Mechanisms of neuromuscular block

A review article

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THE NORMAL MECHANISM OF NEUROMUSCULAR TRANSMISSION

The anatomy of the neuromuscular junction can be briefly summarized as follows. As the nerve approaches the motor endplate it loses its myelin sheath and bifurcates into fine nerve terminals. Electron microscopy shows that these contain large numbers of vesicles, particularly in relation to the synaptic area. At the endplate the muscle fibre surface is shaped into a gutter receiving the nerve ending. Within the gutter are deep folds. The surface of the gutter and the folds can be shown histochemically to be lined with cholinesterase. There is a gap of 200–300Å, partly occupied by basement membrane material, between the nerve ending and the post-synaptic surface.

The original evidence for the theory of chemical transmission by acetylcholine at the vertebrate neuromuscular junction can only be briefly mentioned here. It was established that a particular choline ester, acetylcholine, is synthesized and stored in the motor nerve and is released as a result of a propagated nerve impulse. Acetylcholine specifically depolarizes the endplate region, leading to a propagated action potential and muscle contraction. Curare, long known to block transmission, was shown also to antagonize these actions and to exert competitive antagonism against acetylcholine on striated muscle *in vitro*. The effect of eserine (physostigmine) and other anticholinesterases in reversing curare block was satisfactorily interpreted as causing an increase in the effective local concentration of acetylcholine at the endplate, which shifts the competitive balance between curare and acetylcholine in favour of the latter.

For a deeper understanding of the transmission process, it is necessary to discuss in more detail: (1) the properties of the muscle membrane and the control of its ionic permeability; (2) acetylcholine action and, (3) the acetylcholine receptors and, (4) acetylcholine synthesis and release. This discussion will include reference to more recent work which has both endorsed and greatly extended the theory of chemical transmission and has been admirably reviewed by Katz[54].

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The normal muscle membrane: ionic distribution and movements, membrane potential and conductance changes[42,76]

The membrane of the striated muscle cell shares the general properties of the limiting membranes of excitable cells. One way of viewing such membranes is to consider them as lipid partitions containing discrete sites or systems at which lipid–insoluble groups can cross. At least three groups of such sites can be distinguished. (1) Active transport systems, *i.e.* metabolically driven pumps such as the sodium pump where sodium is transported either alone or in exchange for a coupled potassium movement. (2) Specific carrier systems involved in facilitated transport, handling organic acids, bases such as choline[4,70], or uncharged substances such as glucose. (3) Finally, and of particular relevance for this discussion, there are believed to be aqueous 'channels' or hydrophilic sites through which small ions can move. Here an ion moves according to both its concentration gradient and the electrical potential gradient; the term 'electrochemical potential gradient' represents the resultant. The main ions concerned for striated muscle are sodium, potassium and chloride.

The state of opening or closing of these channels influences the ionic distribution between the cell and its surroundings. Three main factors govern this ionic distribution. First is the fact that in the resting state the sodium permeability (P_{Na}) of the membrane is low, whereas the potassium permeability (P_K) and the chloride permeability (P_{Cl}) are high. Second, the amount of sodium inside the cell is kept low by the active transport mechanism mentioned above. Thirdly, the presence within the cell of large non–permeant anions, especially proteins and nucleic acids, together with the high external concentration of weakly permeant sodium ions produces an asymmetry in the distribution of the permeant ions. The Gibbs–Donnan rule predicts that under these conditions the permeant potassium and chloride ions will become distributed so that their concentrations bear the relation $\frac{[K^+]_i}{[K^+]_o} = \frac{[Cl^-]_o}{[Cl^-]_i}$

The net result is that potassium ions are about fifty times more concentrated inside than outside muscle fibres, whereas sodium ions are roughly thirty times more concentrated outside. There is thus a substantial potassium concentration gradient from inside to outside together with a high potassium permeability (P_K). Potassium ions tend therefore to diffuse outwards; but since sodium ions cannot readily move inwards in exchange, the process stops when, as the result of the movement of a minute amount of potassium ion, the outside has become sufficiently positively charged relative to the inside. In general, if P_K is much greater than P_{Na}, the membrane potential is given by $E_m = \frac{RT}{F} \log_e \frac{[K^+]_o}{[K^+]_i}$

(approx. 90mV, inside negative, for skeletal muscle fibres). Chloride too is highly permeant and is distributed according to a similar relationship, but because of its opposite charge is more concentrated outside. If we now suppose that P_{Na} becomes much greater than P_K, the membrane potential E_m will change to $\frac{RT}{F} \log_e \frac{[Na^+_o]}{[Na^+_i]}$ *i.e.* to approximately 60mV, inside positive. Thus the membrane potential depends on the ratio of sodium permeability to potassium permeability. The effect of chloride (the permeability to which, in muscle cells, changes little) is negligible in the steady state, but tends to damp down changes in membrane potential.

The membrane can also be considered electrically as a resistance which has a capacitance in parallel. The convention for the sign of current flow is such that the potential at any point of a resistance falls in the direction of the current flow. Thus if current is passed outwards through a membrane the outside is less positive, and the membrane is 'depolarised'. Likewise if a cathode (negative) is placed at one point on a muscle and an anode (positive) is placed at another, the membrane becomes depolarised under the cathode and hyperpolarised under the anode. The membrane is not passive and such changes in membrane potential alter the sodium and potassium permeabilities. 'Permeability' is however a rather complicated quantity and it is more straightforward to speak of the conductances for these ions (G_{Na} and G_K). Conductance is simply the reciprocal of resistance and represents the ratio between the current flowing and the applied change in potential difference. The measured conductance can be used, given certain assumptions, to estimate the number of channels open to a particular ion. Depolarising a membrane affects the values of G_{Na} and G_K as follows. There is an initial increase (activation) followed by a slower decline (inactivation). These changes take place faster for G_{Na} than for G_K; so that during depolarisation a point can be reached (the threshold) at which the current carried inwards by sodium ions becomes greater than that carried outwards by potassium ions, giving a net inward movement of positive charge which depolarises the membrane still further. This gives rise to the action potential, which is terminated partly by the eventual rise in G_K and partly by the inactivation of G_{Na}. The result of the whole process is that a small triggering depolarisation at one point can lead to a regenerative depolarisation at that point. But the lipid nature of the membrane gives it 'capacitance', that is, the ability to hold charge, so that the local depolarisation spreads a short distance along the fibre and falls to $1/e$ of its value in a distance called the space constant, which is about 1mm for a mammalian muscle fibre. The neighbouring areas that are thus slightly depolarised produce in turn a regenerative response and as a result the action potential propagates along the whole length of the fibre.

The effects of certain drugs on the action potential

A normal action potential lasts for only a few milliseconds and, as we have seen, its peak, when P_{Na} greatly exceeds P_K, approaches the sodium 'equilibrium potential'. Drugs that interfere with the sodium and potassium 'channels' alter the time course of the action potential. Thus low concentrations of tetrodotoxin (TTX) block sodium channels[40,51]; this reduces the height of the action potential. Conversely, tetraethylammonium (TEA) ions block the potassium channels and [3,41,74] to reduce the rise in G_K during depolarisation and thus prolong the action potential. The falling phase of the action potential can in fact become so prolonged as to lead to the production of a second action potential, giving rise to the repetitive firing associated with TEA. Veratrine alkaloids, such as germine, are also able to produce prolonged action potentials and repetitive firing, but apparently in a different way, by delaying the closure of sodium channels after depolarisation[40,96]. Finally, drugs such as procaine affect both sodium and potassium channels. The action potential is more sensitive to the effects on sodium channels, so that it is reduced in height or abolished.

Acetylcholine–induced conductance change

Acetylcholine released by a nerve volley depolarises the postsynaptic membrane, by opening sodium and potassium channels so that the conductance to each of these ions increases. It is believed that acetylcholine produces this action by interacting with specific receptor sites, controlling the channels. These channels are different from those involved in the action potential and they are not affected by tetrodotoxin [51,56].

The most important effect of these increases in conductance, remembering that G_K is already high, is to allow sodium ions to enter, reducing the inner negatitivity of the membrane, *i.e.* depolarising it. In this sense acetylcholine acts like a cathode and the depolarisation that it produces will lead to the usual changes in G_{Na} and G_K of the surrounding membrane. Normally this depolarisation is sufficient to exceed the threshold for propagation of an action potential. But if this threshold is not reached, all that occurs is a so-called 'endplate potential'[28]. The time course of the endplate potential depends on the electrical characteristics of the membrane, and on the activity of the cholinesterase of the endplate region. An interesting recent discovery is that procaine, in addition to its other effects, produces a characteristic prolongation of the tail of the endplate potential[67]; there is suggestive evidence that procaine delays the closure of the sodium channels after acetylcholine's action[36].

Creese & Maclagan[20] and Taylor *et al*[90,91] have shown that during the action of decamethonium in the rat, entry of decamethonium into the muscle fibre occurs at the endplate region, with subsequent lateral diffusion within the fibre. This may mean that the channels opened by depolarising

drugs permit the movement of ions other than sodium, including quaternary compounds and even the depolarising drug itself. Although it is known that depolarising drugs delivered electrophoretically inside the cell neither depolarise nor interfere with the response to extracellular drug, yet the presence of a quaternary compound over a period of hours or days inside the fibre may have unforeseen effects.

The acetylcholine receptor

The acetylcholine 'receptor' mediating these conductance changes is located on the outside of the membrane. Receptors can be detected, by electrophoretic application of acetylcholine, not only under the nerve endings but beyond this immediate region even up to 1mm away. The fact that sensitivity to acetylcholine spreads beyond the region where cholinesterase is located is part of the evidence that cholinesterase is not the acetylcholine receptor. When a muscle is denervated, new receptors appear and ultimately cover the whole of the fibre[5,72]. This contributes to the hypersensitivity of denervated muscle to acetylcholine and other drugs.

Attempts have been made to investigate the properties of receptors and even to isolate the receptor material. Waser[98] has shown that C^{12}–labelled curare alkaloids become localised at the endplate region to the extent of about 5×10^6 molecules per endplate. But a similar localisation of labelled curare did not occur in the region of new receptors in denervated muscle. It is possible that some of the uptake of labelled curare may be due to the affinity of curare for mucopolysaccharides which are present in the basement membrane at the endplate region, rather than to uptake by physiological acetylcholine receptors[14,101]. Work on the acetylcholine receptor of smooth muscle[80,86] showed that uptake by receptors of tritiated atropine could be detected, but uptake by non–receptor sites also occurred and the number of receptor sites was such that it would be a major technical feat to extract the receptor material.

From the chemical structure of potent blocking agents attempts have been made to deduce, by the 'principle of complementariness', a corresponding receptor structure; but apart from the conclusion that an anionic group must be present in the receptor, the arguments remain speculative. The fact that uranyl ions, which have a high affinity for phosphate groups, compete successfully with acetylcholine at the receptor[75] suggests that the anionic group concerned could be phosphate.

It has been found that at lower temperatures the action of acetylcholine at the endplate is increased[39]. Similarly, low muscle temperatures enhance the neuromuscular blocking action of depolarising drugs and antagonise curare block[6]. Possible mechanisms involved in these effects of temperature include alterations in the reaction of drug with receptor or binding sites, in membrane potential and conductance, and in cholinesterase activity.

It has been argued that the receptor is likely to be a polypeptide or

protein[38]. This view is compatible with the demonstrated presence, near the 'active site', of a disulphide bond, whose reduction leads to reversible inhibition of sensitivity to acetylcholine[95]. Hexamethonium is an antagonist at the normal receptor but depolarises at the reduced one[53].

Desensitisation

The acetylcholine receptor of striated muscle and its associated system display a remarkable property known as desensitisation. This appears as the waning of a stimulant effect or the development of repolarisation (usually partial but under some circumstances complete) in the continued presence of the depolarising drug. The rate of this repolarisation increases with concentration of drug[27,59,92]. Models have been proposed for desensitisation in terms of receptors, in which the receptor can exist either in its normal state or in a conformationally different desensitised state. Katz & Thesleff[59] concluded from a kinetic analysis, particularly from the fact that the rate of offset of desensitisation could be faster than that of onset, that a model such as

$$\begin{array}{ccc} D+R & \rightleftharpoons & DR \\ \uparrow\downarrow & & \uparrow\downarrow \\ D+R^1 & \rightleftharpoons & DR^1 \end{array}$$

was required, where D is the drug, R the normal receptor, R^1 the desensitised receptor and DR or DR^1 the drug–receptor complexes of each kind. Acetylcholine action has been shown[87] to change the affinity of the receptor for certain blocking agents and this may be connected with the mechanism of desensitisation. The receptors in denervated muscle desensitise at abnormal rates[5,72]. The extent of desensitisation appears to vary both between species and between individuals. Repolarisation occurs more readily *in vitro*[66,82]. The ionic environment may affect the tendency of receptors to become desensitised. Thus Manthey[68] showed that repolarisation occurred faster in high external calcium concentrations.

Acetylcholine synthesis and release

For the synthesis of acetylcholine in nerve endings, it is known that there is required acetylcoenzyme A, ATP and a supply of choline. The supply of choline may be rate-limiting, since choline is not synthesised locally, but derives from plasma. Uptake into the nerve ending occurs mainly by a saturable 'carrier' system[4,70,85]. There has been some uncertainty about the site of choline acetylation. Recent work on the solubility of choline acetylase suggests that choline acetylation occurs chiefly in the soluble cytoplasm, followed by concentration of acetylcholine in the vesicles of the nerve ending[35].

Choline deficiency in the nerve endings can be produced by certain drugs, notably the hemicholinium, HC3, which competes with choline for the 'carrier' system. By its use considerable light has been thrown on

acetylcholine metabolism in the cat's superior cervical ganglion[7]. There are three main fractions of acetylcholine in the ganglion. First, a fraction remaining even after prolonged stimulation in the presence of HC3 and unaffected by treatment with an anticholinesterase. This probably represents the acetylcholine in intraganglionic axons. Second is the so–called 'surplus' acetylcholine, which appears when an anti–esterase is applied, disappears in the absence of a cholinesterase inhibitor and is normally not used in transmission. It can probably be regarded as spilling over from vesicles or as a cytoplasmic acetylation which is not transferred to the vesicles. The third category, 'depot' acetylcholine, is the important one for transmission and constitutes 85% of the normal acetylcholine content. It is steadily depleted by excitation if choline for further synthesis is not available, but is otherwise kept very constant. The evidence indicates that part of the depot fraction is more readily releasable than the rest.

If a micro–electrode is inserted at an endplate to record the post–synaptic membrane potential, small (1mV) depolarisations are seen with an average frequency of about 1/sec, occurring at random intervals[29]. These are due to the spontaneous release of 'quanta' of many thousands of molecules of acetylcholine and are called 'miniature endplate potentials' (m.e.p.p.'s); they provide another way of monitoring acetylcholine release which, although it is indirect, avoids the technical difficulties of perfusion and bio–assay. They are thought to correspond to the vesicles seen in the presynaptic nerve ending. An endplate potential results normally from a synchronised release of about 300 of these 'quanta' and it consists of a transient acceleration of m.e.p.p.'s occurring shortly after the action potential reaches the nerve terminal. The m.e.p.p. frequency is very sensitive to presynaptic depolarisation and the relationship between the presynaptic membrane potential and the amount of transmitter released shows the following three general characteristics[54,55,57,58,63].

(1) Release increases exponentially with increased intensity of depolarisation.

(2) Release increases with duration of depolarisation.

(3) The resting level of membrane potential is important; conditioning depolarisation reduces, and hyperpolarisation increases, the release produced by a given depolarising pulse.

The amount of transmitter released by a nerve impulse is thus very sensitive to the presynaptic action potential height and duration and to the existing level of presynaptic membrane potential.

Another factor influencing the release of acetylcholine by depolarisation is the concentration of extracellular cations, especially calcium and magnesium. Ca^{++} increases the amount released; very little can be released in its absence. Mg^{++} antagonises this action and the two ions behave as though competing for some reactive site in the membrane[44,45].

Repetitive stimulation

If the endplate potentials are recorded during a tetanus, their size is seen to fall from an initial maximum towards a plateau level. This fall is due to a reduced output of transmitter[69]. This may be the result of depletion, either of presynaptic acetylcholine stores[12] or of a component of the system producing release[47]. The process of quantal release may be described by the equation m=np; where m is the number of quanta released by an action potential (the "quantal content") and constitutes a fraction (p) of an available population (n)[69]. After a tetanus there is a period of post-tetanic potentiation during which transmitter output, m, is raised by an increase in p that outlasts the reduction in n[64,69].

NEUROMUSCULAR BLOCK

The mechanisms involved in neuromuscular transmission which have been outlined above obviously present many possibilities for interference with transmission. An earlier review in this journal may be consulted for the older approaches to the subject[77]; there are two recent reviews[52,94]. Rather than list all the theoretical possibilities, we shall consider mechanisms of block in three groups: (1) interference with acetylcholine release; (2) change of the sensitivity of the endplate to released acetylcholine; (3) change of the threshold for propagation from the endplate to the rest of the muscle fibre.

Interference with acetylcholine release

Choline transport. Neuromuscular block can be produced by HC3; it develops faster, the higher the rate of nerve stimulation and it can be temporarily reversed by giving choline[26]. Onset is always slow, since the existing stocks of acetylcholine must be used up before block appears; and the importance of this type of paralysis in anaesthesia is probably greater as a contributory factor than as a primary cause. Thus certain blocking agents with a tubocurarine-like action begin to show, after a fairly prolonged exposure with repeated stimulation, an HC3 like effect. It is not improbable that most quaternary blocking agents can interfere with choline transport to some extent; the effect has already been demonstrated, on choline transport into red cells, for tubocurarine, dimethyltubocurarine, decamethonium and suxamethonium[4,70a]. Further, MacIntosh[65] has provided interesting evidence that abdominal operations are associated with a fall in blood choline levels, a condition that of course favours HC3 like action. It may well be, therefore, that apnoeas which follow abdominal surgery combined with prolonged use of relaxants are in part due to a functional choline deficiency. If this could be shown, choline infusion would provide a simple treatment.

Neuromuscular paralysis of this type could be considered for treatment of chronic spastic states; and this was explored by Bowman & Rand[10] using triethylcholine. With this substance, a progressive muscular weakness, exacerbated by exercise, can be produced. But an interesting complication arises, for it shares one of the actions of the closely related tetraethylammonium, namely the production of repetitive firing in nerves[88]. TEA has been found, indeed, to *increase* acetylcholine output from the neuromuscular junction[18] and it has long been known to produce sensory paraesthesiae and to antagonise both tubocurarine and decamethonium block[62]. This approach is, therefore, hardly ripe for clinical exploitation. Another interesting possibility suggested by Bowman & Rand is that triethylcholine may become acetylated in the nerve ending, to form a 'false transmitter'. Thus paralysis by triethycholine could arise, not only from deficiency of acetylcholine store, but also from release of a substance both ineffective and antagonistic to acetylcholine. But proof of these possibilities is still lacking.

Control and release. In the original evidence for the theory of chemical transmission at the neuromuscular junction there was nothing that suggested a presynaptic action by drugs such as acetylcholine, tubocurarine or anticholinesterases. But as early as 1940, there was evidence that, in the presence of an anticholinesterase, a single motor nerve volley or the injection of acetylcholine would evoke a repetitive antidromic discharge in the motor nerve[71]. Riker and his colleagues[89] have subsequently produced many examples of such antidromic discharge, associated with facilitation of the muscle twitch, in response to many quaternary compounds.

Hubbard *et al*[43,46] have used micro-electrodes in rat muscles to study these actions. Acetylcholine was found to increase the excitability of the nerve endings and this was associated with a *reduction* in transmitter output as judged by the quantal content of endplate potentials. Hubbard's interpretation is that acetylcholine can act directly to depolarise the nerve probably at the nearest node of Ranvier. It is unlikely that it depolarises the terminals in rats, since the frequency of m.e.p.p.'s is not increased. Since the rat is less susceptible to twitch potentiation and fasciculations with depolarising drugs, the presynaptic actions of acetylcholine may be more complex in other species. As Riker has pointed out, the presence of presynaptic receptors for acetylcholine would make some of the original evidence for chemical transmission open to more than one interpretation. But Hubbard's findings suggest that the presynaptic actions of depolarising drugs are of pharmacological interest (perhaps contributing to fasciculations and to neuromuscular block) but of unknown physiological importance[43].

The conditions at the neuromuscular junction after administration of an anticholinesterase are even more intricate. The drug itself may act on the terminal, or acetylcholine released by the nerve ending and preserved through cholinesterase inhibition may do so; the postsynaptic region may

be depolarised, either directly or through accumulating acetylcholine, so that the nerve ending lies in an electrical potential gradient; potassium ions released from the depolarised muscle fibre may be accumulating in the region; some anticholinesterases may have TEA$^+$–like actions on the presynaptic action potential; and antidromic activity at one terminal may lead, by an 'axon reflex', to orthodromic activity in another branch of the same motor unit, leading to 'reverberation' between endplate regions. The situation is notoriously difficult to disentangle[8].

Calcium and magnesium. Since a fall in the Ca^{++}_o/Mg^{++}_o ratio depresses acetylcholine release, it would be worth testing whether changes in the ratio occur clinically. It is also possible that drugs may act at this level; for instance by interfering with the binding of Ca^{++} to some negatively charged molecule at the membrane surface, or by interfering with the effect of the complex in causing an enhanced quantal release.

Other agents

There are other indications that new ways of controlling acetylcholine output may be discovered. Botulinum toxin[11] reduces output and does this neither by interfering with nerve impulse conduction nor by reducing the size of the acetylcholine quanta; in some way it selectively reduces the resting and evoked release of normal quanta. In another tissue, the parasympathetic cholinergic endings of Auerbach's plexus, morphine and catecholamines depress acetylcholine output[78,81,84]. Noradrenaline increases transmitter–output in skeletal muscle[9]. This may be linked to its phenol structure and recalls the anti–curare effect of phenol and catechol[73]. The catecholamine effect on the motor nerve endings is thought to be an α–effect[9,49] and contributes to the anti–curare action; it contrasts with a β–effect on the postsynaptic membrane, leading to a hyperpolarisation and a deepening of curare block[9]. These results, together with the effect of catecholamines on muscle contraction, account for the very diverse results recorded. Finally, the ability of drugs such as bretylium to depress catecholamine release by adrenergic nerves, without reducing catecholamine content of the nerve endings, may point to yet another type of interference.

Change of acetylcholine sensitivity

Competitive antagonism. Recent work confirms the classical conception that tubocurarine and similar substances compete with acetylcholine for specific receptors at the motor endplate. The main advance has been in analysing the kinetics of the process. Although tubocurarine has a fairly slow onset and offset *in vivo*, it was found by electrophoretic application *in vitro* that the rate of combination and dissociation with the receptors was to be measured in seconds or less[22]. Waud[99] has confirmed this and

provided evidence that even if the time course of the drug-receptor interaction itself is rapid, access to, or escape from, the region of the receptors provides a substantial rate limitation. Other rate-affecting processes, include binding to protein or acidic mucapolysaccharides[14], renal excretion and metabolism. The general position seems to be that the firmness of binding of curare alkaloids at the motor endplate is much less than that of, say, atropine at the muscarinic effector site. But competitive neuromuscular blocking agents *in vivo* still resemble atropine-like substances in that they have a slower onset and more persistent action the more potent they are[82].

Desensitisation. The decline in sensitivity of a cholinoceptive region on continued exposure to acetylcholine or a similar antagonist has been mentioned above. This is proving to be a more complicated type of phenomenon than expected. Thus Flacke & Yeoh[34] have found, with leech muscle, that the antagonism by tubocurarine to suxamethonium and decamethonium is unusual; initially the antagonist action of tubocurarine is almost negligible, but with subsequent tests the responses to suxamethonium or decamethonium steadily dwindled, as though tubocurarine had exaggerated the process of desensitisation by these drugs. Gallamine did not show this peculiarity. It is too early to interpret these results; but it seems possible that significant differences will be revealed in the interaction of the various depolarising drugs with different antagonists and that muscles will themselves vary in their responses.

Change of propagation threshold

The analysis of the actions of endplate depolarising drugs immediately brought to light a variety of stimulant effects analogous to those of acetylcholine itself, and attributable to the endplate depolarisation[83]. But in themselves these do not explain how synaptic block could be produced, nor even why the overt signs of stimulation (fasciculations and potentiation of twitch) are quite transient, although the depolarisation persists. It was further found[13], however, during the block of cat's gracilis muscle *in vivo* produced by decamethonium, (1) that the endplate region provided a barrier to the passage of a directly evoked muscle action potential; (2) that the size of endplate potential required to propagate was larger than in the curarised muscle, and (3) that the endplate region itself became electrically less excitable. From these results it was concluded that a new mechanism of block was present, namely a decrease of electrical excitability of the membrane of the endplate region as a result of the persisting depolarisation. Further evidence for this causal relationship was obtained by finding that the block was reduced by passing current through an anode on the depolarised endplate, a procedure which would increase a curare block[13].

This type of action at the endplate has been likened to cathodal block

in other tissues, in which the block is known to be largely due to an inactivation of the sodium conductance by the depolarisation[15,42]. Theoretically the rise in potassium conductance associated with the depolarisation will also reduce excitability, but the effect may be much smaller[1]. The depolarisation also produces an output of potassium ions which can accumulate in the tissue spaces and contribute to the spread of depolarisation. This will lead to further sodium inactivation and thus an increase in the area of inexcitability. This may explain the observations of Churchill–Davidson & Richardson[16], that block by decamethonium was reduced in a functionally sympathectomised or vasodilated arm, compared with a normal arm; since the extent of accumulation of potassium in the tissue spaces will depend on the blood flow to the tissue.

It may be noted that the occurrence of desensitisation during block by a depolarising drug will reduce the depolarisation and hence the contribution by cathodal block to the interference with transmission; at the same time the desensitisation itself contributes to the block. This is one possible mechanism for the so-called 'dual block'[102], or phase II block[48]. Gibberd [37] found that potassium loss from rat diaphragm was associated with an increased liability to phase II block.

The safety factor of neuromuscular transmission

It is relatively easy to suggest mechanisms of neuromuscular block, more difficult to prove their existence and to define their properties and very difficult indeed to determine the contribution to block by the various mechanisms in any given practical situation. In this situation, consideration of the 'safety factor' of transmission provides a useful general approach. It is known that acetylcholine output is lower at high than at slow rates of motor nerve stimulation; it is also known that normal transmission does not begin to fail until quite high rates of excitation are reached. It follows that at slow rates of stimulation considerably more acetylcholine is released per volley than is required for successful transmission: *i.e.* there is a substantial safety factor. It is not easy to measure this by direct determination of transmitter release; but the safety factor can be estimated pharmacologically by measuring the extent to which a depolarising drug is antagonised by a dose of tubocurarine that causes some given degree of interference with transmission[82,82a]. By such a method it is found, for instance, that with cat tibialis stimulated $^1/_{10}$ sec, tubocurarine must produce a 4–5-fold antagonism to depolarising drugs before *any* neuromuscular block is present; and for 95% block, the antagonism must be 15–20-fold. This implies that for the most sensitive muscle fibres, 4–5 times as much acetylcholine is released as is needed for threshold action; or to express it in terms of receptors[82a], that 75–80% of the receptors must be occluded before the threshold is reached. With other muscles in the cat and in the dog, rabbit and rat, safety factors for the

most sensitive fibres indirectly excited at slow rates range between 3 and 5 and for the least sensitive fibres from 10 to 20.

The existence of a safety factor has obvious practical consequences; for instance the action of drugs is far from terminated at the time when transmission is apparently normal and there is scope for a considerable 'subthreshold' action, only detectable by a tetanus or by potentiation of other drugs. In addition it explains the properties of muscles partially blocked by competitive drugs, such as the fall of tension during a tetanus and the sensitivity of the depth of block to anticholinesterases, catecholamines, previous tetanisation, anaesthesia and a wide range of drugs.

Thus if we consider a muscle whose fibres vary in their safety factor from, say, 5 to 20, a dose of tubocurarine occupying 90% of receptors will block transmission to those fibres with safety factors less than 10. Any procedure that halves the safety factor will deepen the block to include even those fibres with a normal safety factor of 20; whereas doubling the safety factor will relieve block in all fibres with a normal safety factor of 5 or more. As an example, the output of transmitter per volley during a tetanus may be half that during slow rates of stimulation; consequently the first shock of a train of stimuli will be transmitted to many more fibres than the last shock and the tension will fade from an early maximum. One can thus see how, once partial block has been produced, the effect of various procedures on the level of block depends on the variation of safety factor between different fibres. If variation is small, then small changes of safety factor have a big effect. On the other hand if variation is very large, then a partial block will seem to be relatively insensitive to procedures modifying the safety factor. Three possible mechanisms for increasing the variation can be cited. Synapses with extensive nerve endings might have larger safety factors than those with restricted nerve endings. A drug which increased the safety factor by interacting with the nerve ending would therefore tend to increase it more in those junctions in which the safety factor was already high, thus increasing the range of variation of safety factor. Such an argument might also apply to the action of anticholinesterases if cholinesterase density varies at different junctions. Finally depolarising drugs will increase the variation of safety factor. Slightly depolarised fibres become *more* excitable, *i.e.* their safety factor is greater than normal. On the other hand, deeply depolarised fibres become less excitable; if the depolarisation is sufficient, the propagation threshold may rise above the maximum depolarisation achievable by acetylcholine and the safety factor becomes zero. It is likely that this underlies the sustained tetanic tension and general insensitivity of partial depolarisation block.

It is evident that there is no *logical* necessity that 'depolarisation block' must always be coupled with these effects; and there is some danger in classifying a block as 'depolarising' or 'competitive' solely on character-

istics such as the response to an anticholinesterase, the behaviour during and after a tetanus and interaction with other drugs. Thus, were the safety factor eroded from some cause, it would be possible for block to arise from quite a small rise in propagation threshold produced by a depolarising drug, without greatly increasing the variation in safety factor; such a block would show many of the characteristics of a 'curare' block. If one allows for the fact that some blocking agents may be 'partial agonists'[82] (*i.e.* possessing (limited) ability themselves to depolarise as well as an ability to compete) and that drugs may interfere with transmission presynaptically as well as post-synaptically, it is inevitable that many situations will occur clinically whose underlying mechanisms can only, at present, be guessed. To interpret the effect of neuromuscular blocking drugs clinically, it is necessary therefore not only to assess contributions due to depolarisation, competitive antagonism, desensitisation and presynaptic action produced by the drugs used, but also to know the safety factor of transmission in the absence of such drugs.

SPECIAL TOPICS

Pharmacogenetics of suxamethonium (succinylcholine)

An interesting aspect of suxamethonium action is that its brevity is largely due to the action of pseudocholinesterase, and individuals were soon recognised in whom this enzyme was deficient and in whom suxamethonium had a prolonged action. Synthesis of the enzyme is under control of a single gene; as a result, instead of the more usual continuous variation associated with multifactorial inheritance, there exist three main and readily distinguishable groups: those homozygous for the normal gene with normal enzyme and normal suxamethonium response; those heterozygous, about 3–4% of the population, with lower plasma esterase than normal, in whom suxamethonium action may be slightly prolonged; and those homozygous for a defective gene, about 1 in 2,000–3,000 of the population in whom suxamethonium has a much longer action[50].

A further genetic aspect arises when a depolarising drug is given to individuals suffering from certain muscular diseases. In dystrophia myotonica or myotonia congenita, suxamethonium can produce a muscular spasm which, once evoked, resists any neuromuscular blocking agent, since it arises beyond the motor endplate; it may respond to quinine. In idiopathic recurrent rhabdomyolysis, the muscle fasciculations can lead to myoglobinuria and renal failure.

TEA and germine as antagonists to neuromuscular block

Kensler[61] showed that tetraethylammonium (TEA) ions were able to antagonise block produced both by tubocurarine and by decamethonium, that the effect was still present after a maximal antagonism to curare by

neostigmine had been produced and that neostigmine could still exert its action after TEA had exerted its full effect. These findings are recalled by the recent interest in veretrum alkaloids, particularly germine diacetate, which (as mentioned earlier) resemble TEA in causing repetitive firing of excitable tissues, although by a different mechanism. Veratrine itself is unsatisfactory for clinical use since it is strongly hypotensive and emetic, but germine diacetate is if anything pressor and has a much reduced emetic action. It retains, however, the ability to induce paraesthesiae, thereby resembling TEA; but possesses the advantages that it lacks the ganglion–blocking action of TEA and does not have so transient an action[30]. At the neuromuscular junction, effects similar to those described by Kensler for TEA have been obtained[33]. In addition it has been directly verified that repetitive firing is produced in muscles treated with germine[31] and it has also been shown that myasthenic patients are strengthened by it[32]. The effect of germine can be shown in curarised patients or denervated muscles stimulated directly; any effect on nerve terminals therefore contributes little, if at all, to the action. An action of this type requires sufficient transmission for at least some action potentials to be present and thus cannot be used alone to relieve complete block.

The attraction of germine therapeutically is that its action is independent of and can supplement, anticholinesterase action. In anaesthetised subjects, abdominal movement or hiccough occurred occasionally. Germine itself is too scarce to be generally available, but drugs like it may be developed. The main problem in their clinical assessment would presumably be (1) to determine whether there are any conditions of anaesthesia or administration of other drugs which will annul the repetitive firing; and (2) to assess the clinical importance of the sensory stimulation and emetic effect.

Myasthenia gravis and carcinomatous myopathy

The aetiology of myasthenia gravis is still obscure (see the Symposium on Myasthenia Gravis[2]). A characteristic of the disease is the response to repetitive nerve stimulation. Thus at frequencies about 3 per second, normal muscle responds with a train of well sustained action potentials and twitches, but in the myasthenic there is a progressive reduction in the responses. This resemblance to curare block can be explained by a reduction in the safety factor of transmission in myasthenia, and led Mary Walker[97] to introduce eserine as a treatment. The patients are also abnormally sensitive to tubocurarine and it was suggested that they might be a circulating curare–like substance. Convincing evidence for this has not been obtained, so that the cause of the reduction in safety factor is still obscure. There appears to be no overactivity of endplate cholinesterase. In micro–electrode studies of relatively 'uninvolved' myasthenic muscles [24,93] the only defect seen was that the miniature endplate potentials were about one–fifth of normal size. This could arise either because the amount

of acetylcholine in a quantum was reduced or because the postsynaptic responsiveness to released acetylcholine was reduced. In these muscles the depolarisation of the endplates by low concentrations of depolarising drugs was not significantly different from normal and it was suggested that the acetylcholine content of a quantum might be low, perhaps due to a disturbance in the synthesis or storage of acetylcholine.

This theory does not explain the finding of Churchill–Davidson & Richardson[17] that muscles of myasthenics are not blocked by doses of decamethonium normally producing 90% block. These authors found that the more diseased muscles were less resistant and the block produced in these was usually reversible by edrophonium.

Overtly 'involved' muscles have been studied *in situ* by Desmedt[23] and shown to differ from normal curarised muscle in showing a prolonged post–tetanic depression. Desmedt likened the condition to HC3 poisoning. But the same comparison might apply to any situation in which the size of the depot acetylcholine is reduced. Moreover the muscles studied by Elmquist *et al*[24] did not show post–tetanic depression and further differed from HC3–treated muscles in other details; the size of miniature end–plate potentials did not fall during prolonged repetitive stimulation and did not increase during rest in the presence of choline). Interpretation is difficult since it is not clear which features of the disease are primary and which might represent compensatory reactions. Histologically, changes have been seen in myasthenic muscle, such as elongation of end–plates[100]. In more severely 'involved' muscles inflammatory changes are seen[2]. Pathologically myasthenia is often associated with thymoma and there is often evidence of an auto–immune reaction involving striated muscle[2]. Although myasthenia gravis is itself a fairly uncommon disease, a myasthenic condition accompanies malignant disease, particularly pulmonary neoplasm[21, 62].

CONCLUSION AND SUMMARY

In this review an attempt has been made to bring to bear some of the advances in physiology and pharmacology on the problem of neuromuscular block. While this makes the subject more complicated and more difficult in some respects, it also makes it more definite. Types of block have been split into three categories. The first, that involving presynaptic mechanisms, used to receive merely cursory treatment. But now we can consider in detail the role of choline uptake and acetylcholine synthesis; the discovery of quantal release of acetylcholine has provided a new tool both for studying mechanisms of output and for quantifying output in a way impossible by classical assay; and modern electrophysiology is throwing a flood of light on the physiology of the nerve terminal and its responses.

The second category of block, involving changes in chemical sensitivity of the postsynaptic membrane, has also undergone changes of emphasis. The old concept of competition at acetylcholine receptors has been exploited to allow measurement of the safety-factor of transmission, an approach which itself can be conceptually helpful. We must now add to this the phenomenon of desensitisation, whose role and mechanism still leave much to be explored.

Finally, the third category of block, interference with the propagation threshold, can be discussed more precisely in the light of our knowledge of the response of excitable membranes to depolarisation or application of drugs.

It is also true, however, that most of the techniques involved in these developments are not likely to be available for direct use in clinical circumstances for many years. Yet certain pointers for clinical development can be mentioned. First, one must mention that the mere recording of muscle twitches under clinical conditions is not likely to yield much further dividends. On the other hand, a careful study of tetanic and post-tetanic responses under different conditions of block would be very helpful; and if a technique for measuring depolarisation can be developed, then much-needed information, particularly as to the extent to which desensitisation is concerned in neuromuscular block in man, could be obtained. Second, study of choline metabolism in man, especially in relation to quaternary drugs, could well be fruitful. Each of these approaches could improve our understanding of prolonged apnoea. Third, the development of veratrine-like drugs, though difficult, might turn out rewarding. Fourth, there are many drug-interactions hardly touched on in this review which deserve further analytic study. Among these could be mentioned the effect of anaesthetics in augmenting curare-like effects and the effect of substances such as neomycin which can both reduce acetylcholine release and antagonize its postsynaptic action[25]. It is possible, too, that drugs are used clinically which interfere with the presynaptic effects of blocking drugs. Related to this are the still poorly-understood mechanisms involved in the muscle pain produced by suxamethonium. It has been found that small doses of curare-like drugs, stop the fasciculations and thiopentone, if given only a few minutes before suxamethonium, has been found[19] to reduce both fasciculations and pain – even though fasciculation and pain do not necessarily go together. Another simple question, still unsettled, (see ref. 79 for one pilot study), is whether depolarising drugs increase the serum potassium in man as they do in animals; experiments on this point, under a variety of clinical conditions, might throw a good deal of light on the depolarising process itself, on the cause of muscle pain[60] and on interactions with other drugs. Finally, there are many pointers, such as the morphological changes in myasthenia, the changes in carcinomatous myopathy and the variation in neuromuscular responses with age or

genetic constitution, which taken along with recent advances, suggest that there is much to be found out about the trophic interactions between nerve and muscle and about the development, genetic control and immunology of the components of the junctional region. The anaesthetist, with his access to abnormalities occurring genetically or created by disease, is well placed to advance our knowledge in this field.

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The references in this paper do not follow our usual form of presentation. It has been decided, after discussion with the authors, that an exception to our rule would facilitate the choice of additional reading to accompany a review of this nature.

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