this and what consequences does it have for drug design?
4. Suggest how the binding interactions holding acetylcholine to the active site of acetylcholinesterase might aid in the hydrolysis of acetylcholine.
5. Explain how the following diester could act as a prodrug for pilocarpine.

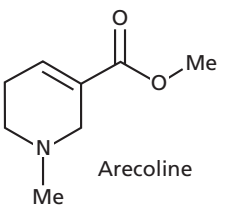


Diester prodrug for pilocarpine



Pilocarpine analogue

6. What advantage do you think the pilocarpine analogue shown might have over pilocarpine itself, and why?
7. Arecoline has been described as a cyclic 'reverse ester' bioisostere of acetylcholine. What is meant by this and what similarity is there, if any, between arecoline and acetylcholine?
8. Arecoline has a very short duration of action. Why do you think this is?
9. Suggest analogues of arecoline that might have better properties, such as a longer duration of action.



Arecoline

10. Neuromuscular blocking activity for tubocurarine is associated with a pharmacophore where the distance between two charged nitrogen atoms is 1.15 nm. Decamethonium can adopt a folded conformation where the N–N separation is 1.14 nm. Octamethonium is an analogue of decamethonium which contains an eight-carbon bridge between the charged nitrogens. The fully extended conformation is the most stable conformation and corresponds to a N–N distance of 1.157 nm. Discuss whether octamethonium is likely to be more active than decamethonium.
11. An electrostatic gradient has been proposed that guides acetylcholine into the active site of the acetylcholinesterase enzyme. Can you foresee any problems associated with the presence of such a gradient? It has also been proposed that there may be a 'back door' into the active site. What do you think this means, how could it occur, and why would it be necessary?
12. Research is being carried out to design Alzheimer's drugs that will inhibit both acetylcholinesterase and butyrylcholinesterase, despite the fact that the former enzyme is more effective at catalysing the hydrolysis of acetylcholine. Why do you think this approach is considered relevant? What might be the disadvantages of such an approach?

FURTHER READING

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Titles for general further reading are listed on p. 763.