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Neuromuscular block

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Descriptions of the South American arrow poisons known as curares were reported by explorers in the 16th century, and their site of action in producing neuromuscular block was determined by Claude Bernard in the mid-19th century. Tubocurarine, the most important curare alkaloid, played a large part in experiments to determine the role of acetylcholine in neuromuscular transmission, but it was not until after 1943 that neuromuscular blocking drugs became established as muscle relaxants for use during surgical anaesthesia. Tubocurarine causes a number of unwanted effects, and there have been many attempts to replace it. The available drugs fall into two main categories: the depolarising blocking drugs and the nondepolarising blocking drugs. The former act by complex mixed actions and are now obsolete with the exception of suxamethonium, the rapid onset and brief duration of action of which remain useful for intubation at the start of surgical anaesthesia. The nondepolarising blocking drugs are reversible acetylcholine receptor antagonists. The main ones are the atracurium group, which possess a built-in self-destruct mechanism that makes them especially useful in kidney or liver failure, and the vecuronium group, which are especially free from unwanted side effects. Of this latter group, the compound rocuronium is of especial interest because its rapid onset of action allows it to be used for intubation, and there is promise that its duration of action may be rapidly terminated by a novel antagonist, a particular cyclodextrin, that chelates the drug, thereby removing it from the acetylcholine receptors.
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Introduction

Conventionally, it is understood that the term 'neuromuscular block' specifically refers to block of transmission by those drugs that interact with acetylcholine receptors located on the postjunctional face of the motor endplates of striated muscles. As exemplified by curare, these drugs are unusual and perhaps unique in that knowledge of their mechanism of action and their use as tools in physiological experiments preceded their widespread clinical use by almost a century. The converse is the case with most drugs of ancient origin (e.g. morphine, digitalis, aspirin) in that their therapeutic use long preceded the discovery of how they work. Neuromuscular blocking drugs, in particular curare and its most important purified alkaloid tubocurarine, are a prime example illustrative of the close liaison that has been and continues to be essential between physiology and pharmacology, for many physiological discoveries concerning cholinergic transmission in striated muscle and autonomic ganglia have depended upon the use of curare or tubocurarine.

Neuromuscular transmission and nondepolarising blocking drugs

Curare is one of the names coined by South American Indians to describe the plant-derived poisons that they used to coat the tips of their hunting arrows or blow-pipe darts. The poison is little absorbed after oral ingestion and hence the meat from animals killed with curare is harmless. The hunters developed what must be a very early example of a crude bioassay, in that they assessed the strength of their curare preparations in terms of the time it took to paralyse their prey. They referred to one-, two-, and three-tree curares. With a strong curare, a monkey fell paralysed while climbing the first tree. Conversely, a weak curare allowed the monkey to leap to two more trees in its attempted escape.

From the time of Columbus's voyages, exaggerated tales abound about the powers of the native South American weapon poisons. Such tales are given in the writings of 16th, 17th and 18th century explorers, including Sir Walter Raleigh. Portions of two main poisonous plants were used in the preparation of curares. These were the twisted vines of *Chondrodendron*, particularly *C. tomentosum*, and species of *Strychnos*, especially *S. toxifera*. The development of appropriate analytical methods enabled determination of the structure of the curare alkaloids, of which there are many. In Oxford, in 1935, King examined the constituents of museum specimens and was able to identify and characterise the first samples of (+)-tubocurarine (still called *d*-tubocurarine in the U.S.A.). *C. tomentosum* is a rich source of (+)-tubocurarine. King made a minor error in his proposed structure in that he deduced that both nitrogens were quaternised. In fact, as Everett and his co-workers showed 35 years later, one of the nitrogens of tubocurarine is tertiary, although its pK_a value is such that in most molecules it is protonated and therefore charged at body pH values.

In Paris, the French physiologist, Magendie, received samples of curare from Napoleon III and from the explorer

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von Humboldt on the latter's return to Europe. Magendie, who worked before the development of anaesthetic techniques, often used curare to immobilise animals for his physiological experiments. Claude Bernard worked in the same laboratory and was familiar with Magendie's use of curare. The classical experiments of Claude Bernard and his pupil Vulpian in the middle of the 19th century to locate the site of action of curare between the motor nerve and the muscle, that is, at the motor endplate, are familiar to all. At that time, Kühne and others had described the existence of the motor endplate. For a brief history of the early discovery and pharmacology of the curare alkaloids, an article by Bowman (1983) may be consulted.

The next important pharmacological step in the development of the concept of the mechanism of action of curare probably derived from the experiments of Langley in 1906 and 1907 in Cambridge. Langley studied the actions and interactions of nicotine and curare on chicken and frog muscles, including chronically denervated frog muscle. Nicotine stimulated the muscle in small doses and blocked the effect of nerve stimulation in large doses. More recently, the blocking action of nicotine was extensively studied in the cat by Paton & Savini (1968) and shown to be a consequence of depolarisation. The mechanism of block by depolarisation is dealt with later. In Langley's experiments, curare blocked the stimulant action of nicotine in both innervated and chronically denervated muscles, showing that its actions were exerted mainly on muscle rather than on the nerve endings, yet it did not affect the response of the muscle to direct stimulation. The concept of a lock-and-key mechanism for enzyme–substrate interaction had already been put forward by Fischer at the end of the 19th century, and du Bois-Raymond and Elliott soon thereafter had at least contemplated the idea of chemical transmission at sympathetic nerve endings. Langley concluded that nicotine and curare combined with a specific 'receptive substance' in the muscle, and he deduced that the nerve impulse crossed the junction, not by an electrical discharge, but by the secretion of what we now call a neurotransmitter. However, it took more than 30 years before Dale and his co-workers in London established that transmission at the neuromuscular junction is brought about by acetylcholine. In these experiments, especially those of Dale *et al*. (1936), curare, or rather the then recently available tubocurarine, was an important tool, since it blocked the responses of the muscle both to nerve stimulation and to the putative transmitter acetylcholine. Additionally, these experiments showed that the main action of tubocurarine was to block the effects of acetylcholine rather than to impair its release from the nerve, and so the concept of specific cholinoceptors present on the motor endplate membrane was becoming firmly established. Full references to the earlier workers in the field are given in Bowman (1983; 1990).

The eventual isolation and characterisation of these nicotinic acetylcholine receptors, involving irreversible ligand binding with snake toxins, immunological and electrophysiological techniques, and the knowledge of their synthesis, turnover and destruction is a fascinating story that has been reviewed many times (see, e.g., Colquhoun, 1986; Bowman, 1990; Vincent & Wray, 1990).

Postjunctional nicotinic receptors

We now know that the postjunctional receptors are located essentially only on the shoulders of the folds of the postjunctional face of the motor endplate, at a density of around 10,000 per μm^2. The structure of a motor endplate nicotinic receptor is shown in Figure 1. They are of the cys-loop superfamily of transmitter-gated ion channels, each composed of five protomers, and they have the stereochemistry when viewed from the junctional cleft and in anticlockwise order: α_1, ε, α_1, δ, and β_1. Before innervation, a so-called γ protomer is present in place of the ε protomer. Innervation turns on the gene for the synthesis of the ε protomer, which replaces the γ protomer. Each α-subunit possesses a binding site for acetylcholine – actually at the $\alpha\varepsilon$ and $\alpha\delta$ borders. Binding of acetylcholine to one facilitates binding to the other, but both have to be bound to activate the receptor. We also know that the lipid composition of the membrane around the receptors is important. It must contain about 50% cholesterol molecules for correct receptor function. The receptors at the innervated neuromuscular junction are not free to float about in the plasma membrane. They are anchored to the cytoskeleton by mechanisms involving a chorus of proteins including agrin and rapsyn.

A patch-clamp electrode applied to an adult mammalian receptor shows that acetylcholine produces a series of rectangular current pulses of equal amplitude, but different durations, carried by Na^+ through the open channels (Colquhoun, 1986). The characteristics of the single current pulse at 37°C and at normal resting membrane potential of a human receptor are a current amplitude of around 3.5 pA, a conductance of around 60 pS, and a mean duration (i.e. a mean channel open time) of around 6.5 ms. A change in the concentration of acetylcholine produces a corresponding change in the frequency of channel opening, but amplitude, conductance and mean open time do not change. In the presence of functional acetylcholinesterase, as in the intact neuromuscular junction, any one acetylcholine molecule survives long enough to open only one receptor ion channel at the most. The endplate current of Na^+ flowing across the postjunctional endplate membrane in response to acetylcholine released by a nerve impulse is therefore the summed response of the thousands of activated individual channels, each opened only once for a few ms. The endplate current depolarises the endplate membrane to produce the endplate potential of about 40 mV.

If tubocurarine as well as acetylcholine is present in the patch-clamp pipette, the effect of acetylcholine is not abolished, but instead the frequency with which it opens the receptor channel is reduced, the effect resembling that of a reduction in acetylcholine concentration. Unlike an irreversible receptor antagonist such as α-bungarotoxin, tubocurarine does not inactivate the receptor but merely reduces the probability of acetylcholine activating it. Obviously, despite its continued presence in the biophase, tubocurarine does not remain in contact with the receptor, but rather it repetitively associates with and dissociates from its binding site. This is the action expected of a reversible competitive receptor antagonist, and Jenkinson (1960) in fact showed that tubocurarine is a competitive antagonist of acetylcholine when studied under equilibrium conditions. Equilibrium conditions cannot be attained *in vivo*, and purists object to the term 'block by competition' on these grounds. For this reason, the less precise terms, 'nondepolarising block' and 'nondepolarising blocking drug', are used to distinguish drugs of the tubocurarine type from the depolarising blocking drugs and their effects, which are described below.

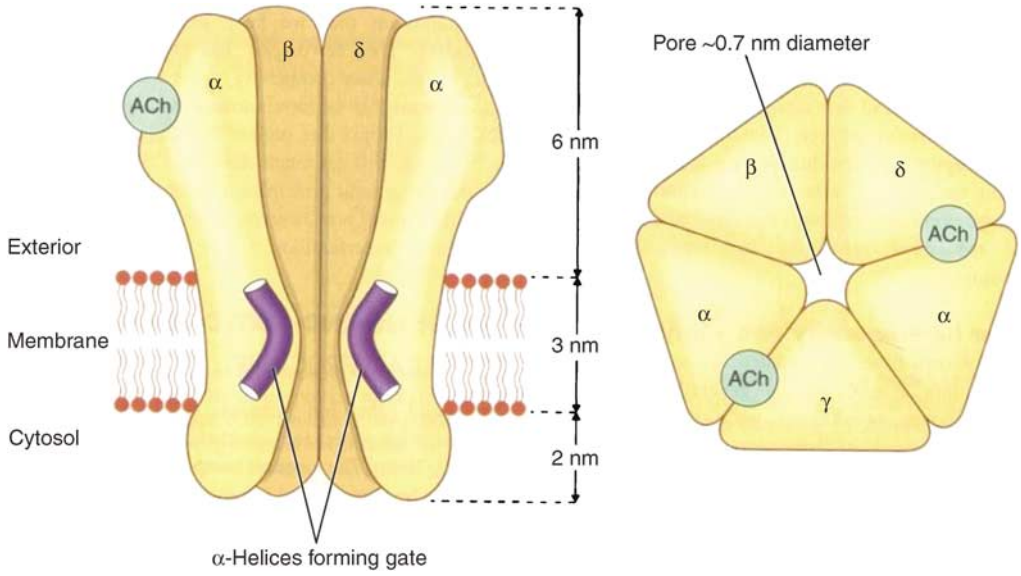

Figure 1 Structure of the motor endplate nicotinic acetylcholine receptor in side-view (left) and plan-view (right). The five protomers form a cluster surrounding a central transmembrane pore, the lining of which is formed by the M2 helical segments of each subunit. These contain a preponderance of negatively charged amino acids, which make the pore cation selective. There are two acetylcholine (ACh)-binding sites in the extracellular portion of the receptor at the interfaces between the $\alpha\gamma$- and $\alpha\delta$-subunits. The second ACh-binding site is invisible in the side-view because it is hidden by the α-subunit. When ACh binds, a conformational change is transmitted through the receptor proteins that results in the opening of the channel pore. The receptor shown is a foetal receptor. When the motor nerve grows out to innervate the muscle fibre, it switches off the RNA that specifies the γ-subunit and switches on the RNA that specifies an ε-subunit. The figure is reproduced with permission from *Pharmacology* 5th Edition (2003) by Rang, H.P., Dale, M.M., Ritter, J.M., and Moore, P.K. Churchill Livingstone/Elsevier Science, Edinburgh. The figure is based on original studies by N. Unwin.

Depolarising blocking drugs

Bacq & Brown (1937), in London, showed that when junctional acetylcholinesterase is inhibited, acetylcholine itself, either injected or released from the motor nerve by high-frequency stimulation, can cause neuromuscular block. In 1948, Barlow & Ing, in Oxford, and, independently, Zaimis, in London, synthesised a series of polymethylene bistrimethyl ammonium salts (chlorides or iodides) of the general structure: $(CH_3)_3N^+(CH_2)_n\text{-}N^+(CH_3)_3$, known as the methonium compounds. Zaimis (1950) delayed for 2 years before publishing her synthetic method. Among the methonium compounds, that in which $n=10$, called decamethonium (Figure 2), produced a neuromuscular block that differed in its characteristics from that produced by tubocurarine (Paton & Zaimis, 1949), but in fact resembled that produced by excess acetylcholine. Burns & Paton (1951) later showed that a similar type of transmission block could be produced in the gracilis muscle of the anaesthetised cat by applying a cathodal (depolarising) electrode to the endplates. The blocks produced either by the cathodal electrode or by decamethonium or acetylcholine could be reversed by applying an anodal (hyperpolarising) electrode to the endplates. Burns & Paton (1951) further showed that during the block a zone of inexcitability was present around the endplate region. The depolarised endplate acts as a current sink for adjacent regions of the muscle fibre membrane, and local circuit currents flow into it from the surrounding normally polarised membrane. Outward flowing currents in the surrounding membrane briefly open the sodium channels in that region but subsequently inactivate them. With the normal method of intravenous administration of a depolarising drug, inactivation of voltage-dependent sodium channels keeps pace with the depolarization, so that no initial contraction is produced before the block. Block by endplate depolarisation is therefore analogous to the production of a prolonged refractory period in the region of the membrane immediately surrounding the depolarised endplate. The ability of decamethonium and other nicotinic agonists to depolarise the endplates has been confirmed by others, especially by the elegant experiments carried out by Wray (1981).

Block by depolarisation is, therefore, a real phenomenon, but it is frequently complicated by the secondary production of actions in addition to simple postjunctional nicotinic receptor activation. Hence, the block may exhibit two components, which are often referred to as Phases I and II. The mechanism underlying the transmission block changes to one that is apparently not a direct consequence of endplate depolarisation. The propensity to undergo this change differs under different circumstances and is species dependent and even muscle dependent within a species. Several explanations have been suggested for the apparent change in mechanism of action. These include receptor desensitisation, channel block, the possibility that the drugs are not full agonists but are partial agonists, nerve ending depolarisation and consequent reduction in acetylcholine release, inhibition of acetylcholine synthesis, and stimulation, consequent upon the endplate depolarisation, of an electrogenic sodium pump that repolarises the endplate membrane despite continuing block. These possible mechanisms have been discussed extensively elsewhere (Bowman, 1990).

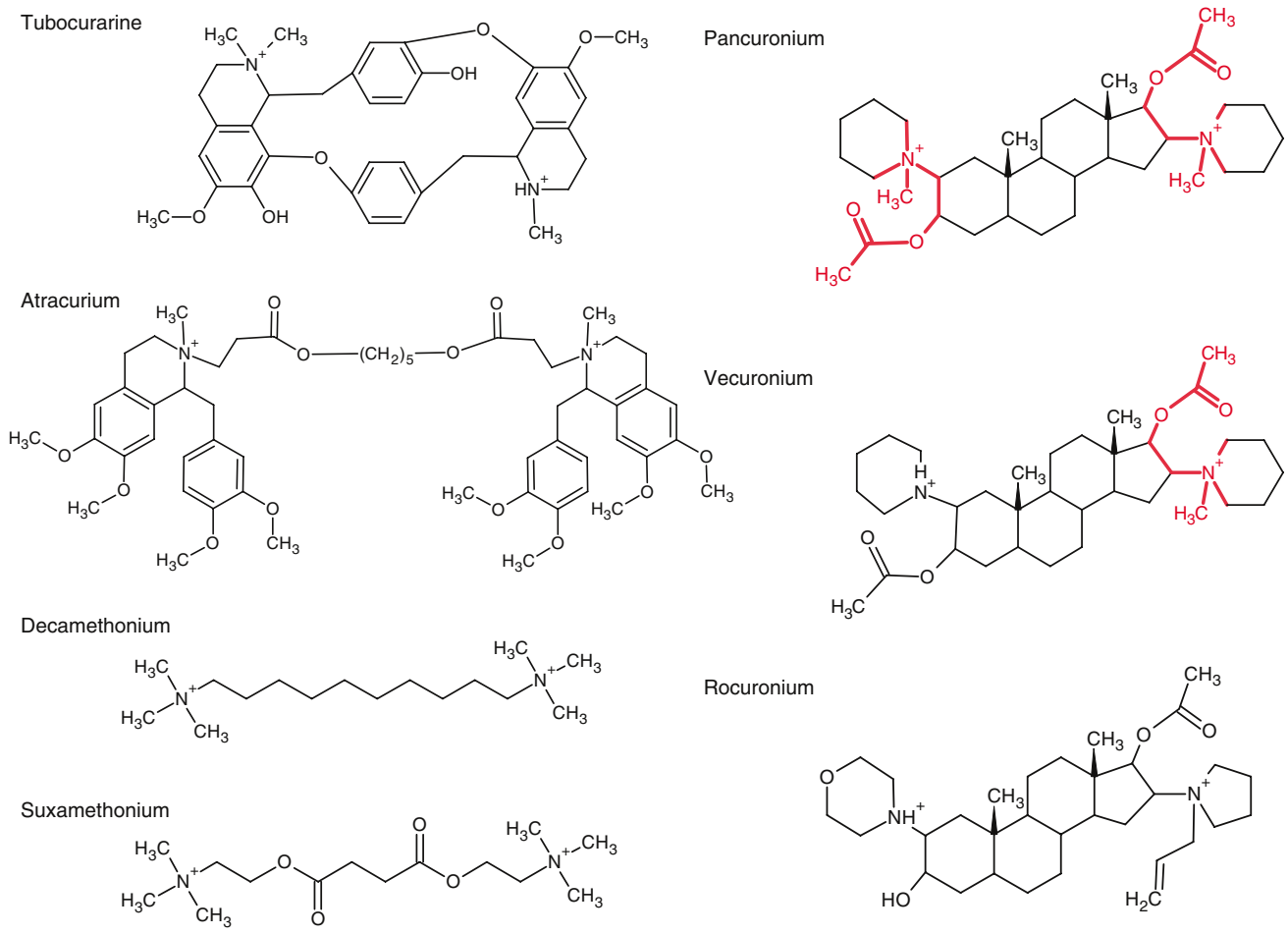

Figure 2 Structures of some neuromuscular blocking drugs. The acetylcholine moieties in pancuronium and vecuronium are shown in red.

Structure–action relations

Shortly after Claude Bernard's work on curare, Crum Brown and Fraser in Edinburgh, around 1868, showed that the methiodides and other quaternised salts of several alkaloids (including brucine, codeine, morphine, strychnine, atropine and coniine) all shared the common property, unlike the parent compounds, of paralysing skeletal muscles in a way that resembled the action of curare. This demonstration of the correlation between the quaternary ammonium function and neuromuscular blocking activity seems to have been the first valid structure–action relationship to be established in any pharmacological field. The subsequent observation that tubocurarine contains two positively charged nitrogens (albeit that one is a protonated tertiary amine; see Figure 2) led to the idea that most potent compounds contain two quaternary centres. An exception that has not yet been explained is the mono-nitrogen, tertiary amine dihydro-β-erythroidine, which shows considerable neuromuscular blocking potency, yet is greatly weakened in potency by quaternisation (Waser, 1972).

In general, a bisquaternary structure gives some selectivity for nicotinic acetylcholine receptors over most visceral muscarinic receptors (M_2 muscarinic receptors are often an exception), and an interonium distance of around 1–1.4 nm generally increases potency at the neuromuscular junction to the extent that ganglion blocking potency is negligible with neuromuscular blocking doses. However, here tubocurarine is an exception in that it has substantial ganglion blocking activity in neuromuscular blocking doses, probably because its molecules are flexible enough to allow them to interact with the ganglionic receptor. Although it clearly plays an important part, the actual function of the second charged nitrogen site in the molecule is not understood. It cannot be that the two charged centres allow each molecule to bridge two cholinoceptors, or the two α-subunits of the same receptor, because autoradiographic studies with labelled α-bungarotoxin and other studies show that the distances between receptors and the distances between the acetylcholine binding sites on the α-subunits of any one receptor are much greater than the 1–1.4 nm distance between the positively charged centres of each molecule. The most likely explanation, at least in relation to non-depolarising acetylcholine antagonists, seems to be that the second charged centre of the molecule must combine with an accessory anionic site that is not involved in binding acetylcholine (Hucho & Changeux, 1973). Occupation of each site must mutually facilitate appropriate two-point binding. This possibility is compatible with stoichiometric data that show tubocurarine binding is prevented by acetylcholine on a 1 : 1 basis (Neubig & Cohen, 1979).

Although depolarising blocking drugs are also synthesised with two onium centres (e.g. suxamethonium and decamethonium, Figure 2), it is not actually necessary for depolarising

activity (cf., acetylcholine itself, carbachol, nicotine, dimethylphenylpiperazinium and even the simple tetramethylammonium ion, all of which possess only one charged nitrogen). With depolarising drugs, it thus appears that two onium centres are preferable for entirely different reasons, connected with selectivity for muscle nicotinic receptors, and perhaps with lack of micelle formation.

On the basis of the large number of neuromuscular blocking drugs that had been obtained from plant sources or synthesised at the time, Bovet (1951) classified neuromuscular blocking drugs into two broad classes: (i) the *pachycurares* (Greek pachys = thick) are bulky molecules with the bulk residing either in the molecular unit bearing the charged onium centres, or in the substituents on the onium centres, or both; these have nondepolarising activity and (ii) the *leptocurares* (Greek leptos = slender) are thin flexible molecules with the substituents on the onium centres limited to methyl groups. These generally have depolarising activity. Hence, it is not difficult for a medicinal chemist to synthesise a compound that will possess neuromuscular blocking activity. The difficulties lie in creating a molecule that does not interact with receptors other than the nicotinic receptors of striated muscle, and that behaves pharmacokinetically and metabolically in human beings in an appropriate way insofar as the onset of action, duration and offset of action are concerned. These characteristics are determinable only in intact nerve muscle preparations, in whole animals and, finally, in human beings. They are not helped by studies on isolated cells or receptors.

Nicotinic receptors on motor nerve endings

Neuromuscular blocking drugs have played a large part in the acquisition of evidence for the existence of nicotinic receptors at the nerve endings. The matter remains controversial, but it has been proposed that two groups of such receptors exist. One group of preterminal receptors, the original evidence for their existence stemming largely from the work of W. Riker and his co-workers (see Bowman, 1990), is thought to be located in the region of the first node of Ranvier. This is the same region at which the presence of neuronal Na^+ channels ceases (Brigant & Mallert, 1982). These receptors are blocked by neuromuscular blocking drugs, including α-bungarotoxin. They are stimulated by nicotinic agonists, including acetylcholine released by nerve stimulation. The latter, however, occurs only when junctional acetylcholinesterase is inhibited, and for this reason it has been proposed that one of the functions of the junctional enzyme is to protect the nerve from this action of the transmitter. Stimulation of these preterminal receptors gives rise to antidromic impulses in the motor nerve as well as to orthodromic excitation and transmitter release (see, e.g., Blaber & Bowman, 1963). Since the transmitter does not excite these receptors when cholinesterase is normally functional, they are judged to have pharmacological but not physiological importance.

A second group of nicotinic receptors is thought to be located on the neuronal membrane close to or at the nerve terminals. These are pharmacologically different from the first group in that they are not blocked by α-bungarotoxin. They are blocked by reversible neuromuscular blocking drugs and also by hexamethonium. By using specific monoclonal antibodies, the presence of nicotinic receptors containing α_3-protomers has been demonstrated on motor nerve endings (Tsuneki *et al*., 1995). Such receptors, which, in the open state, are relatively selective for Ca^{2+}, are known to be insensitive to α-bungarotoxin. It is proposed that these nerve terminal autoreceptors play a physiological role in mobilising acetylcholine from the reserve to the readily releasable pool of transmitter, in order that release may keep pace with the demands of the high frequencies of nerve impulses that are characteristic of transmission to striated muscle (see, e.g., Bowman, 1989). Blockade of these autoreceptors by tubocurarine and similar drugs is thought to account for the so-called 'tetanic fade' that occurs during partial nondepolarising neuromuscular block. The evidence for, and the possible roles of, prejunctional nicotinic receptors have been reviewed several times, most recently by Bowman (1996).

Development of clinically useful neuromuscular blocking drugs

In the early years of anaesthesia, a sufficiently high and potentially dangerous dose of anaesthetic agent (ether, chloroform) was required in order to paralyse reflex muscle movements. In 1912, a German surgeon, Dr Läwen, used 'curarine', a partially purified material from calabash curare, as an adjunct to anaesthesia. He found that the safety of surgical anaesthesia was improved if only sufficient anaesthetic agent to produce unconsciousness was administered coupled with curarine to paralyse reflex actions. Dr Läwen's observations seem largely to have been ignored. About 28 years later, two Canadian anaesthetists, Griffith and Johnson, successfully used 'Intocostrin', a standardised extract of *C. tomentosum,* as a muscle relaxant in surgical anaesthesia. From this time on, the use of neuromuscular blocking drugs has become a standard technique employed by anaesthetists all over the world (see Bowman, 1990).

An effective means of reversing a tubocurarine block with an anticholinesterase agent was known long before the drug was used clinically as a muscle relaxant. Indeed, reversal of block by an anticholinesterase drug formed part of the evidence that the block is competitive in nature. Block by depolarisation is not reversed because the blocking drug and the persisting transmitter acetylcholine have essentially the same mechanism of action. Pal (1899) showed that physostigmine was capable of reversing block produced by curare, and from the time of the initial clinical use of neuromuscular blocking drugs in surgical anaesthesia the use of anticholinesterase agents, particularly neostigmine and pyridostigmine, has been standard procedure for restoring neuromuscular transmission. Such drugs are not ideal, however, for they produce their own range of side effects, particularly associated with excessive parasympathetic activity. Furthermore, there is a ceiling to their antagonistic action, the limit being imposed by the amount of transmitter acetylcholine that is released. Generally speaking, an anaesthetist would not attempt to reverse a nondepolarising block with an anticholinesterase drug until spontaneous recovery is well under way.

These days, the safe technique of so-called 'balanced anaesthesia' is largely used. In this technique, a relatively small dose of anaesthetic is given to produce unconsciousness, an analgesic is administered to abolish pain, and a neuromus-

cular blocking drug is injected in order to prevent reflex muscle movement. Neuromuscular blocking drugs, by themselves, have no effect at all on consciousness or pain sensation. Dr Frederick Prescott (father of our own Laurie Prescott!) was the first known individual voluntarily to receive tubocurarine in the absence of any other drug. He could not even change his facial expression to signal his distress. He describes his traumatic experience in a paper in the *Lancet* (Prescott *et al*., 1946). These days, the skill of the anaesthetist is required to ensure that sufficient anaesthetic is given to maintain the patient in an unconscious state. There are a number of reports from patients who have regained consciousness during an operation, while still totally paralysed. Their experiences resemble that of Dr Prescott! There is even a report of a U.S. 'death row' prisoner, in one of those states that continue with the barbaric death penalty, regaining consciousness but with maintained paralysis after having had a 'lethal injection'. For unexplained reasons, since presumably no surgical reflex-inducing procedure is carried out, such injections contain a neuromuscular blocking drug, as well as an intravenous anaesthetic and other toxic substances.

In the early days, curare extracts had to be standardised by biological assay, for which Holaday's rabbit head-drop method or Bülbring's isolated phrenic nerve-hemidiaphragm preparation from the rat (Bülbring, 1946) were used. Once the use of tubocurarine had been established as an adjunct to anaesthesia, the difficulty of obtaining it from its natural sources, together with some obvious disadvantages in its clinical use (Paton, 1959), soon led to attempts to find substitutes for it, either by modifying its structure or those of related alkaloids, or by synthesising new compounds. The depolarising blocking drug, decamethonium, synthesised and tested by Paton & Zaimis, had a brief clinical use, as had a few other depolarising drugs that were subsequently developed (e.g. carbolonium and the Russian drug dioxonium). However, their side effects, uncertainty of mechanism of action, and the difficulty of reversing their action, soon led to their abandonment.

The depolarising drug, suxamethonium (Figure 2), had been known as a chemical since 1906, but it was only during the era of depolarising blocking drugs in the 1950s that Brücke and Foldes and their co-workers (see Bowman, 1990) encouraged its clinical use as a muscle relaxant. Owing to its range of unwanted effects, the use of suxamethonium is now virtually restricted to single dose use, where its rapid onset of action and brief duration remain useful for intubation at the start of an operation. What is required, however, is a nondepolarising type of muscle relaxant with a time course of action like that of suxamethonium.

Newer neuromuscular blocking agents

By the early 1960s, relatively few compounds from the vast number synthesised were available to the anaesthetist, the main ones of the nondepolarising type being tubocurarine and its trimethyl derivative (metocurine), gallamine, laudexium, benzoquinonium and alcuronium. Generally speaking, as the complexity of surgical procedures increased, anaesthetists became dissatisfied with the available neuromuscular blocking drugs because of their side effects and inappropriate time course of action.

Armed with little more than the structure : action data described above, pancuronium (Figure 2) was designed on the drawing board by Hewitt and co-workers in the early 1960s (see Buckett *et al*., 1968). A steroid skeleton was chosen on which to append the quaternary nitrogens because, being a relatively rigid structure, an appropriate interonium distance of 1.1 nm was present in every molecule. It is a bulky molecule of Bovet's pachycurare type and therefore has a nondepolarising mechanism of action. Acetylcholine moieties were inserted into the molecule in the hope of increasing affinity for the receptors and of providing a possible substrate for inactivation by cholinesterases. It was first tested in animal experiments by Buckett *et al*. (1968). Pancuronium remains one of few drugs logically and successfully designed on the drawing board. At the time of its development, pancuronium was the most potent neuromuscular blocking drug available, and the fact that, after about 40 years, it is still used, reflects the success of its rational design. Nevertheless, pancuronium does have unwanted effects that can be disadvantageous in some types of operative procedure, and its onset of action and recovery rate are slow.

Around the time that Claude Bernard, in the mid-19th century, was studying the site of action of curare in the frog, the German chemist, W. Hofmann, working in London, demonstrated what came to be known as a Hofmann elimination reaction. He showed that if a quaternary ammonium compound is treated with NaOH and the solution is boiled, a carbon–nitrogen bond is broken and a tertiary amine is eliminated leaving an olefine. A hundred years or more later, in Glasgow, J.B. Stenlake and co-workers developed the opinion that a Hofmann elimination reaction in a neuromuscular blocking drug would occur at body pH and temperature providing that an appropriate electron withdrawing group and other features were inserted into the molecule. They worked with bisbenzyl-isoquinolinium molecules of the tubocurarine type. The main achievement of their studies was the now widely used compound, atracurium (Figure 2), which spontaneously breaks down in the body to inactive compounds through a Hofmann elimination reaction (Stenlake, 1982; Hughes & Payne, 1986). This self-destruct mechanism means that the termination of neuromuscular blockade is not dependent on metabolism or excretion, and therefore the drug is especially useful in patients with kidney, liver or multiorgan failure. *Cis*-atracurium is one of the optical isomers of atracurium. It is somewhat more potent than atracurium and its propensity to cause histamine release is less. In other respects, it is similar to atracurium and it has gained widespread use in anaesthetic practice.

Other improvements in neuromuscular block focused on two other aspects of clinical action, cardiovascular side effects and the rate of onset of block. Advances in these areas are discussed below.

Muscarinic receptor block

Over 50 years ago, rudimentary evidence that muscarinic acetylcholine receptors were not a homogeneous group was beginning to accumulate. Perhaps the first indication was the observation of Riker & Wescoe (1951), who showed that gallamine blocked cardiac muscarinic receptors (now called M_2 receptors) without affecting those of the gut or of most other peripheral tissues. It is now recognised that muscarinic receptors exist in five subtypes, but gallamine has relatively

selective affinity for the M_2 subtype. Pancuronium, like gallamine, although to a lesser extent, blocks muscarinic M_2 receptors and therefore blocks the cardiac vagus. It also blocks neuronal reuptake of noradrenaline ($Uptake_1$). During surgical operations, these effects combined may lead to a mild tachycardia and hypertension that is undesirable in certain procedures.

The best prediction of effective dose, time course of effect and cardiovascular side effects in man is, in our many years' experience, given by studies in the chloralose-anaesthetised cat. A large number of aminosteroidal compounds related to pancuronium were synthesised, after discussion, by industrial chemists under the direction of the late David Savage at Organon in Scotland. We tested these compounds in anaesthetised cats for their ability to block contractions of hind limb muscles, and to affect the arterial blood pressure, the responses to stimulation of the centrally cut efferent cardiac vagus, and to a muscarinic agonist, and the contractions of the nictitating membrane to preganglionic stimulation. Effective compounds selected from this basic screening programme were then subjected to other tests involving electrophysiological analysis, biochemical tests, and metabolism and excretion studies.

After testing a large number of such compounds it became clear to us that in this series of compounds, the abilities to block cardiac M_2 receptors and neuronal noradrenaline reuptake are associated with the presence of an acetylcholine moiety at the ring A end of the molecule. The simple analogue of pancuronium that differs only in that the ring A nitrogen is tertiary (thereby having lost its acetylcholine moiety) is about equipotent with pancuronium in its neuromuscular blocking action, yet it is nearly 100 times less potent in its ability to block the cardiac vagus and it has little or no action on noradrenaline uptake. This is the effective compound vecuronium (Figure 2, Durant *et al*., 1979; Bowman & Sutherland, 1986; Miller, 1986), which is widely used in anaesthetic practice. It is free from cardiovascular effects in human patients. The effects of vecuronium in the anaesthetised cat are illustrated in Figure 3. For contrast, the effects of a drug that does block the cardiac vagus and which also blocks $Uptake_1$ in neuromuscular blocking doses are shown in Figure 5.

It is noteworthy that the two most widely used classes of neuromuscular blocking drugs in the world today, the atracurium group and the vecuronium group, which virtually share the world market, have their origin in the same University (the University of Strathclyde in Glasgow). They were developed simultaneously in secret, each research group in different departments being sworn to secrecy by their industrial sponsors.

Rapid onset and offset

A desirable feature in a new nondepolarising neuromuscular blocking drug is a rapid onset of action that by itself permits intubation without the need for an initial paralysis, separately produced by suxamethonium. Brief duration and rapid recovery (or at least rapid reversibility by another agent) are also desirable properties so that spontaneous breathing quickly recommences in those rare instances when intubation turns out to be impossible.

In studying more than a hundred related aminosteroidal compounds, it became clear to us that rapid onset of action was generally associated with lack of potency. Our early observation of this effect is reported by Bowman *et al*. (1988). Strictly speaking, *in vivo,* rapid onset is associated with a high plasma C_{max}, but with a series of chemically related compounds, rank order of potency is largely the same as rank order of C_{max}. The relationship is of course a general one; there will be occasional exceptions especially in so far as dose is concerned. If, for example, a particular compound has a volume of distribution that is greater than expected because of some unsuspected pharmacokinetic characteristic, such as excessive binding to plasma proteins or to cartilage, then its

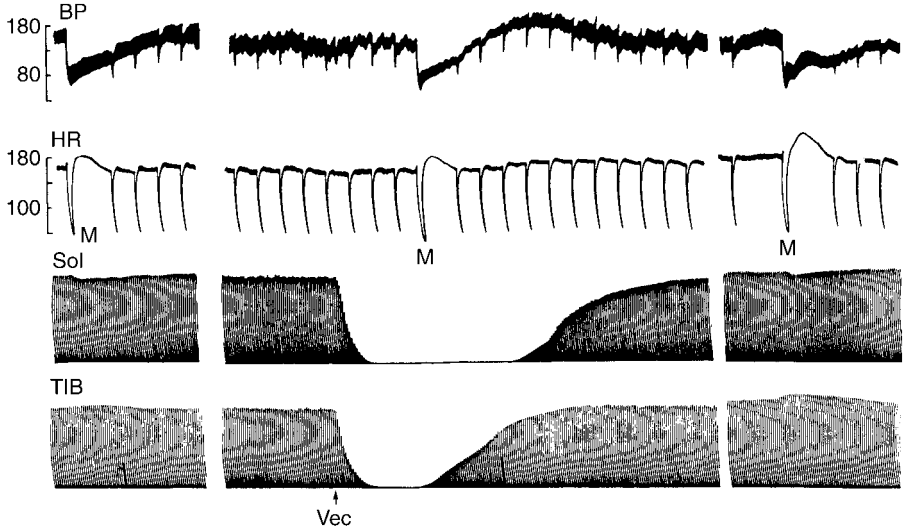

Figure 3 Records from a cat under chloralose anaesthesia, with both vagi cut. Records from above downwards are arterial blood pressure (BP), heart rate (HR, beats min^{-1}), maximal twitches of the soleus (S) and tibialis anterior (T) muscles evoked by stimulation of the motor nerve at a frequency of 0.1 Hz. Every 100 s, the peripheral end of the right vagus nerve was stimulated (8 Hz for 5 s) to produce falls in heart rate. At M, 10 $\mu g\,kg^{-1}$ methacholine was injected intravenously. At Vec, 40 $\mu g\,kg^{-1}$ vecuronium was injected intravenously. Note that vecuronium was without effect on responses to vagal stimulation and to methacholine. Reproduced with permission from Bowman & Sutherland (1986).

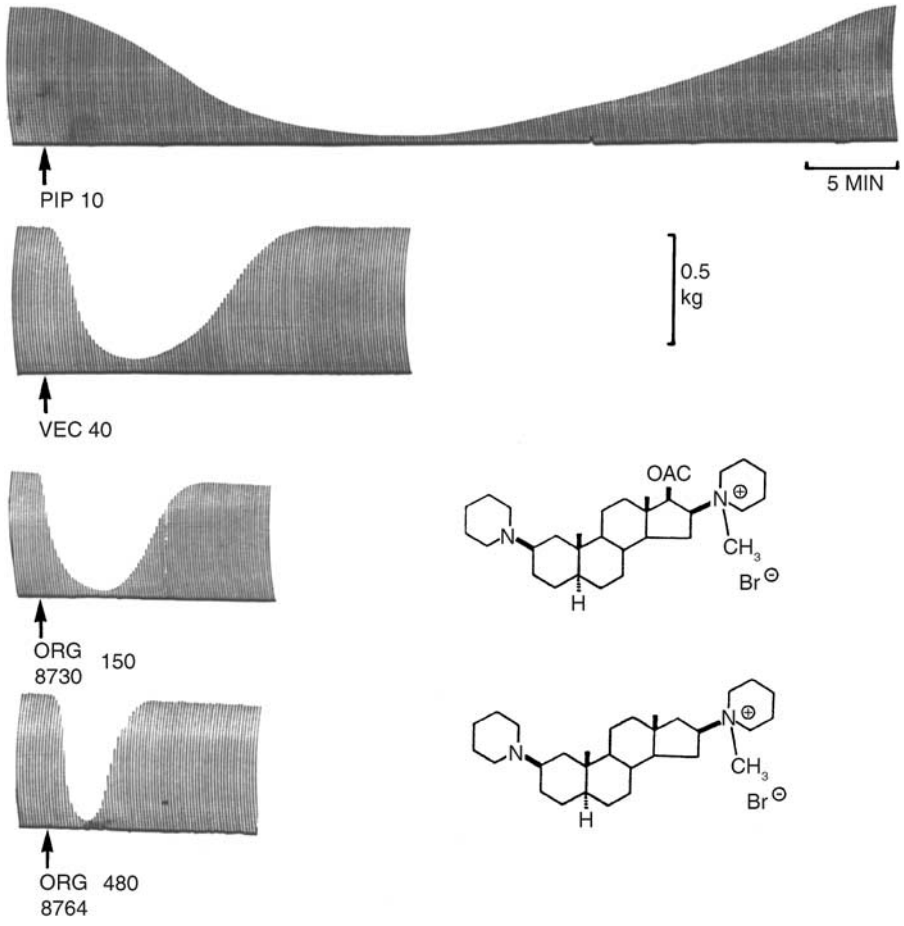

Figure 4 Records from cats under chloralose anaesthesia. Maximal twitches of tibialis anterior muscles evoked by stimulation of their motor nerves at 0.1 Hz in four different experiments. Typical blocks (approx. 90%) are shown in response to pipecuronium (PIP), vecuronium (VEC) and two other Organon compounds (8730 and 8764, whose structures are shown on the right of the record). The numbers denote the doses in $\mu g\,kg^{-1}$. Generally, highly potent drugs have slower onset and longer duration than less potent drugs. Slightly modified and reproduced with permission from Bowman (1990).

concentration in the junctional cleft will be lower than supposed from the dose injected and consequently its speed of onset will be slower.

A graph of potency vs onset time of a large range of chemically related drugs is a rectangular hyperbola. At one end of the graph, the curve becomes asymptotic towards the circulation time, because time of onset cannot be shorter than the circulation time. At the other, highly potent, end, the curve must become asymptotic towards the minimum number of molecules of infinitely high affinity necessary to block sufficient endplate receptors to prevent muscle activation. Of course, rapid onset of block can also be achieved by giving a huge dose of a potent drug, but this occurs only at the expense of very prolonged duration that will last until the biophase concentration falls below the blocking level. For rapid offset coupled with rapid onset, a drug that lacks potency is necessary, unless a large dose of a potent drug happens to be metabolised or redistributed at an extremely high rate, in which case recovery will be swift. The general observation relating rapid onset and offset to lack of potency is illustrated for four compounds in Figure 4.

The results showed that it should be possible to produce a nondepolarising type of drug with a time course of action resembling that of suxamethonium. Indeed, in the cat, the compound referred to as Org 8764, illustrated in Figure 5, is in fact faster in onset and shorter in duration than suxamethonium. The problems are that the greater the dose required, the more expensive is the drug to produce and the more likely it is to produce unwanted side effects. This last is illustrated by the compound Org 8764 (Figure 5). Here, it is seen that the effective dose is so high that in a neuromuscular blocking dose, the drug also blocks the response of the heart to vagus stimulation, and it causes tachycardia and a rise in blood pressure (shown in other experiments to be a consequence of an M_2 receptor blocking action coupled with an $Uptake_1$ blocking action). Such a compound is not worth developing for clinical use.

A compromise is required that is acceptable in terms of expense and side effects and yet which is fast enough in onset to permit intubation. The compound called rocuronium (Org 9426, Figure 2) is such a compromise (Muir *et al*., 1989). It is widely used by anaesthetists in many countries. It retains the tertiary nitrogen at the ring A end of the molecule, thereby minimising its cardiovascular effects. However, it is one of the rare exceptions to the general rule, for despite its rapid onset of action, its duration is similar to that of vecuronium.

Redistribution or metabolism of the drug is an over-riding determinant of the commencement of recovery, because

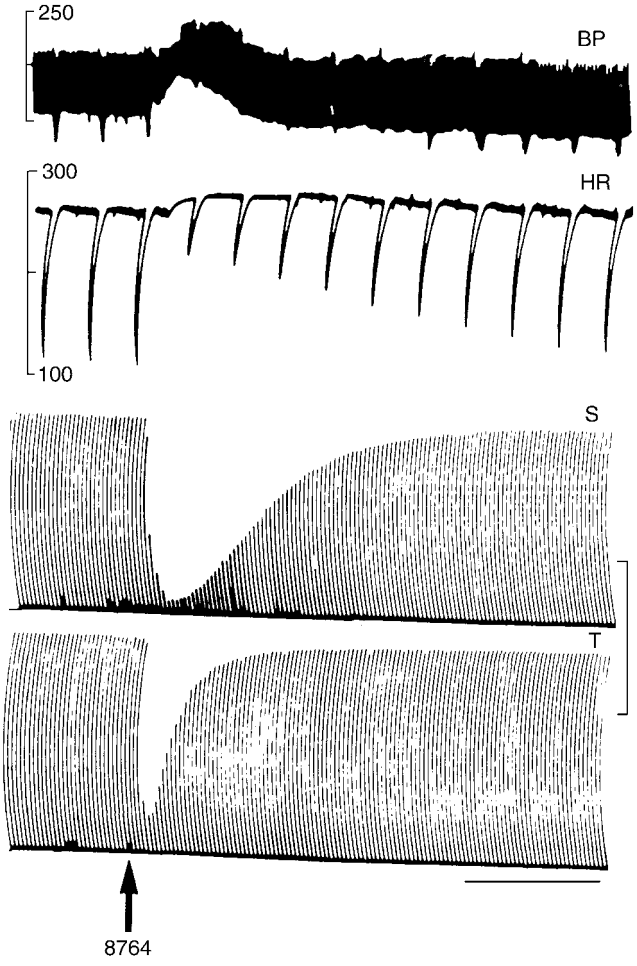

Figure 5 Records from a cat under chloralose anaesthesia, with both vagi cut. Records are as in Figure 1. Calibrations: time, 5 min; tension, 0.5 kg weight for tibialis anterior and 0.2 kg weight for soleus. At 8764, 300 μg kg^{-1} of ORG 8764 (see Figure 4 for structure) was injected intravenously. Note that the drug produced a short-lasting neuromuscular block accompanied by block of the bradycardia produced by vagal stimulation. This was shown in other experiments to be a consequence of an M_2 muscarinic receptor blocking action. With intact vagi, this would result in tachycardia. Despite the cut vagi, it also caused a rise in heart rate and in blood pressure, which other experiments showed to be a consequence of an $Uptake_1$ blocking action. Reproduced with permission from Bowman *et al.* (1988).

recovery cannot begin while the plasma concentration, and therefore the biophase concentration, remain above the blocking level. However, the fact that removal from the plasma is not the only factor determining the slope of the recovery curve has been emphasised by Feldman *et al.* (1990), who showed that in their human so-called 'isolated arm' experiment, the rate of recovery from block by vecuronium on releasing the cuff was slow even though this would almost instantly dilute the blocking drug out of existence in the plasma. Likewise, in the isolated phrenic nerve-diaphragm preparation of the rat bathed in physiological salt solution, changing the bath fluid for drug-free solution (analogous to abruptly emptying the plasma), does not cause rapid recovery, say, from tubocurarine block; several washes are necessary. Yet, it might be supposed that changing the bath fluid for drug-free solution creates a concentration gradient essentially similar to, but in the reverse direction from, that pertaining during onset of block. However, onset is always much faster than offset. It cannot be argued that slow offset is a consequence of slow release from receptors, because reversible, competitive neuromuscular blocking drugs do not work in that way. The interaction of a neuromuscular blocking drug with its receptors is a dynamic one, as is clearly illustrated by patch clamp studies of single receptor activity, which show that the blocking drugs repetitively and rapidly combine with and dissociate from receptors. Tubocurarine, for example, repetitively occupies and dissociates from receptors on a sub-millisecond time scale as Sheridan & Lester (1977) showed, so that release from receptors by itself cannot account for the slow recovery over many minutes seen in the 'isolated arm' or isolated rat diaphragm.

Factors involved in the rate of recovery from block have been discussed (Bowman 1997). They might include the following: (1) buffered diffusion consequent upon repetitive receptor binding and unbinding as the drug molecules 'bounce' their way across the receptor region while escaping from the junctional cleft. (2) The two acetylcholine binding sites on each receptor complex do not have the same affinity for the same blocking drug. Likewise there is evidence that the binding ability of the hypothetical accessory binding site differs from that of the receptor binding sites to different extents with different drugs. It is possible that these different binding capacities influence the rate of escape from the biophase. (3) Blocking drugs combine to different extents to the mucopolysaccharide of the basement membrane in the cleft. This binding might possibly act as a gradually diminishing depot from which the receptors are temporarily resupplied, hence delaying offset to different extents with different drugs. Which, if any, of these possibilities accounts for the relatively slow recovery from rocuronium is not yet known.

This relatively slow recovery with rocuronium would be a drawback in relation to failed intubation, were it not for the work on reversal agents by Bom and his co-workers in Scotland (Epemolu *et al.*, 2003). They have shown that a particular α-cyclodextrin (an α-cyclodextrin is a ring of six linked sugar molecules) has a powerful ability rapidly and selectively to reverse the effect of rocuronium from virtually any depth of block. The compound is known as sugammadex. The outside of the ring-like molecule is hydrophilic and the molecule is therefore very water soluble and so it is rapidly excreted *via* the kidneys. The inside of the ring is hydrophobic and it acts to chelate the rocuronium molecules and literally remove them from the receptors. Sugammadex is currently undergoing advanced clinical trials.

Rocuronium, with its rapid onset of action, can be used in rapid sequence induction where the aim is to intubate the trachea quickly and safely in patients who are at risk of aspiration of gastric contents. The promise is that sugammadex can then be at hand quickly to reverse the block in those rare instances in which intubation proves to be impossible. Hence, the need for suxamethonium, with its propensity to produce unwanted complications, may be avoided.

Final comments

It might well be that apart from marginal and expensive improvements, as much as possible has already been achieved

in the field of neuromuscular blocking drugs. Such is the prolonged development phase for new drugs that the molecules that might become clinically useful in say 10 years time are already known. Yet, who knows what advances in surgical techniques there might have been by then. Muscle relaxants as we know them today might rarely be required, and, when they are, the existing drugs should be adequate. Hence, it is my view that attempts at any further advance will be initially for academic research, rather than a commercial proposition.

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