Doughnuts and holes: molecules and muscle relaxants*

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The site and mechanism of action of neuromuscular blocking drugs have puzzled investigators for over a century. In the early years it was speculated that the fine endings of the nerves to the muscles bore the key spots, but this view changed early in this century when Langley showed that curare antagonized the action of nicotine on denervated muscles and concluded that the sites of action had to be on the muscle. As scientific insight grew, so did knowledge of curare and its receptor, but always there was a search for the single, or at least the principle, site of drug action. In this aspect, laboratory research diverged from clinical experience because those who used the relaxants knew that they were dealing with complicated agents, and it took little reflection to think that there might be more than one site or mechanism of action.

Modern investigations are beginning to bring clinical and laboratory experience together. The experimental work clearly shows that muscle relaxants have several sites and mechanisms of action and that their relative importance can change with changes in dose, activity in a neuromuscular system, disease, and interaction with other drugs. Although it is apparent that the relaxants can have actions on synapses outside the neuromuscular junction, e.g., in the heart or ganglia, the neuromuscular junction is the centre of interest because it is here that the most desired effects take place and the techniques for experimental investigation are most advanced. Furthermore, although it is well established that drugs have effects on prejunctional receptors in motor nerve endings, the majority of interest follows the lead established by Langley and focuses on the receptors in the membrane of the muscle (Figure 1).

Junctional and extrajunctional receptors

Occurrence

Muscles are now known to have at least two distinct receptors that respond to muscle relaxants; the receptors that normally are present in large numbers in the endplate membrane of normal adult junctions and another type that appears throughout the muscle whenever there is deficient stimulation of the muscle by the nerve. The latter, called extrajunctional receptors, are not found in significant numbers in normally active adult muscle, but they become more numerous and contribute to the clinical actions of relaxants, especially to those of succinylcholine, whenever motor nerves are less active than normal,[1] e.g., in patients with denervated muscles, burned muscles, or underactive muscles, such as those of individuals with spinal cord injury or a stroke, or even in muscles in limbs immobilized in casts.

Both types of receptors are made in the muscle cell, but apparently by systems that are under different control mechanisms. Extrajunctional receptor synthesis is repressed in the normal adult, but when these receptors are allowed to be made, they are made more rapidly and are destroyed more rapidly ($t_{\frac{1}{2}}$ 10 to 30 hours vs 7 to 14 days) than the receptors in the endplate.[2] Of more practical importance, the extrajunctional receptors are inserted into the membrane all over the muscle instead of being confined to the endplate. Although they are less densely spaced than the latter, they are placed into an area of membrane enormously greater than that of the endplate; hence, pharmacologic effects in response to drug action on them can be significant. Despite differences between them and some pharmacologic characteristics that may have significant consequences when relaxants are used clinically (see following text), the two kinds of receptors are similar and are used interchangeably in many kinds of experiments.

The receptors of the endplate are remarkable in that they seem to have arisen early in evolution; there seems to be little difference between the receptors of human beings, frogs, electric fish, or other organisms.[3–5] This continuity across evolutionary lines greatly facilitates research in this area; while the ultimate object of inquiry may be the receptors of human beings, research is far more readily carried out on lower species or on cells grown in culture. Some of the most valuable creatures have been electric fish of the *Torpedo* family. These fish

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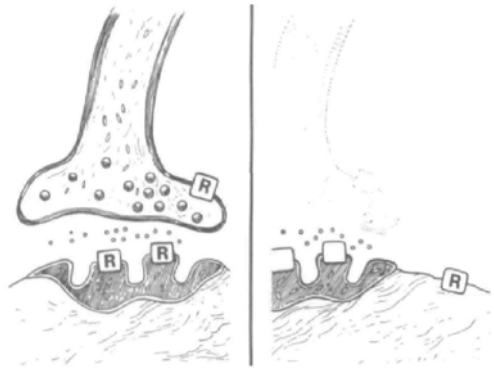

FIGURE 1 Nicotinic cholinergic receptors (R) on postjunctional folds (bottom left) at the motor endplate of skeletal muscle, on motor nerve endings (top left), and in extrajunctional membrane (right) in immature or denervated skeletal muscle.

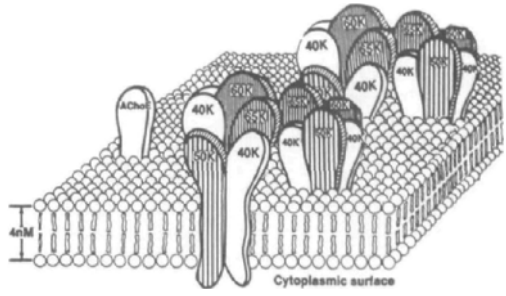

FIGURE 2 Sketch of postjunctional nicotinic acetylcholine receptors with an acetylcholinesterase (AChoE) molecule nearby.

have such enormous numbers of acetylcholine receptors in their electric organs that it is practical to isolate receptors chemically and to study their morphologic and biochemical properties in the laboratory. It also has been possible to use these receptors and those from higher organisms, including human beings, to study in exquisite detail their physiologic and pharmacologic responses to acetylcholine and related agonists and antagonists.

From studies of isolated receptors and of receptors *in situ*, it is possible to describe the appearance of receptors, their chemical composition, and even the sequence of nucleic acids that guides their synthesis. It also is possible to measure the electrical activity started by the binding of acetylcholine to a single receptor and to describe the interaction of relaxants and other drugs with receptors.

Electron micrographs of receptors *in situ* show them to be in the endplate membrane, particularly on the shoulders of the junctional folds, which places them precisely opposite to the acetylcholine release sites in the nerve ending.[6] This area of the membrane is so rich in receptors (10,000 to 20,000/μm^2) that the surface appears to be paved with them.[7] They appear as discrete ring- or rosette-shaped particles, 8 to 9 mm in diameter with a central pit. Both the ring and the pit are believed to be essential for function. The ring is the top view of a cylinder of protein and contains the sites to which acetylcholine and other drugs bind, while the pit is the mouth of a channel in the centre of the cylinder through which ions pass across the cell wall. The receptor particles occur in pairs and the pairs are in tidy rows across the surface. Each receptor is believed to operate independently, but there are links between the members of a pair[8] and they may cooperate with each other in some way.

Chemistry

These receptors have been isolated, purified, and analyzed; their structure is known in detail. The key features are sketched in Figure 2. Each receptor is a protein with a molecular weight of about 250,000 daltons that is made up of five subunits, which are designated alpha, beta, gamma, and delta. There are two alpha units, weighing about 40,000 daltons apiece, and one each of the others, weighing about 50,000, 60,000, and 65,000 daltons, respectively.[9] The receptor complex is approximately 11 nm in length, one half of which protrudes from the extracellular surface of the membrane. The protein passes entirely through the membrane but extends only about 2 nm into the cytoplasm.[10-12]

Each of the five subunits is linear, and the subunits are arranged longitudinally so that the combination is potentially capable of forming a tube or channel[13] that allows cations to flow along the concentration gradient. Sodium and calcium move into the muscle while potassium moves out. This tube is opened when two acetylcholine or other agonist molecules attach to the alpha units, one on each, and cause the subunits to rotate into a new conformation. In the open conformation the channel in the tube is large enough to pass all physiologic cations and even slim organic cations such as decamethonium, but it does not pass either anions or large organic cations.[14] The acetylcholine binding areas on the 40,000-dalton alpha units are the site of competition between cholinergic agonists and antagonists. Both agonists and antagonists are attracted to the site and either may occupy it. When both alpha unit sites are occupied by an agonist, the protein molecule undergoes a conformation change to form a channel through which ions flow (Figure 3). This current depolarizes the adjacent membrane. Both alpha units must be occupied simultaneously by an agonist;[15,16] if only one of them is occupied by an agonist, the channel remains closed. This is the basis for the prevention of depolariza-

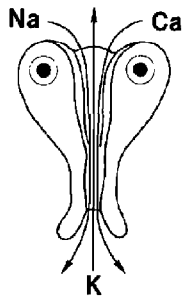

FIGURE 3 Sketch of the open configuration of the acetylcholine receptor with a molecule of ACh attached to each of the agonist binding sites (adapted from Horn and Brodwick[10]).

tion by antagonists. Drugs like tubocurarine act because they bind to either or both alpha units and, by doing so, they prevent acetylcholine from binding and opening the channel. This interaction between agonists and antagonists is competitive, and the outcome, transmission or blockade, depends on the relative concentrations and binding characteristics of the drugs involved.

Noncompetitive drug actions

The extracellular end of the tube is much larger than the part where the protein crosses the membrane; thus large molecules can enter the tube but not cross it. Drugs that do this can act like plugs in a funnel and prevent or impede the normal flow of ions through the tube, thereby producing a phenomenon called channel blockade. Since channel blockade prevents the flow of physiologic ions, it prevents depolarization of the endplate and can block neuromuscular transmission. Channel blockade is a familiar feature of local anaesthetic action on the sodium channels of nerves, yet it is only a recently recognized, but potentially important, feature of drugs that act at the neuromuscular junction. Channel blockade of neuromuscular transmission prevents depolarization, but since the action is not at the acetylcholine site on the alpha subunits, it is not via a competitive antagonism of acetylcholine.[17–20]

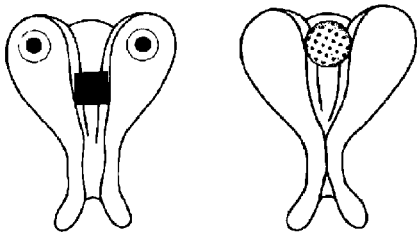

FIGURE 4 (Left) open channel blockade; (right) closed channel blockade.

Two major types of channel blockade can occur: open channel blockade and closed channel blockade (Figure 4). In the former the drug enters a channel that has been opened by reaction with acetylcholine but cannot penetrate all the way through. When in the channel it impedes the flow of physiologic ions and thus prevents depolarization from occurring. Most of these drugs exhibit two characteristics: (1) they are use dependent, meaning that since they can enter the channel only when it is open, the intensity of their effect depends upon how often the channel is opened, i.e., how often the system is used; and (2) they are driven by the electrical interaction between the potential across the membrane and the charge inherent in their molecular structure, which means that only cationic drugs are involved and that the intensity of effect varies with the chemical structure of the molecule. In addition, drugs that penetrate the opened channel may bind temporarily to some point on the wall of the channel, which means that the duration of the blockade also may vary with the molecule.

Closed channel blockade is more difficult to study experimentally so less is known about it, but there are drugs that can react around the mouth of the channel and by their presence prevent physiologic ions from passing through the channel. Since the reaction is around the mouth of the channel, the process can take place whether or not the channel is opened and so is not use dependent. This type of blockade is believed to be part of the pharmacology of, for example, tricyclic antidepressants, piperocaine, naltrexone, and naloxone.[19,21]

Figures 2 and 3 suggest that the receptor molecule is rigid and fixed in shape, but this is not the case. The receptor is made of large flexible macromolecules that are capable of existing in a number of states depending upon the electrical and chemical milieu. Several states of practical importance are illustrated in Figure 5. The three in the top row are the traditional ones that are important to normal receptor function. The resting receptor at the left is free of agonists, so its channel is closed and no ions can flow through to depolarize the membrane. The channel remains closed for an instant after two agonist molecules bind, but then the molecule undergoes the conformation change that results in an active receptor and an open, current passing channel. This is the basis of normal neuromuscular transmission.

The receptors in the bottom row of the figure depict another situation: receptors that bind agonists but that cannot undergo the conformation change that opens the channel. Receptors in these states are termed *desensitized*: i.e., they are not sensitive to the channel opening

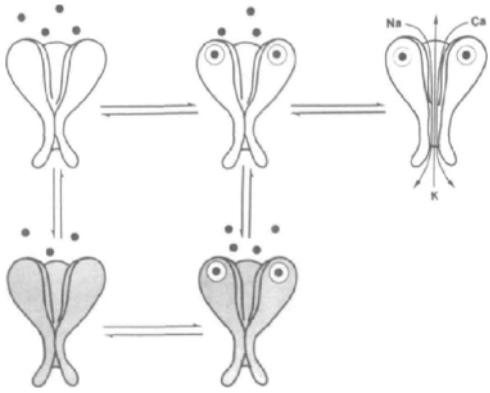

FIGURE 5 Representations of several receptor conformations. Normal forms are in the top row; desensitized forms are below.

actions of agonists. Desensitized receptors can bind agonists (or antagonists); indeed, they usually bind them with exceptional avidity, but the binding does not result in activation of the receptor or opening of the channel.

Desensitization has practical consequences because, as suggested in Figure 5, normal and desensitized receptors are in equilibrium. One form can change into another, but when receptors are desensitized they are not available to participate in the normal processes of neuromuscular transmission. Since the presence of desensitized receptors means that there cannot be the usual numbers of normal receptors, the production of desensitized receptors weakens the intensity of neuromuscular transmission. If so many receptors are desensitized that there are not enough normal ones remaining to depolarize the motor endplate, neuromuscular transmission will not occur. Even if only some of the receptors are desensitized, the intensity of neuromuscular transmission will be impaired and the system will be more susceptible to blockade by conventional antagonists such as tubocurarine or pancuronium. A significant number of drugs cause or promote desensitization[22–25] (Figure 6), and the phenomenon probably is involved in many of the familiar interactions between relaxants and other drugs used during anaesthesia.

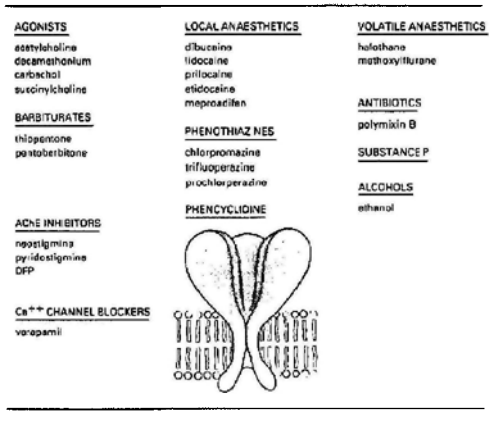

FIGURE 6 Some drugs that may promote desensitization.

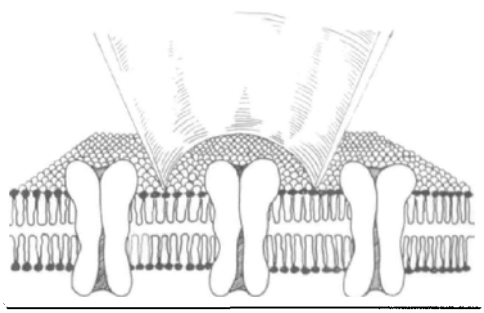

FIGURE 7 Patch clamp technique for recording current flow through a single acetylcholine receptor.

Physiology and pharmacology

Just as we can visualize individual receptors and analyze them biochemically, so can we measure their electrical function. The most direct method is patch clamping,[26] a method in which a glass micropipette is used to probe the membrane surface until a single receptor is encompassed. The tip of the pipette is sealed by the lipid of the membrane, and electronic apparatus is arranged to clamp the membrane potential and to measure the current that flows through the channel of the receptor. The solution in the pipette can contain acetylcholine, tubocurarine, or another drug or mixture of drugs. The arrangement is illustrated in Figure 7.

Figure 8 contains schematized examples of records that are obtained in this way. The top tracing is produced when an agonist, suberyldicholine, an analogue of succinylcholine but more stable than it under experimental conditions, is in the pipette and can react with sites on the receptor. In this situation, randomly occurring descending rectangular pulses are recorded. Each pulse is caused by current flowing across the membrane while two agonist molecules are attached to the receptor, activating it and opening the channel. The pulse stops when one or both agonist molecules detach from the receptor and the channel closes. The current that passes through each channel is minuscule, only a few picoamperes (about 10^7

ions per second), but each neuromuscular junction contains several hundred thousand receptor/channels. The current that flows when many are opened at once, as for example in response to a burst of acetylcholine from the nerve ending, is substantial and can be more than enough to depolarize the entire region and create the endplate potential that triggers muscle contraction.

Thus, the receptor and its channel make a powerful amplifier, the current carried by two acetylcholine ions is converted into a current carried by tens of thousands of sodium, calcium, and potassium ions. The receptor also is a switch. It is closed and off until acetylcholine is present; then it snaps open and passes current. When acetylcholine leaves, the channel snaps shut and cuts off the current.

The middle tracing (Figure 8) typifies an experiment in which both an agonist (e.g., acetylcholine) and tubocurarine are used simultaneously. In this situation, sometimes two agonists attach to the receptor. If this happens, the receptor activates and opens its channel to permit ions to flow and a descending pulse to be recorded. At other times one or two tubocurarine molecules may attach to the receptor, in which case the receptor is not available to agonists; therefore, the channel stays closed until the tubocurarine leaves. Since the channel is not opened, no current flows and no pulse is recorded. The result is a record of normal-appearing agonist-induced pulses of current, but there are fewer pulses than if tubocurarine was not present.[27] Because current flows through each channel less often, the amount flowing through the endplate at any instant is reduced from normal. This results in a smaller endplate potential and, if carried far enough, a block of transmission. This is a molecular description of the classical competitive interaction between acetylcholine and tubocurarine to reduce neuromuscular transmission.

The bottom tracing (Figure 8) is taken from an experiment in which the pipette contained a mixture of suberyldicholine and QX 222, a derivative of lidocaine. The QX 222 does not compete with an agonist for the receptor, so the channel is open just as often as in the control. However, current does not flow steadily even though the channel remains open.[28] It is thought that this occurs because the QX 222 molecule rapidly and repeatedly hops into and out of the channel. When the molecule is in the channel the flow of physiologic ions is blocked and no current flows, but when the drug is out of the channel the pathway is clear and current flows. This is typical of the channel blocking action of the local anaesthetics and other open channel blocking drugs. In practical terms, the channel opens and closes as before, but because of the intermittent block caused by the drug in the channel, the total current per channel opening is less than that of

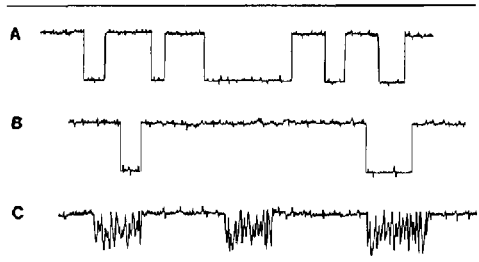

FIGURE 8 Traces represent patch clamp recordings of ion flow through a single receptor in the presence of an agonist (A), an agonist and tubocurarine (B), and an agonist and a quaternary local anaesthetic, QX 222 (C) (adapted from Neher *et al.*[27]).

the control. Summed over the entire endplate, this reduces the total endplate current and the endplate potential and so weakens neuromuscular transmission. It also may be noted that since tubocurarine and QX 222 act at different places, receptor site and channel, respectively, the effects of the two can add to reduce current and neuromuscular transmission.

It is now known that channel block is extremely common at neuromuscular junctions; many drugs can and do get into the receptor/channel's mouth and hinder the flow of current.[17,21] Many of these reactions occur with concentrations of drugs used clinically and thus may contribute to the phenomena and drug interactions that are seen in anaesthetized patients.

Particularly interesting is the fact that muscle relaxants themselves may cause channel block in addition to acting competitively with acetylcholine on the alpha unit sites.[29] All muscle relaxants are cations and are capable of entering the channel. Even though the drugs may act at both sites, a given drug may prefer to act at one or the other site, i.e., there are differences in preferred site of action. In this sense, it takes substantially greater concentrations of pancuronium to affect channels than receptor sites, whereas gallamine acts at both places at all concentrations. Tubocurarine seems to be in between; at low doses, which clinically produce minimal blockage of transmission, the drug is essentially a pure receptor blocker while at high doses, which produce complete or near complete blockade, the drug also affects channels. Decamethonium is particularly interesting because as an agonist it opens channels, but as a cation it enters and blocks them.[30] In fact, as a long slim cation, decamethonium is capable of going all the way through the open channel to enter the muscle cytoplasm.[31]

Figure 9 illustrates some of the classical actions of acetylcholine and muscle relaxants on endplate receptors. The top section depicts a section of membrane

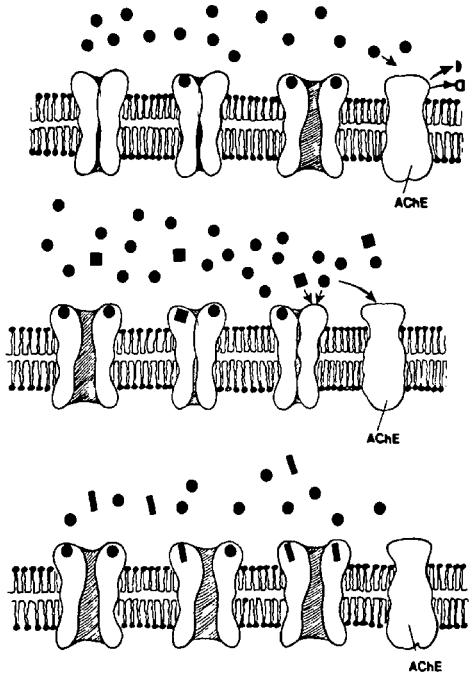

FIGURE 9 Representation of the classical actions of acetylcholine and muscle relaxants on endplate receptors. Symbols: ● = acetylcholine; ◗ = choline; ⟨ = acetate; ■ = d-tubocurarine; ▮ = decamethonium.

exposed to acetylcholine. Some receptors have attracted two molecules of acetylcholine and current flows through the channel, while some channels remain closed because the receptor does not have two molecules of agonist on it. The sketch also contains a representation of acetylcholinesterase, which may destroy acetylcholine by hydrolyzing it to acetate and choline.

The middle section (Figure 9) depicts a system exposed to a modest concentration of tubocurarine in the junctional cleft. Some receptors attract two acetylcholines and open the channel to depolarize that segment of membrane. Others attract one acetylcholine and one tubocurarine. No current will flow through these channels. The third receptor is particularly interesting because it has acetylcholine on one alpha unit and nothing on the other. What will happen depends upon which of the molecules above it wins the competition for the vacant site. If acetylcholine wins, the channel will open and the membrane will be depolarized. If tubocurarine wins, the channel will stay closed and the membrane will not depolarize. This is where cholinesterase plays a role. Normally the

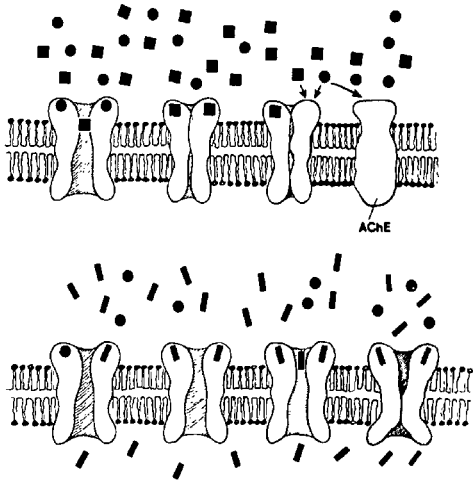

FIGURE 10 Representations of the effects of exposure to high and prolonged concentrations of muscle relaxants. Symbols: ● = acetylcholine; ■ = d-tubocurarine; ▮ = decamethonium.

enzyme destroys acetylcholine and removes it from the competition for a receptor, so normally tubocurarine wins the competition and transmission is blocked. If, however, an inhibitor such as pyridostigmine is added, the cholinesterase cannot destroy acetylcholine. This lets the agonist stay in the cleft where its increased concentration tips the competition between acetylcholine and tubocurarine to favour the former. This improves the chances of two acetylcholines binding to a receptor, even though tubocurarine is still in the environment. This is a molecular explanation of how reversing drugs can overcome a neuromuscular block produced by nondepolarizing relaxants.

The third panel (Figure 9) depicts the immediate effects of a modest dose of a depolarizing relaxant, decamethonium. Since it is an agonist, its reaction with receptors is like that of acetylcholine and the membrane is depolarized. Since it is not susceptible to acetylcholinesterase, it is not destroyed and removed from the cleft, so the depolarization persists and leads to neuromuscular blockade.

Figure 10 illustrates the results of exposure to high and prolonged concentrations of muscle relaxants. The top portion represents tubocurarine. In this case there is a much greater concentration of relaxant, and the molecular situation is very different from that depicted previously. The centre receptor has two tubocurarines and is closed. The left one is opened by acetylcholine, but a tubocurarine molecule in the channel prevents current from flowing to depolarize the membrane. The right

receptor already has one tubocurarine, so it makes no difference whether acetylcholine or tubocurarine binds to the other site; the channel cannot open in either case. When neuromuscular blockade is produced by tubocurarine in doses of this magnitude, inhibiting cholinesterase cannot be expected to be as beneficial as it is against low concentrations of the relaxant.

The bottom portion of the figure depicts some results of exposure to a high concentration of a depolarizing relaxant, decamethonium in this case. Decamethonium and acetylcholine are both agonists, and two molecules of either one, or a combination of the two, can open the channel and depolarize the membrane; thus some channels are opened and depolarize the membrane as before. Decamethonium has entered some channels (third receptor from the right). This blocks current flow and prevents depolarization of this membrane segment. some receptors, like those at the far right, have desensitized in the presence of decamethonium: their channels cannot be opened to carry current. Also, decamethonium is a slim molecule that can pass through opened ion channels; the sketch contains decamethonium molecules that have entered the muscle cytoplasm to cause mischief there. In this situation neuromuscular block is a complex of many things. some phenomena prevent current flow and thus produce a nondepolarizing blockade, but the total effect consists of depolarizing and nondepolarizing actions, channel effects, receptor desensitization, and intracellular effects. These events are part of phase II block but are not the complete explanation of the syndrome, which at present is not well understood.

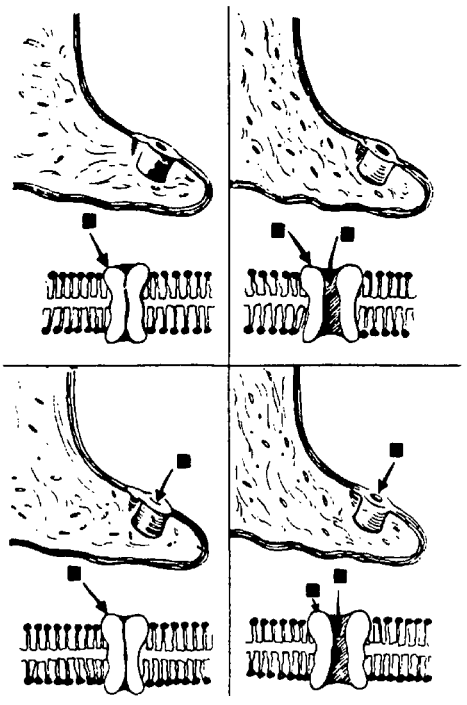

FIGURE 11 Sketch of some actions of d-tubocurarine at prejunctional and postjunctional receptors of the neuromuscular junction during changes in dose and frequency of stimulation: (upper left) low dose, low frequency; (upper right) high dose, low frequency; (lower left) low dose, high frequency; (lower right) high dose, high frequency.

Prejunctional receptors

Much less is known about cholinergic receptors in other organs, but the general principles probably apply. Both receptor block and channel block are basic phenomena that can occur whenever a drug and the receptor/channel have characteristics appropriate for an interaction. Some of the most interesting of these drug actions occur at the motor nerve terminal. This structure is known to have cholinergic receptors and it has been postulated that these influence the release of transmitter. In many aspects these postulates are still controversial, but the prejunctional receptors seem to be different from postjunctional ones in their chemical binding characteristics, the nature of the ion channel they control, and their preferential block during high-frequency stimulation.[6,32]

In its action on the prejunctional receptor/channel, tubocurarine seems to block the entry of sodium but not calcium. Accordingly, it interferes with the mobilization of acetylcholine from synthesis sites to release sites rather than interfering with release per se. There may be a specific receptor, a mobilization receptor, that is affected by tubocurarine to slow mobilization and cause failure of transmission at high frequency,[33] or it may be that tubocurarine blocks the flow of sodium needed for the mobilization process by causing block of cholinergic sodium channels in the nerve ending.[6] Practically, the two ideas have the same outcome: prejunctional receptor or receptor/channel blockade diminishes the release of acetylcholine from nerves stimulated at high frequency and this contributes to the weakening of transmission caused by muscle relaxants.

Overview

Figure 11 offers a framework within which to consider some of these actions of the muscle relaxants. The particular example is tubocurarine and the figure is limited to the neuromuscular junction, but a similar scheme potentially could be drawn for other muscle relaxants and other organs. The figure shows how site and mechanism of action relate to the concentration of the drug and

the stimulus imposed on the nerve. At low doses (e.g., those that produce minimal blockage of transmission) and at low frequencies (e.g., the one shock every few seconds favoured by investigators and those monitoring neuromuscular transmission during anaesthesia), tubocurarine acts predominantly at the receptor site to compete with acetylcholine. This is the process described for so long by numerous texts.

In more strenuous situations additional things take place. If the dose is increased, the drug enters the ion channel of the endplate to add channel block. This further weakens neuromuscular transmission and diminishes the efficacy of reversing agents. If the rate of nerve stimulation is increased, e.g., to tetanizing frequencies, then the nerve is stressed and the capacity to sustain transmitter release is diminished by the prejunctional action of tubocurarine to reduce mobilization. This causes waning of the amount of transmitter released and diminishes the capacity of acetylcholine to compete with the concurrent actions of tubocurarine at the postjunctional receptor. The sketch at the bottom right summates these events by illustrating a rapidly stimulated junction exposed to a high concentration of tubocurarine. In this situation all mechanisms, prejunctional and postjunctional, are operative and contributing to the blockade of neuromuscular transmission.

Extrajunctional receptors do not participate in the scheme sketched because they are not present in significant number at normal adult neuromuscular junctions. But, as noted previously, they are made rapidly if normal nerve activity to the muscle is not maintained, and they can strongly affect certain clinical uses of the relaxants.

The extrajunctional receptors are very responsive to agonists, such as acetylcholine or succinylcholine, and poorly responsive to antagonists, such as tubocurarine or pancuronium. Since agonists acting on these receptors cause substantial flows of ions across the muscle membrane, they can allow enough potassium to be released to elevate the serum concentration and lead to adverse cardiac affects. Because these receptors are not as strongly affected by nondepolarizing relaxants as are normal receptors, the hyperkalemic effect of succinylcholine may be reduced but not prevented by prior administration of tubocurarine or a related drug. Indeed, the opposite has been observed; tubocurarine can act as an agonist on these receptors; it opens receptor/channels[34] and so causes depolarization and contraction of denervated muscle.[35]

Since extrajunctional receptors are formed rapidly after the slackening of neural influence on muscle and are degraded soon after the neural influence returns, mixtures of normal and extrajunctional receptors are present in many clinical situations and may account for some of the quantitative differences in response to relaxants seen among various clinical states.

The new knowledge of receptors, ion channels, and muscle relaxants seems to complicate things; certainly it was easier to remember when we only thought of a competition between cholinergic agonists and antagonists for receptors in the endplate. However, the newer observations encompass a broader range of sites and mechanisms of action for both agonists and antagonists and in doing so they are more realistic. We intuitively recognize that no drug has only one site or one mechanism of action. We know that the muscle relaxants are not exceptions to this rule, and in recognizing their complexity we begin to bring our theoretical knowledge closer to explaining the variety of things that are seen when these drugs are administered to living human beings.

Acknowledgement

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