Neurotransmitters, Drugs and Brain Function.
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6 Acetylcholine (ACh)

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INTRODUCTION

As outlined in Chapter 1, acetylcholine was the first neurotransmitter to be discovered and isolated. Its mode of action at peripheral synapses and in particular at the neuromuscular junction has been extensively studied and it is clearly the NT at all first synapses outside the CNS, whether this be at sympathetic or parasympathetic ganglia of the autonomic nervous system, the adrenal medulla or neuromuscular junctions in skeletal muscle. In these instances it transmits fast excitation through nicotinic receptors linked directly to the opening of Na^+ channels. At parasympathetic nerve endings, such as those of the vagus on smooth and cardiac muscle and secretary cells, as well as just those few sympathetic nerve endings to sweat glands, it is also the neurotransmitter. In these instances it has much slower excitatory or inhibitory effects mediated through muscarinic receptors utilising second messenger systems.

By contrast, the central actions of ACh are perhaps still less well understood than those of some more recently discovered NTs like dopamine and GABA. It does not appear to have a clear primary function but often an important supporting role. Attempts to understand its central actions were not encouraged by the knowledge that even those anticholinergic drugs that clearly cross the blood–brain barrier have few marked central effects and handicapped by the difficulty in measuring its release and turnover, or mapping its pathways.

Until the recent development of appropriate HPLC techniques capable of detecting pmol amounts (see Flentge *et al*. 1997) ACh could only be measured chemically by relatively lengthy and expensive procedures (e.g. gas chromotography), which were not always very sensitive, or by bioassays. Although the latter, using muscle preparations that responded to ACh, such as the dorsal muscle of the leech, the rectus abdominus of the frog or certain clam hearts, were reasonably sensitive they were tiresome and not easily mastered. Thus studies on the release and turnover of ACh have not been as easy as for the monoamines.

Similarly, cholinergic nerves could only be visualised indirectly. Staining for cholinesterase, the metabolising enzyme for ACh, gave some information on the location of cholinergic synapses, where it is found postsynaptically rather than in nerve terminals, but it is not specific to cholinergic nerves. Fortunately choline acetyltransference (ChAT), which acetylates choline in the synthesis of acetylcholine, is specific to cholinergic nerve terminals and its labelling by immunochemistry has much facilitated the mapping of cholinergic pathways (Fig. 6.7).

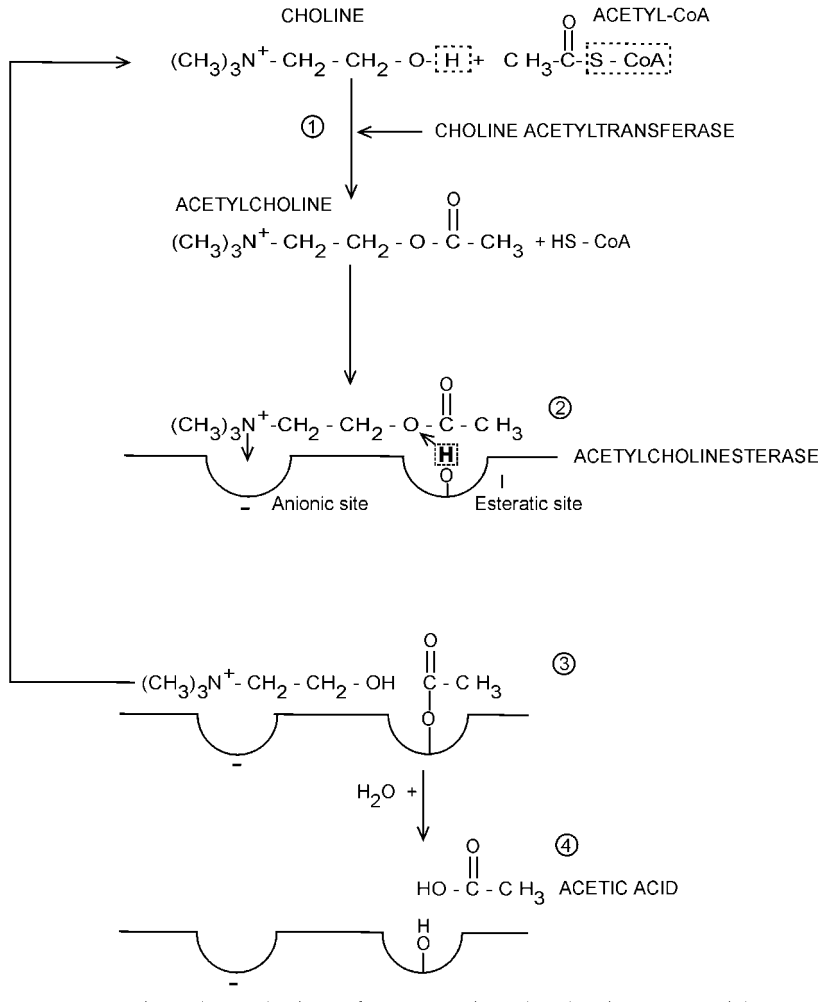

Figure 6.1 Synthesis and metabolism of acetylcholine. Choline is acetylated by reacting with acetyl-CoA in the presence of choline acetyltransferase to form acetylcholine (1). The acetylcholine binds to the anionic site of cholinesterase and reacts with the hydroxy group of serine on the esteratic site of the enzyme (2). The cholinesterase thus becomes acetylated and choline splits off to be taken back into the nerve terminal for further ACh synthesis (3). The acetylated enzyme is then rapidly hydrolised back to its active state with the formation of acetic acid (4)

In contrast to all this negativity, it must be acknowledged that more is known about the structure and function of cholinergic receptors and synapses, especially the nicotinic ones, than for the receptors of any other NT. It is unfortunate that nicotinic synapses are not very common in the CNS.

NEUROCHEMISTRY

The basic biochemistry of the synthesis and destruction of ACh is outlined in Fig. 6.1 and put into the context of the cholinergic synapse in Fig. 6.2.

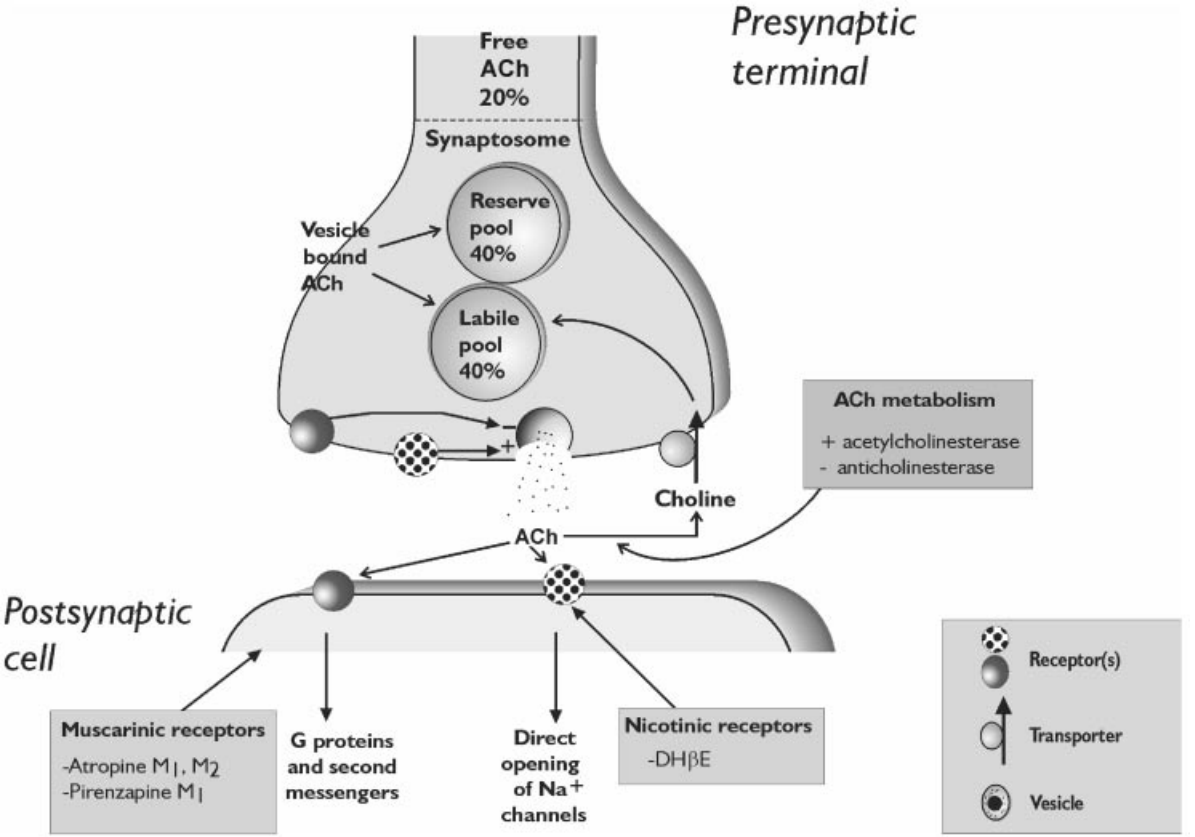

Figure 6.2 Diagrammatic representation of a cholinergic synapse. Some 80% of neuronal acetylcholine (ACh) is found in the nerve terminal or synaptosome and the remainder in the cell body or axon. Within the synaptosome it is almost equally divided between two pools, as shown. ACh is synthesised from choline, which has been taken up into the nerve terminal, and to which it is broken down again, after release, by acetylcholinesterase. Postsynaptically the nicotinic receptor is directly linked to the opening of Na^+ channels and can be blocked by compounds like dihydro-β-erythroidine (DHβE). Muscarinic receptors appear to inhibit K^+ efflux to increase cell activity. For full details see text

SYNTHESIS

Acetylcholine is synthesised in nerve terminals from its precursor choline, which is not formed in the CNS but transported there in free form in the blood. It is found in many foods such as egg yolk, liver and vegetables although it is also produced in the liver and its brain concentration rises after meals. Choline is taken up into the cytoplasm by a high-affinity ($K_m = 1–5\,\mu M$), saturable, uptake which is Na^+ and ATP dependent and while it does not appear to occur during the depolarisation produced by high concentrations of potassium it is increased by neuronal activity and is specific to cholinergic nerves. A separate low-affinity uptake, or diffusion ($K_m = 50\,\mu M$), which is linearly related to choline concentration and not saturable, is of less interest since it is not specific to cholinergic neurons.

The reaction of choline with mitochondrial bound acetylcoenzyme A is catalysed by the cytoplasmic enzyme choline acetyltransferase (ChAT) (see Fig. 6.1). ChAT itelf is synthesised in the rough endoplasmic reticulum of the cell body and transported to the axon terminal. Although the precise location of the synthesis of ACh is uncertain most of that formed is stored in vesicles. It appears that while ChAT is not saturated with either acetyl-CoA or choline its synthesising activity is limited by the actual availability of choline, i.e. its uptake into the nerve terminal. No inhibitors of ChAT itself have been developed but the rate of synthesis of ACh can, however, be inhibited by drugs like hemicholinium or triethylcholine, which compete for choline uptake into the nerve.

STORAGE AND RELEASE

ACh is not distributed evenly within the neuron. If brain tissue is homogenised in isotonic salt solution containing an anticholinesterase, about 20% of the total ACh is released into solution, presumably from cell bodies, and it is found in the supernatant fraction on centrifugation. The remaining 80% settles within the sedimenting pellet and if this is resuspended and spun through a sucrose gradient it is all found in the synaptosome (nerve ending) fraction. After analysis of the synaptosomes and further centrifugation about half of this ACh, i.e. 40% of the original total still remains in the spun-down pellet. This is referred to as the firmly bound or stable 'pool' of ACh since it is not subject to hydrolysis by cholinesterase during the separation procedure. The other half (again 40% or the original) is found in the supernatant and undergoes hydrolysis unless protected by anticholinesterases. While it is generally assumed that some of this latter ACh was always in the synaptosomal cytoplasm probably half of it (20% of the original) comes from disrupted vesicles. This mixture of vesicular and cytoplasmic ACh is called the labile pool and is probably the most important source of releasable ACh, and also where newly synthesised ACh is found. Thus in studies in which tissue has been incubated with labelled precursor choline not only is this pool (fraction) heavily labelled but since most of the released ACh is also labelled it is assumed to come from this pool. With the passage of time there is interchange of ACh between the labile and the so-called fixed pool and in the absence of adequate resynthesis, i.e. blockage of choline uptake, it is likely that ACh will be released from the latter source as well.

Morphological evidence has also been obtained for two distinct vesicles with one designated VP_1 (see Whittaker 1987; Zinnerman *et al.* 1993) being larger but less dense than the other (VP_2). It is the latter which are thought to be incompletely filled with

ACh and considered to be the vesicles in the labile releasable pool. The evidence for and the actual mechanism of the vesicular release of ACh, mostly gained from studies at peripheral synapses, has been covered in Chapter 4.

Apart from inhibiting the uptake of choline and hence its availability for ACh synthesis, with hemicholinium (see above), there are no drugs that directly affect the actual storage or release of ACh. Some experimental tools have, however, been used such as vesamicol, which appears to block the packaging of ACh into its vesicles and thus initiates the slow rundown of releasable vesicular ACh. Some toxins also inhibit ACh release.

Botulinum toxin produced by the anaerobic bacillus *Clostridium botulinum* is unbelievably toxic with a minimum lethal mouse dose of 10^{-12} g. Its occurrence in certain, generally preserved, foods leads to an extremely serious form of poisoning (botulism) resulting in progressive parasympathetic, motor and eventually respiratory paralysis and death. There are no antidotes and mortality is high. Despite this frightening profile, the toxin is finding increasing therapeutic use in relieving some forms of localised muscle spasm such as those of the eyelids (blepharospasm). Obviously it has to be injected directly into the muscle in carefully calculated small amounts. Provided this is achieved its firm binding and slow dissociation ensures a local effect that can last a number of weeks.

Beta-bungarotoxin, a protein in cobra snake venom, also binds to cholinergic nerves to stop ACh release while α-bungarotoxin (from the same source) binds firmly to peripheral postsynaptic nicotinic receptors. The combined effect ensures the paralysis of the snake's victim.

While there is no active neuronal uptake of ACh itself, cholinergic nerve terminals do possess autoreceptors. Since these are stimulated by ACh rather than by the choline, to which ACh is normally rapidly broken down, it is unlikely that they would be activated unless the synaptic release of ACh was so great that it had not been adequately hydrolysed by cholinesterase.

ACh is widely distributed throughout the brain and parts of the spinal cord (ventral horn and dorsal columns). Whole brain concentrations of 10 nmol g^{-1} tissue have been reported with highest concentrations in the interpeduncular, caudate and dorsal raphé nuclei. Turnover figures of 0.15–2.0 nmol g^{-1} min^{-1} vary with the area studied and the method of measurement, e.g. synthesis of labelled ACh from [^{14}C]-choline uptake or rundown of ACh after inhibition of choline uptake by hemicholinium. They are all sufficiently high, however, to suggest that in the absence of synthesis depletion could occur within minutes.

METABOLISM

Released ACh is broken down by membrane-bound acetylcholinesterase, often called the true or specific cholinesterase to distinguish it from butyrylcholinesterase, a pseudo- or non-specific plasma cholinesterase. It is an extremely efficient enzyme with one molecule capable of dealing with something like 10 000 molecules of ACh each second, which means a short life and rapid turnover (100 μs) for each molecule of ACh. It seems that about 50% of the choline freed by the hydrolysis of ACh is taken back into the nerve. There is a wide range of anticholinesterases which can be used to prolong and potentiate the action of ACh. Some of these, such as physostigmine, which can cross the blood–brain barrier to produce central effects and neostigmine, which does not readily

do so, combine reversibly with the enzyme. Others such as the pesticide, disopropylphosphofluorate (DYFLOS), form an irreversible complex requiring the synthesis of new enzyme before recovery. Recently longer acting but reversible inhibitors such as tetrahydro aminoacridine have found some use in the therapy of Alzheimer's disease (Chapter 8). The manner in which acetylcholinesterase is thought to bind to and react with ACh and how drugs may inhibit it are shown in Fig. 6.3.

In addition to its vital role in the metabolism of ACh, acetylcholinesterase has been shown somewhat surprisingly to be released in the substantia nigra, along with DA,

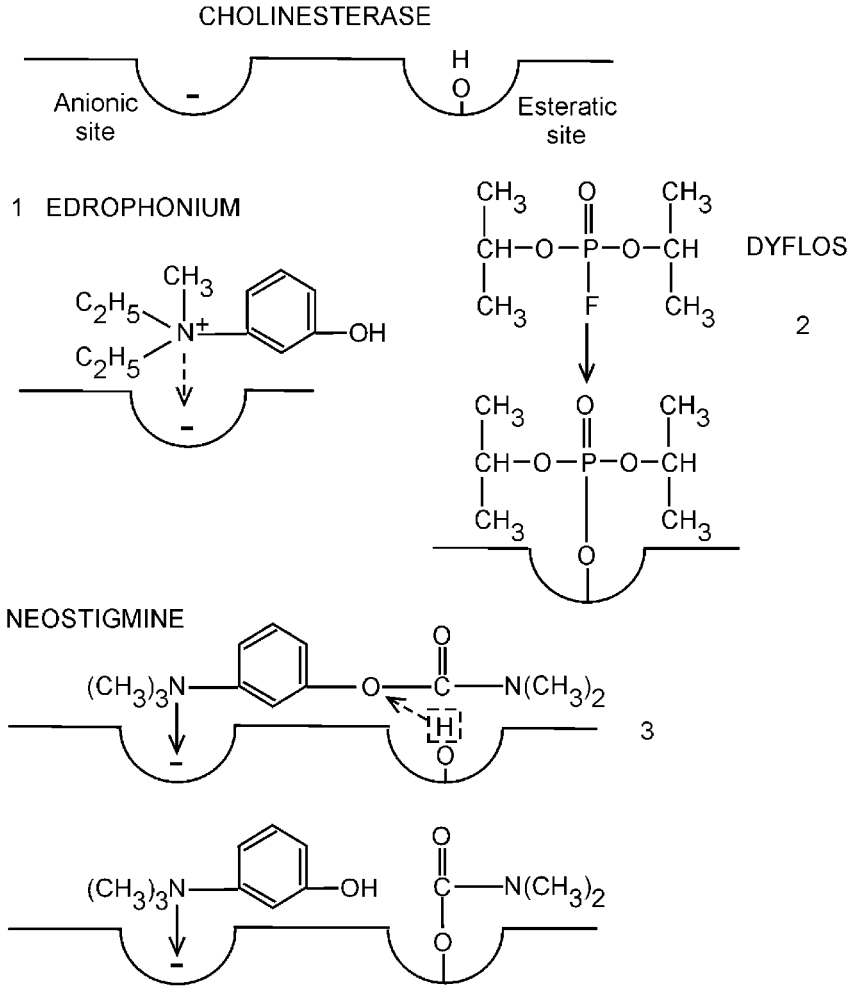

Figure 6.3 Modes of action of the anticholinesterase drugs. Cholinesterase, which has both an anionic and an ester site (Fig. 6.1), can be inhibited by drugs acting reversibly and irreversibly. Edrophonium is a short-acting inhibitor that binds reversibly with the anionic site (1) while DYFLOS reacts almost irreversibly with the esteratic site (2). Since hydrolysis of the enzyme is negligible new enzyme must be synthesised to overcome the effect of this very toxic compound. Clinically useful anticholinesterase like neostigmine have a medium duration of action ($\frac{1}{2}t = 1$ h). It binds to both sites on the enzyme (3) with the result that neostigmine itself is hydrolysed but the transfer of its carbamyl group to the enzyme's esteratic site produces a carbamylated enzyme which recovers by hydrolysis much more slowly (min) than after its acetylation by ACh (ms)

presumably from the soma and dendrites of DA neurons. Its function there is uncertain but purified preparation of the enzyme infused into the substantia nigra cause not only hyperpolarisation of the neurons, due to the opening of K^+ channels, but also a variety of motor effects in rats that are not related to its enzymatic activity and the turnover of ACh (see Greenfield 1991).

RECEPTORS

CLASSIFICATION AND STRUCTURE

As already mentioned, ACh acts on two distinct receptors: (a) nicotinic receptors, which mediate fast synaptic events and (b) muscarinic ones controlling much slower changes. This classification was originally based on the use of antagonists since atropine blocked only the slower events and curare only the fast ones. Their naming derives, perhaps unfortunately, from the fact that muscarine mimics the slow effects and nicotine the fast ones, initially anyway. As might be expected, the cholinergic receptors have been cloned and their structures established. In the CNS the muscarinic receptors outnumber the nicotinic possibly by 100:1, and, not surprisingly, they have been studied more extensively.

Nicotinic

Those receptors at the neuromuscular junction and in the electric organ of *Torpedo* have been studied much more than those in the CNS, but they all have similar characteristics. The peripheral receptor has four different protein subunits α, β, γ and δ but is pentameric with the α always doubled. In the CNS the receptors are less complex. Most have just two subunits α and β, but are again pentameric with 2α and 3β subunits situated around and forming the ion channel. Several variations of the α subunit, from $\alpha2$–$\alpha9$ ($\alpha1$ in periphery) and three in the β give the possibility for a number of different heteromeric receptors, although $\alpha4$ and $\beta2$ predominate and receptors with the configuration $\alpha4_2$ and $\beta2_3$ (2 of $\alpha4$ and 3 of $\beta3$) show the highest affinity for ACh. Homomeric assembled receptors of just $\alpha7$ subunits are also found. Each subunit folds into a four-transmembrane domain (m_1 m_2 m_3 m_4) with the m_3–m_4 loop linkage in the cytoplasm and the terminal amine and carboxyl groups extracellular (Fig. 6.4). (The accepted scheme for subunit and configuration numbering is outlined in Chapter 3).

The amino-acid sequence of each subunit is known and they are characteristic of a NT receptor that directly gates ion channels. Activation of the receptor requires 2 ACh molecules to combine with two α subunits. Pharmacologically it is not easy to distinguish between central and peripheral nicotinic receptors, let alone their variants. Those in the CNS are more like those found in peripheral ganglia than at the neuromuscular junction and are more readily blocked by dihydro-β-erythroidine than curare. Receptors containing $\alpha4\beta2$ subunits (the majority) are also not blocked by α-bungarotoxin but bind a shorter kappa or 'neuronal' bungarotoxin. Those receptors with the $\alpha7$ subunit, for which α-bungarotoxin has high affinity, will, however, bind that toxin.

Although drugs may not be able to distinguish between the subclasses of nicotinic receptor the last few years has seen the breeding of knock-out mice in which most of the

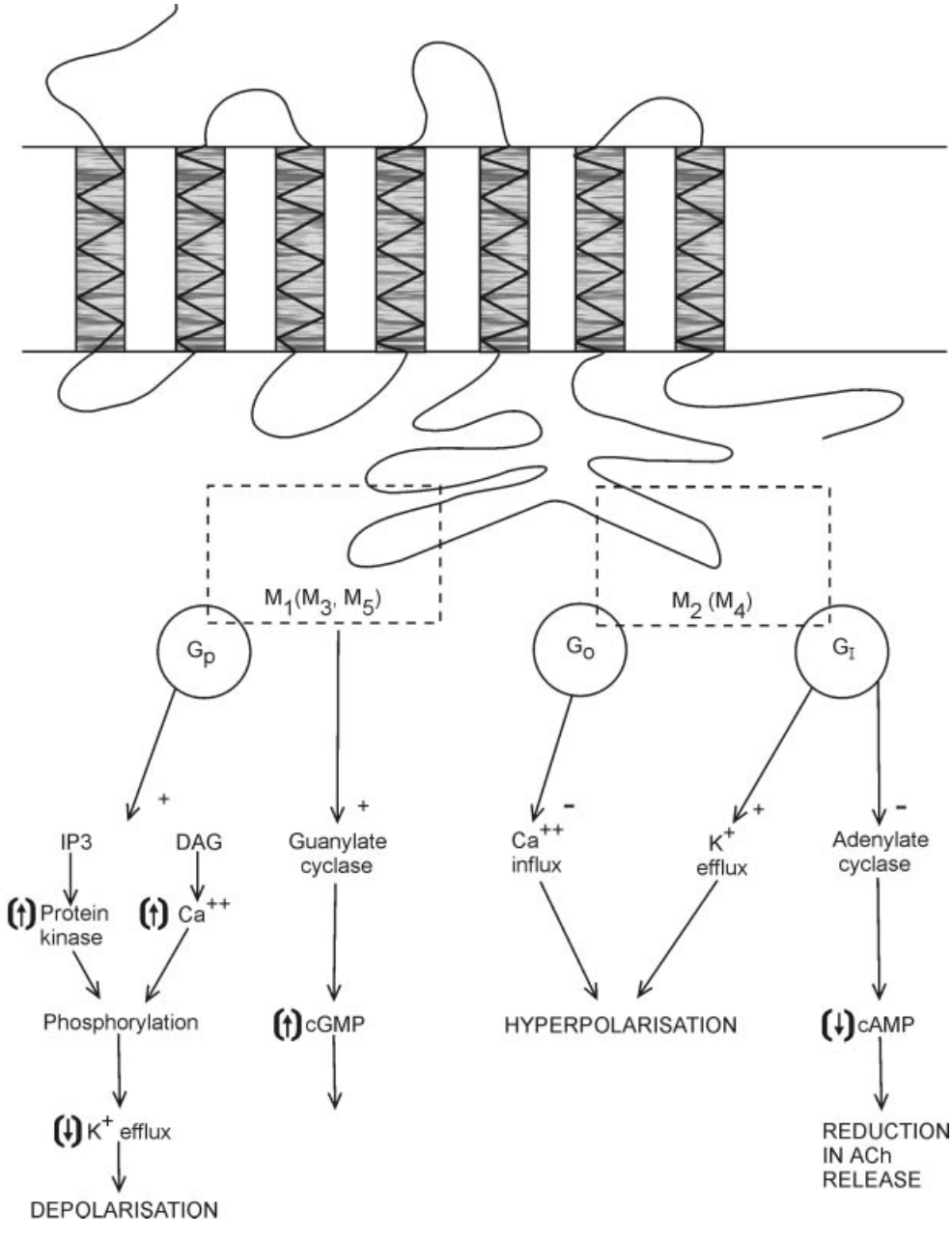

Figure 6.4 Schematic representation of the muscarinic receptor. All muscarinic receptors have seven transmembrane domains and the major difference between them is within the long cytoplasmic linkage connecting the fifth and sixth domains. This implies different G-protein connections and functions. Some possibilities are shown although the position of the M_1 and M_2 boxes is not intended to indicate their precise structural differences within the loop

mamalian nAChR subunits have been selectively deleted (see Cordero-Erausquin *et al.* 2000). While only those mice lacking subunits found mainly in peripheral nicotinic receptors (e.g. $\alpha 3$ and $\beta 4$) do not survive, others show little change in spontaneous behaviour but some reduced responses (less antinociception) to nicotine.

Muscarinic

Despite the wide variety of effects associated with the activation of muscarinic receptors on different peripheral organs it appeared that they were either identical or very similar because known antagonists, like atropine, were equally effective against all muscarinic responses. A decade ago, one drug, pirenzepine, was found to be a hundredfold more active against ACh-induced gastric acid secretion than against other peripheral muscarinic effects. The receptors blocked by pirenzapine became known as M_1 and all the others as M_2. Recently some differences between muscarinic M_2 receptors on heart (inhibitory) and those on exocrine glands (generally excitatory) became apparent through slight (fivefold) differences in the binding of some antagonist drugs (tools) such as AF-DX-116 and 4-DAMP. The former was more active on the receptors in the heart, accepted as M_2 receptors, while the glandular ones, blocked preferentially by 4-DAMP, became M_3. Molecular biology has since confirmed the existence of these three receptors and revealed (at the time of printing) two more — M_4 and M_5. The M_1 receptor mediates most of the central postsynaptic muscarinic effects of ACh while the M_2 is predominantly a presynaptic autoreceptor.

The structure of the muscarinic receptor is very different from that of the nicotinic. They are single-subunit proteins which belong to the group of seven transmembrane receptors (like adreno and dopamine receptors) typically associated with second messenger systems. The major difference between muscarinic receptors is in the long cytoplasmic linkage connecting the fifth and sixth transmembrane domain, suggesting different G-protein connections and functions. Thus M_1, M_3 and M_5 receptors are structurally similar and their activation causes stimulation of guanylate cyclase and an increase in cyclic GMP as well as inosotal triphosphate hydrolysis through an increase in G-protein (Gp) (Fig. 6.4).

The M_2 and M_4 receptors also show structural similarities. Through G-protein (G_1) they inhibit cyclic AMP production and open K^+ channels while activation of another G-protein (G_0) closes Ca^{2+} channels. The latter effect will cause membrane hyperpolarisation as will the G_1-induced increase in K^+ efflux. The reduction in cAMP production, although possibly leading to depolarisation, is more likely to explain the presynaptic reduction in ACh release associated with the M_2 receptor.

DISTRIBUTION

Cholinergic receptors should obviously be found where ACh is concentrated and cholinergic pathways terminate. Autoradiography with appropriately labelled ligands does in fact show M_1 receptors to be predominantly in the neocortex and hippocampus (where pathways terminate) and in the striatum where ACh is released from intrinsic neurons. By contrast, M_2 receptors are found more in the basal forebrain where ascending cholinergic pathways originate. Such a distribution is in keeping with the postsynaptic action of the M_1 receptor and the presynaptic cell body (autoinhibition) mediated effects of its M_2 counterpart. Unfortunately the ligands available for labelling

are not sufficiently specific to use this technique to reliably distinguish M_1 from M_3 and M_5 receptors or M_2 from M_4. *In situ* hybridisation studies of receptor mRNA, which detects cell body receptors, is more sensitive and confirms the M_1 dominance in the neocortex, hippocampus and striatum with M_2 again in subcortical areas. Receptor mRNA for the M_3 is, like that for M_1, in the cortex and hippocampus but not in the striatum while that for M_4 is highest in the striatum and low in the cortex. Elucidation of the precise functional significance of such a distribution awaits the arrival of much more specific ligands for the receptor subsets. In their absence a more detailed analysis of the distribution of muscarinic and nicotinic receptors is not justified here but see Hersch *et al.* (1994), Levey *et al.* (1991), Wall *et al.* (1991) and Wess (1996).

Nicotinic receptors have been found and studied predominantly in the hippocampus, cerebral cortex and viatral tegmented area (VTA).

FUNCTION

Activation of nicotinic receptors causes the rapid opening of Na^+ channels and membrane depolarisation. This is a feature of cholinergic transmission at peripheral neuromuscular junctions and autonomic ganglia but while it is found in the CNS, it is not widely observed. Exogenously applied nicotinic agonists have been shown to directly excite neurons through somato-dendritic receptors in various brain regions while the excitatory response of GABA interneurons in the hippocampus and dopamine neurons in the VTA following some afferent stimulation is reduced by nicotinic antagonists (see Jones, Sudweeks and Yakel 1999). Nicotinic receptors also mediate the fast response of ACh released at the endings of collaterals from motoneuron axons to adjacent inhibitory interneurons (Renshaw cells) in the ventral horn of the spinal cord (see below).

More recently much interest has been directed towards presynaptic nicotinic receptors that have been shown to enhance the release of a number of NTs, i.e. ACh, DA, NA, glutamate and GABA, in perfused synaptosomes or slices from various brain regions, as well as DA into microdialysates of the striatum *in vivo*. Thus they can be hetero- and not just autoreceptors (see Wannacott 1997). Since activation of these receptors can actually evoke, and not just facilitate, NT release, they probably work directly on nerve terminals to increase Na^+ influx and initiate sufficient depolarisation to activate voltage-sensitive Ca^{2+} channels, although there could also be an influx of Ca^{2+} itself through the nicotinic gated channel. In fact the high permeability of some neuronal nicotinic receptors to Ca^{2+} ions provides an obvious mechanism for increasing transmitter release. Differences in the sensitivity of the presynaptic receptors to various agonists and antagonists indicate some heterogeneity but their relatively low affinity for nicotine (EC_{50} about 1 μM) and the absence of clear evidence for their innervation means that their physiological role remains uncertain. Their activation by exogenous agonists could, however, have interesting therapeutic applications such as an increase in ACh release in Alzheimer's disease and mAChRs have been found to be reduced in the cortex and hippocampus of such patients.

Although ACh does not have a primary excitatory role like glutamate in the CNS, it does increase neuronal excitability and responsiveness, through activation of muscarinic receptors. It achieves this in two ways, both of which involve closure of K^+ channels (see Chapter 2 and Brown 1983; Brown *et al.* 1996). The first is a voltage-dependent K^+ conductance called the M conductance, Gm or Im. It is activated by any

attempt to depolarise the neuron, when the opening of the M-channel and the consequent efflux of K^+ counteracts the depolarisation and limits the generation of spikes. This current is inhibited by activation of muscarinic receptors and so ACh will tend to keep the neuron partially depolarised and facilitate repetitive firing and bust spiking. This slow cholinergic excitation in hippocampal neurons is shown in Fig. 6.5.

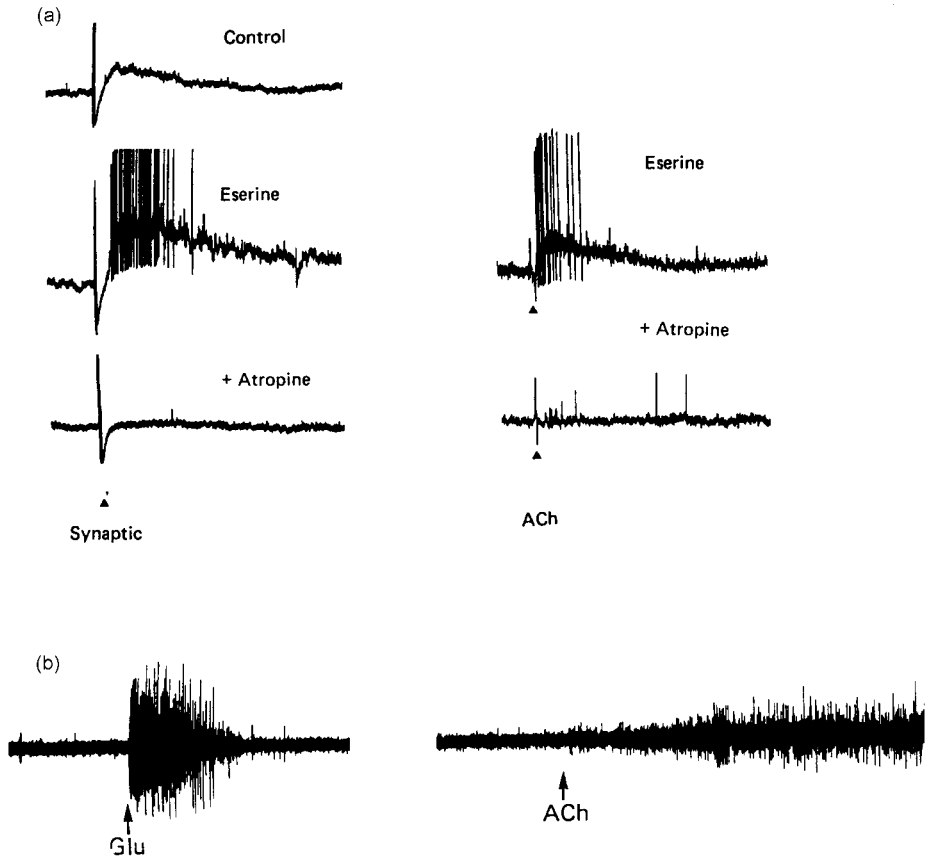

Figure 6.5 Illustrations of the slow excitatory effect of ACh. (a) Electrical stimulation (left-hand traces) of presumed cholinergic fibres (striatum oriens) in the rat hippocampal slice preparation (20 Hz for 0.5 s) induced a short latency epsp followed by an ipsp and a later slow epsp, recorded intracellularly in pyramidal neurons (control). The slow epsp was selectively potentiated by the anticholinesterase drug eserine (2 μM) with the generation of action potentials. This firing and the slow epsp, but not the fast epsp or ipsp, were eliminated by the muscarinic antagonist atropine (0.1 μM). The iontophoretic application of ACh (right-hand traces) in the presence of eserine only produced the slow epsp and superimposed firing which were also antagonised by atropine (resting membrane potentials 57–60 mV). These recordings show that the slow but not the fast epsp is cholinergic. (b) Extracellulary recorded multiunit response in layer V of the guinea pig anterior cingulate cortex slice preparation. Micropipette application of glutamate (10 ms of 1 mM) caused a rapid generation of action potentials up to 100 μV in amplitude while ACh (40 ms of 1 mM) only generated smaller (10–20 μV) potentials more slowly (50 ms) with larger ones (30–50 μV) appearing later. These results again demonstrate the slow excitatory effect produced by ACh compared with the larger and more rapid primary depolarisation of glutamate. ((a) reproduced with kind permission from the *Journal of Physiology* and from Cole and Nicoll 1984 and (b) from McCormick and Prince 1986a)

Studies of the hippocampal neurons have shown a second K^+ current which mediates the long-lasting after-hyperpolarisation following spiking. This is not voltage activated but is switched on by Ca^{2+} entry through channels opened during the initial depolarisation. It is inhibited by activation of muscarinic receptors and so its reduction will also lead to repetitive firing. ACh in fact seems to dampen the inbuilt brakes on cell firing (see also Chapter 2 and Figs 2.5 and 2.6).

M_1 and M_3 receptors mediate the excitatory effects and since this postspike hyperpolarisation is blocked by phorbol esters and is therefore presumably dependent on IP_3 production, one would expect it to be mediated through M_1 receptors (see above), especially as these are located postsynaptically. Unfortunately it does not appear to be affected by pirenzapine, the M_1 antagonist. By contrast, muscarinic inhibition of the M current is reduced by the M_1 antagonist but as it is not affected by phorbol esters is not likely to be linked to IP_3 production, an M_1 effect.

ACh can sometimes inhibit neurons by increasing K^+ conductance and although it has been found to hyperpolarise thalamic neurons, which would normally reduce firing, strong depolarisation may still make the cell fire even more rapidly than normal. This appears to be because the hyperpolarisation counters the inactivation of a low-threshold Ca^{2+} current which is then activated by the depolarisation to give a burst of action potentials (McCormick and Prince 1986b).

AGONISTS AND ANTAGONISTS

Many drugs bind to cholinergic receptors but few of them enter the brain and those that do are not noted for their effects.

AGONISTS

Some agonists, such as methacholine, carbachol and bethanecol are structurally very similar to ACh (Fig. 6.6). They are all more resistant to attack by cholinesterase than ACh and so longer acting, especially the non-acetylated carbamyl derivatives carbachol and bethanecol. Carbachol retains both nicotinic and muscarinic effects but the presence of a methyl (CH_3) group on the β carbon of choline, as in methacholine and bethanecol, restricts activity to muscarinic receptors. Being charged lipophobic compounds they do not enter the CNS but produce powerful peripheral parasympathetic effects which are occasionally used clinically, i.e. to stimulate the gut or bladder.

Pilocarpine, arecoline and, of course, muscarine itself are naturally occurring muscarinic agonists, while oxotremorine is a synthetic one, which, as its name implies, can cause muscle tremor through a central effect.

In view of the preponderance of muscarinic receptors in the CNS and the conceived need to augment the muscarinic actions of ACh in the treatment of Alzheimer's disease, much attention has been given recently to the synthesis of agonists that penetrate the blood–brain barrier, especially those that act specifically on M_1 receptors.

Few drugs, apart from nicotine itself, act specifically on nicotine receptors. One is methylcarbachol, which lacks the muscarinic effects of carbachol and another is dimethylphenylpiperazinium (DMPP), which appears to have some selectivity for the neuronal nicotinic receptor. Neither of them can cross the blood–brain barrier.

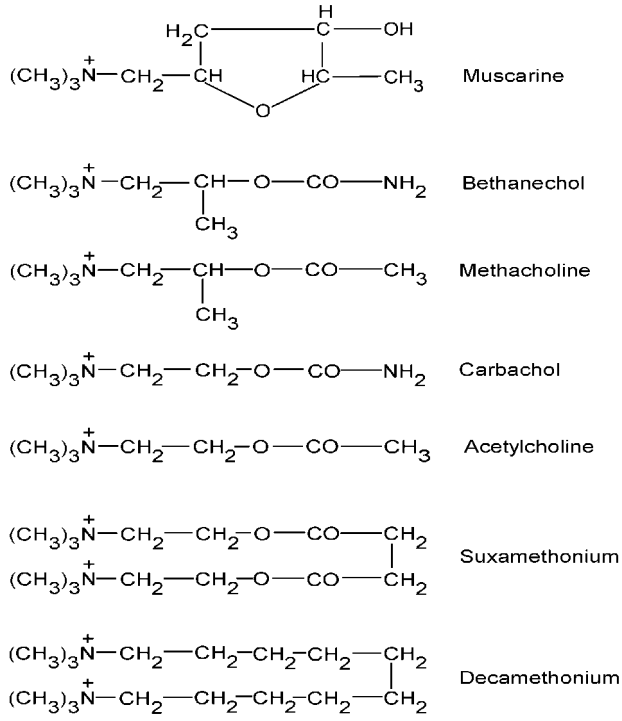

Figure 6.6 Structure of some cholinergic agonists and antagonists. Acetylcholine has the structure to activate both muscarinic and nicotinic receptors. Carbachol retains these actions but is longer acting because it lacks the terminal methyl group and is not so readily hydrolysed by cholinesterase (see Fig. 6.1). Methacholine with the methyl side chain lacks nicotinic activity but can be hydrolysed while bethanechol has a similar action but, like carbachol, is not easily hydrolysed. They show chemical similarities to muscarine. Suxamethonium is like two acetylcholine molecules joined together and has transient nicotinic activity at the neuromuscular junction before desensitising (blocking) those receptors. Decamethonium has a similar but much longer blocking action because, unlike suxamethonium, it is not hydrolysed by plasma cholinesterase

Although nicotine receptors are few in number, the proven ability to stimulate NT release may initiate the search for more effective centrally acting agonists.

ANTAGONISTS

The neuromuscular blocking action of the poison *d*-tubocurarine (curare) has been known for a century. It works by competing with ACh for binding to the nicotinic receptor. Others have been developed such as suxamethonium (succinylcholine) which is essentially two molecules of ACh joined together (Fig. 6.6). Perhaps not surprisingly, it is initially an agonist that causes a depolarisation of muscle fibres and actual twitching, before producing a depolarisation block of transmission. There are a large number of competitive antagonists apart from curare, such as gallamine, pancuronium and atracurium, while decamethonium works like suxamethonium as a depolarising agent. They can be used to produce neuromuscular block and skeletal muscle paralysis in surgery and prevent the damage to limbs that can occur in the electroconvulsive

treatment of depression. An interesting pharmacological distinction between these two classes of neuromuscular blocking agents is that the effect of the competitive receptor blockers like curare can be overcome by increasing the concentration of ACh, which is achieved *in vivo* by giving an anticholinesterase, while the blocking action of the depolarising drugs is not reversed.

Drugs that block the nicotinic receptors on autonomic ganglia, such as hexamethonium, probably do so by actually blocking the Na^+ ion channel rather than the receptor. Generally these receptors appear to resemble the central ones more than those at the neuromuscular junction and dihydro-β-erythroidine is one drug that it is an effective antagonist in both ganglia and the CNS.

In contrast to the nicotinic antagonists and indeed both nicotinic and muscarinic agonists, there are a number of muscarinic antagonists, like atropine, hyoscine (scopolamine) and benztropine, that readily cross the blood–brain barrier to produce central effects. Somewhat surprisingly, atropine is a central stimulant while hyoscine is sedative, as least in reasonable doses. This would be the expected effect of a drug that is blocking the excitatory effects of ACh on neurons but since the stimulant action of atropine can be reversed by an anticholinesterase it is still presumed to involve ACh in some way. Generally these compounds are effective in the control of motion but not other forms of sickness (especially hyoscine), tend to impair memory (Chapter 18) and reduce some of the symptoms of Parkinsonism (Chapter 15).

DRUGS AND THE DIFFERENT MUSCARINIC RECEPTORS

While five different muscarinic receptors have now been distinguished, atropine and the other antimuscarinics discussed above show little specificity for any of them, although pirenzapine is most active at the M_1 receptor. Much effort has been expended in the search for more specific muscarinic agonists and antagonists and while a few compounds have emerged which, from binding studies at least, show some (but never dramatic) selectivity, the results have been somewhat disappointing. As M_1 receptors mediate the postsynaptic excitatory effects of ACh while M_2 cause autoinhibition of its release, then augmenting ACh activity requires an M_1 agonist coupled with an M_2 antagonist capable of crossing the blood–brain barrier as well as an M_1 antagonist that will not. Even then the peripheral effects of the M_2 antagonist such as dry mouth and blurred vision can be unpleasant. Such possible permutations of agonist and antagonists in the treatment of dementia are considered in more detail in Chapter 18.

CHOLINERGIC PATHWAYS AND FUNCTION

Three distinct and basic CNS neuronal systems were referred to in Chapter 1, namely: long-axon neurons, intrinsic short-axon neurons and those in brainstem nuclei with extensively branching and ramifying ascending axons. The ubiquitous nature of ACh as a NT is evidenced by it being employed as such in all three situations to some extent, although for the first it is mainly confined to the periphery where it is released from long-axon preganglionic fibres and somatic motor nerves to skeletal muscle. In the striatum it is released from intrinsic interneurons and in the cortex from the terminals of ascending axons from subcortical neurons in defined nuclei. See Fig. 6.7 for detail.

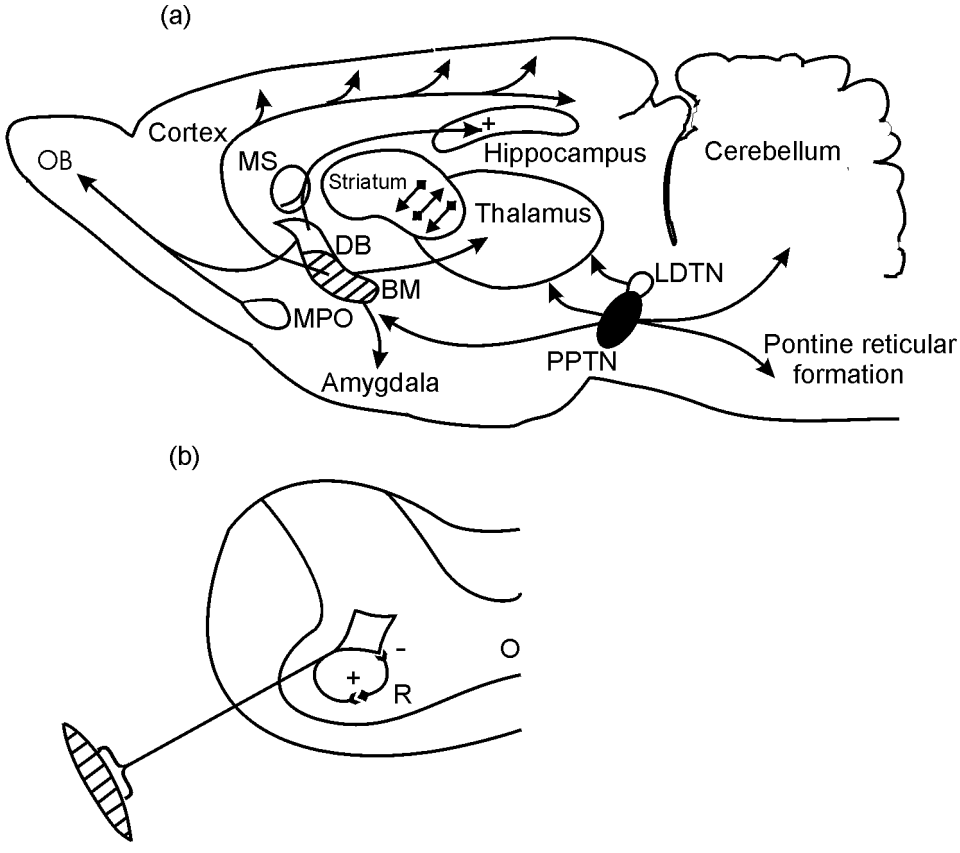

Figure 6.7 Cholinergic pathways. (a) Acetylcholine is found in intrinsic neurons within the striatum but the main pathways are the cortical projections from the nucleus basalis magnocellularis (BM) which also sends axons to the thalamus and amygdala. There are other projections from the medial septum (Ms) and the nucleus of the diagonal band, or diagonalis broco (DB), to the hippocampus and from the magnocellular preoptic nucleus (MPO) and DB to the olfactory bulb (OB). The DM and BM are sometimes referred to as the substantia inominata. Collectively all these nuclei are known as the magnocellular forebrain nuclei (FN). Other cholinergic nuclei are found more caudally in the tegmentum. The paramedian (or pendunculo) pontine tegmental nucleus (PPTN) sends afferents to the paramedian pontine reticular formation and cerebellum but more importantly to the thalamus (lateral geniculate nucleus) and the more cephalic cholinergic neurons in MPO. Activation of neurons in PPTN during REM sleep gives rise to the PGO (ponto–geniculo–occipital) waves (see Chapter 22). There is a smaller lateral and dorsal tegmental nucleus (LDTN) with afferents projections like that of the PPTN, especially to the thalamus, but its role is less clear (see Woolf 1991). In the ventral horn of the spinal cord (b) ACh is released from collaterals of the afferent motor nerves to skeletal muscle to stimulate small interneurons, Renshaw cells (R), that inhibit the motoneurons

SPINAL CORD

Since ACh is the transmitter at the skeletal neuromuscular junction one might also expect it to be released from any axon collaterals arising from the motor nerve to it. Such collaterals innervate (drive) an interneuron (the Renshaw cell) in the ventral horn of the spinal cord, which provides an inhibitory feedback onto the motoneuron. Not

only is ACh (and ChAT) concentrated in this part of the cord but its release from antidromically stimulated ventral roots has been demonstrated both *in vitro* and *in vivo*. Also the activation of Renshaw cells, by such stimulation, is not only potentiated by anticholinesterases but is also blocked by appropriate antagonists. In fact it illustrates the characteristics associated with both ACh receptors. Stimulation produces an initial rapid and brief excitation (burst of impulses), which is blocked by the nicotinic antagonist dihydro-β-erythroidine, followed, after a pause, by a more prolonged low-frequency discharge that is blocked by muscarinic antagonists and mimicked by muscarinic agonists. Thus in this instance although ACh is excitatory, as in other areas of the CNS, the activation of Renshaw cells actually culminates in inhibition of motoneurons. Pharmacological manipulation of this synapse is not attempted clinically and although administration of nicotinic antagonists that are effective at peripheral autonomic ganglia and can pass into the CNS, such as mecamylamine, may cause tremor and seizures, it cannot be assumed that this results from blocking cholinergic inhibition of spinal motoneurons.

STRIATUM

The concentration of ACh in the striatum is the highest of any brain region. It is not affected by de-afferentation but is reduced by intrastriatal injections of kainic acid and so the ACh is associated with intrinsic neurons. Here ACh has an excitatory effect on other neurons mediated through muscarinic receptors and is closely involved with DA (inhibitory) function. Thus ACh inhibits DA release and atropine increases it, although the precise anatomical connection by which this is achieved is uncertain and the complexity of the interrelationship between ACh and DA is emphasised by the fact that DA also inhibits ACh release. In view of the opposing excitatory and inhibitory effects of ACh and DA in the striatum and the known loss of striatal DA in Parkinsonism (see Chapter 15) it is perhaps not surprising that antimuscarinic agents have been of some value in the treatment of that condition, especially in controlling tremor, and that certain muscarinic agonists, like oxotremorine, produce tremor in animals.

CORTEX

Cholinergic neurotransmission has been most thoroughly studied in the cortex where the role of ACh as a mediator of some afferent input is indicated by the finding that undercutting the cortex leads to the virtual loss of cortical ACh, ChAT and cholinesterase. That it is not the mediator of the primary afferent input has been shown by the inability of atropine to block the excitatory effect of stimulating those pathways and the fact that such stimulation causes a release of ACh over a wide area of the cortex and not just localised to the area of their cortical representation (see Collier and Mitchell 1967). Indeed there have been many experiments which show that the release of ACh in the cortex is proportional to the level of cortical excitability, being increased by a variety of convulsants and decreased by anaesthesia (Fig. 6.8). The origins of this diffuse cholinergic input have been traced in the rat to the magnocellular forebrain nuclei (MFN) by mapping changes in cortical cholinesterase and ChAT after lesioning specific subcortical nuclei. The most important of them appears to be the nucleus basalis magnocellularis, similar to the nucleus of Maynert in humans, which projects predominantly to the frontal and parietal cortex and is thought to be affected

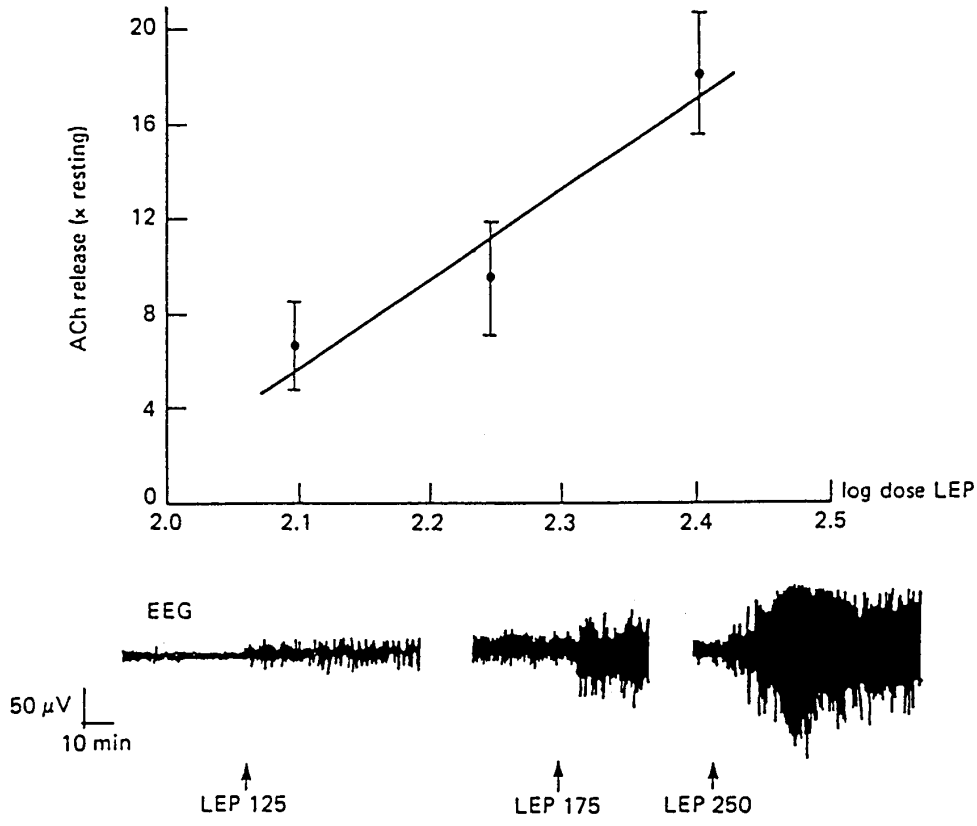

Figure 6.8 ACh release and cortical activity. Correlation between acetylcholine release and EEG activity after injections of leptazol (LEP mg kg^{-1} intravenously) into the urethane anaesthetised rat. ACh was collected in a cortical cup incorporating EEG recording electrodes. Mean values ±SE, $n = 6$ (unpublished data, but see Gardner and Webster 1977)

in Alzheimer's disease. This nucleus, together with the diagonal band, forms the substantia innominata and the dorsal neurons of this band also join with those in the medial septum to provide a distinct cholinergic input to the hippocampus (Fig. 6.7), which may play a part in memory function (see Chapter 18).

There is a second group of cholinergic neurons more caudally in the pontine tegmentum, the pendunculo pontine tegmental nucleus (PPPTN) and a smaller laterodorsal tegmental nucleus (LDTN). Their role in sleep and waking is discussed below and in Chapter 22.

Despite the excitatory effect of ACh in the cortex and its increased release during convulsive activity, antimuscarinic agents have only a slight sedative action (indeed, as emphasised above, atropine may cause excitation) and no anticonvulsant activity, except possibly in reducing some forms of experimentally induced kindling. ACh appears to exert a background excitatory effect on cortical function and while it may not directly stimulate the firing of pyramidal cells it will sensitise them to other excitatory inputs through its muscarinic activity.

AROUSAL AND SLEEP

Such a diffuse excitatory action of ACh in the cortex could fit it for a role in the maintenance of arousal and in fact the forebrain cholinergic nuclei, described above, appear to be innervated by the ventral part of the so-called ascending reticular system or pathway, which originates in a diffuse collection of brainstem neurons (see Chapter 22). If this pathway is lesioned the cortical EEG becomes quiescent but when stimulated it produces a high-frequency low-voltage desynchronised (aroused) EEG, which can be countered by antimuscarinic and potentiated by anticholinesterase drugs. Unfortunately this does not seem to apply to the actual behavioural arousal produced by such stimulation and suggests that ACh does not have a primary and certainly not a unique role in the maintenance of consciousness or sleep, although the firing of forebrain cholinergic neurons increases during the transition from sleep to waking. ACh does, however, feature prominently in one aspect of sleep behaviour.

As we relax in preparation for and pass into sleep, the active desynchronised 'awake' EEG characterised by the low-amplitude (5–10 μV) high-frequency (10–30 Hz) beta waves becomes progressively more synchronised giving larger (20–30 μV) and slower (8–12 Hz) alpha waves, and then even slower (1–4 Hz) and bigger (30–150 μV) delta waves. This so-called slow-wave sleep is interrupted at intervals of some 1–2 h by the break-up and desynchronisation of the EEG into an awake-like pattern. Since this is accompanied by rapid eye movements, even though sleep persists and can be deeper, the phase is known as rapid eye movement, REM or paradoxical, sleep. It is a time when dreaming occurs and when memory may be secured.

Such REM sleep may occur some four or five times during a night's sleep and can occupy 20% of sleep time. More importantly, for this discussion, it can be intensified by anticholinesterases and reduced by antimuscarinics and it is accompanied, and in fact preceded, by burst firing of a group of cholinergic neurons in the pedunculo pontine tegmental nucleus (PPTN). Neurons from this nucleus, which is quite distinct from the nucleus basalis, project to the paramedian pontine reticular formation, the thalamic lateral geniculate body and thus to the occipital cortex, all of which show increased activity during REM sleep to give PGO (ponto–geniculo–occipital) waves. Clearly sleep is not just a passive event and while cholinergic activity may be important in the production of REM sleep it does not appear to be responsible for turning it off or for actually inducing sleep. Many other NTs and neuronal networks come into this (see Chapter 22).

COGNITION AND REWARD

Not only is REM sleep a time for dreaming but it is also believed to be a time for the laying down (consolidation) of memory. This is only one observation among many that implicates ACh in the memory process. Certainly antimuscarinic drugs like atropine are well known to impair cognitive function in both animals and humans. In the former antimuscarinic drugs appear to impair both the acquisition and retention of some learned tasks, as in the Morris water maze. This involves placing a rat in a circular tank of water containing a stand with a platform just below the surface but which is not clearly visible because the vessel walls or water have been made opaque. Generally the rat quickly learns (2–3 trials) to identify the position of and swims to the platform. That ability is impaired by pretreatment with antimuscarinics which increase the number of

trials (possibly tenfold) required before the animal swims directly to the platform and can increase the time to achieve it if given after the task has been learnt.

Perhaps the strongest evidence for the role of ACh in cognitive processes comes, however, from the finding that in Alzheimer's disease there is a reasonably selective loss of cholinergic neurons in the nucleus basalis and that augmenting cholinergic function with anticholinesterase and to some extent by appropriate muscarinic agonists can help to restore memory function in the early stages of the disease. How cholinergic function can facilitate the memory process is uncertain. It is generally thought that the laying down of memory is in some way dependent on the high-frequency discharge of hippocampal neurons in which long-term potentiation or LTP (the persisting potentiated response to a normal afferent input after a prior and short intense activation) plays an important part (see Chapter 18). Unfortunately while NMDA antagonists impair LTP, antimuscarinics do not. Of course, ACh will, by blocking K^+ efflux, increase the likelihood of neurons discharging repetitively.

While it is the muscarinic receptor which is primarily concerned with the cognitive effects of ACh it has recently been shown that part of the cholinergic septal input to the hippocampus innervates excitatory nicotinic receptors on GABA interneurons. Since these appear to synchronise the activity of the main hippocampal glutamate neurons their stimulation could influence hippocampal function and memory process (see Jones, Sudweeks and Yakel 1999). The fact that there is a cholinergic projection from the pedunculo pontine tegmental nucleus to the dopomine neurons of the ventral tegmental area (VTA) and that its excitatory effect is mediated through nicotinic receptors could also implicate them and so ACh, in the reward process. This is thought to be mediated in part through the mesolimbic and mesocortical dopamine pathways arising from the VTA and may offer an explanation for the addictive nature of nicotine and smoking.

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