THE MECHANISM OF ACTION OF MUSCLE RELAXANTS
AND THEIR ANTAGONISTS

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Neuromuscular transmission is the intercellular conduction of excitation. It can be studied under accurately controlled conditions by a wide variety of precise experimental methods. The application of these has not only revealed a mass of information but has served to aid in the elucidation of the mode of action of drugs acting at this site. Moreover, the drugs themselves can be included in the list of tools contributing to the clarification of the over-all picture.

The dearth of knowledge, however, at the molecular level, is so complete that it is unobtrusive. The post-junctional membrane has certain describable attributes; estimates have been made of its electrical capacity, thickness, impedance, and the changes in the latter during depolarization, but its chemical components and their arrangement remain unknown. Likewise the change in this structure resulting in depolarization and the mechanism of restoration of the membrane are not even dimly perceived.

The muscle membrane in the region of the end-plate differs from that elsewhere in several aspects, most interesting of which, from the pharmacological standpoint, is the presence of sites with which acetylcholine and some neuromuscular blocking agents can combine. The chemical nature of these receptors is obscure. What acetylcholine does to them, and how the consequences of reaction between transmitter and receptor produce the sequence of events leading to the initial depolarization is unknown.

If the description of the mode of action of these drugs appears to be simple it is probably because it is incomplete or to some extent illusory. The emphasis in this account is on the more recent developments in the subject. In addition to the contents of this volume recent reviews of general and comprehensive nature have been published as symposia and by Paton and Zaimis, Paton, Riker, Fatt, Katz and del Castillo, and Foldes.

NOMENCLATURE OF MUSCLE RELAXANTS

Although most authors have classified the important muscle relaxants into two groups, those which resemble d-tubocurarine and those which resemble decamethonium, there seems to be two schools of thought. The first refers to the curare-like agents as "competitive" and those like C-10 (decamethonium) as "non-competitive." This terminology is "molecular" in level. The second calls the curare-like group "antidepolarizing" or sometimes "nondepolarizing," while those acting like C-10 are referred to as "depolarizing." The terminology here can be thought of as "physiological" or "cellular" in level of organization.

The term "competitive" comes from the field of enzyme chemistry and in general refers to evidence obtained from the quantitative behavior of the enzyme inhibitors involved. The criteria necessary to establish whether a blocking agent is "competitive" or "noncompetitive" have not been obtained in the case of the interaction of curare with its receptors.

When a stable "competitive" inhibitor blocks cholinesterase it prevents acetylcholine molecules from combining with and being split by the enzyme. When curare reacts with its receptors it prevents acetylcholine from initiating a completely unknown complex process. Curare is assumed to react with the same receptors as acetylcholine but it is unable to cause depolarization itself. It is likely that when its mode of action is clarified, although competition may be present, it will be only a part of a more extensive mechanism. Curare has been shown to block the uptake of a labelled depolarizing agent by voluntary muscle. The term "competitive" offers no insight into this process. One of the basic assumptions underlying the use of the terms "competitive" and

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“noncompetitive” is that curare, C-10 and acetylcholine are presumed to react with the same receptors. If we assume that curare and acetylcholine compete, are we to assume that because C-10 and acetylcholine both depolarize that they do not compete with each other for the receptors? The term does not seem to exclude C-10.

In defining the term “competitive” Paton describes the classical picture of dynamic equilibrium and states that this must underlie neuromuscular block. He points out that during block, both curare and acetylcholine are rapidly combining with and dissociating from their receptors and that the number of receptors occupied by each will depend on the way they compete for receptors during this process. This model needs amplification. Except in the presence of an anticholinesterase, acetylcholine is present for an exceedingly short period of time. In fact del Castillo and Katz have expressed the view that there may be no time for competition; also, they have presented evidence that the release of curare by diffusion from its receptors is slower than was expected.

In the greater time period available to curare for reaction, it will set up a reversible equilibrium with its receptors; combination and release of drug going on concomitantly; with a fraction of the total receptors occupied and the remainder free. The sudden release of acetylcholine would then occupy some of the free receptors and be released and destroyed before any degree of competition could set in.

If curare reduces the reaction of acetylcholine with its receptors in a way remotely similar to the way curare reduces the uptake of labelled depolarizer then it may act in a manner as yet unexplored and not adequately described by the term “competitive.”

Phase I block by C-10 is a depolarization block and is referred to as “noncompetitive” while phase II is curare-like and presumably therefore “competitive.” The assumptions that in phase I block, both C-10 and acetylcholine react with the same group of receptors but do not compete for sites, but that in phase II they do compete is difficult to accept without evidence.

It has been demonstrated that drugs like curare can prevent or reduce the depolarizations produced at the end-plate by acetylcholine, C-10 and similar agents. The term “anti-depolarizing” therefore seems to be a safer description than “competitive” at the present time.

Although the term “depolarizing” does not describe both phases of action of drugs like C-10 and succinylcholine, no term is likely to do so.

That some drugs may be depolarizing in one preparation and antidepolarizing in another may be a fact, but it is not necessarily a defect of terminology, for presumably a name referring to drug action in man is desired, and a good word which will include all animals affected will be hard to find.

Block can also be produced in synapses and at the neuromuscular junction by diminution of the acetylcholine liberated. Agents such as the hemicholinium can do this act presynaptically. It will be simpler if such agents are kept separate from those acting postsynaptically and not combined under the term “nondepolarizing.”

Some Characteristics of Neuromuscular Blocking Agents Labelled with Radioactive Isotopes

Neuromuscular blocking agents labelled with radioactive isotopes are being used increasingly to study the mode of action, distribution, and fate of compounds closely related to d-tubocurarine. C14 tagged representatives of both depolarizing and antidepolarizing drugs are available but the number of the latter is larger. So far only C14 has been used to label the anti-depolarizing drugs while both C14 and T201 have been used in the case of the depolarizers.

Labelled Antidipolarizing Agents. The preparation and use of these agents seems to have been started by Marsh (fig. 1), who prepared d-chondocurarine by adding a labelled methyl group to each of the two nitrogen atoms of d-chondocurarine and d-tubocurarine dimethyl ether by adding a labelled methyl group to each of the two hydroxyl groups of d-tubocurarine. He also prepared d-tubocurarine dimethyl ether from d-chondocurarine by adding labelled methyl groups to both nitrogen atoms and to both hydroxyl groups. The latter preparation gives a product with both the highest potency and radioactivity per molecule of this series. In all cases the methylation was done with radioactive methyl iodide. The syn-
thetic reactions involved are easy to carry out and the yields of labelled product uniformly high. The specific activity achieved depends on the activity of the methyl iodide used and the number of tagged groups introduced per molecule.

The preparation of \(d\)-tubocurarine dimethyl ether from \(d\)-tubocurarine is more convenient because of the greater availability of the starting material, but the radioactivity is only half that obtainable when it is prepared from \(d\)-chondocurine.

Radioactive \(d\)-tubocurarine itself has not been prepared yet because the corresponding tertiary alkaloid, \(d\)-tubocurine, from which it could be prepared is unknown. The structurally and pharmacologically closest labelled derivative is the dimethyl ether of \(d\)-tubocurarine.

A potent antidepolarizing agent labelled with C\(^{14}\) has been introduced by Waser, Schmid and Schmid,\(^{18}\) who prepared it by methylation of nor-Calabash-curarine I with radioactive methyl iodide. With this Waser, Lüthi and Huber\(^{19-22}\) have demonstrated binding of the drug in the region of the motor end-plates of rat diaphragm.

Callamine triethiodide has been introduced by Chagas et al.\(^{23}\) It has six labelled atoms per molecule attached to the three quaternary nitrogen atoms. These workers have also used the dimethyl-\(d\)-iso-chondodendrine labelled by attaching two methyl groups to its nitrogen atoms. It is not a very potent neuromuscular blocking agent, but is an effective ganglionic blocking agent. Chagas and associates have made use of these agents in their work on the binding of curare by substances in the electrical organ of the electric eel and in voluntary muscle of mammals.

**Labelled Depolarizing Agents.** So far, only two of these are available—decamethonium labelled with C\(^{14}\) and a compound closely related to it labelled with I\(^{131}\). The synthesis of the first of these has been described by Waser and Lüthi.\(^ {21}\) It is prepared from decamethylene diamine by methylation with C\(^{14}\) methyl iodide, six labelled methyl groups being added per molecule, giving a high specific activity.

The second compound \(^{10}\) iodochlolinium

\[
\text{CH}_3 \quad \text{CH}_3
\]
\[
\text{I} \cdots \text{CH}_2 \cdots \text{N}^+ \cdots (\text{CH}_2)_{10} \cdots \text{N}^+ \cdots \text{CH}_2 \cdots \text{CH}_2 \cdots \text{I} \quad 2\text{Cl}^-
\]
\[
\text{CH}_3 \quad \text{CH}_3
\]
is related to decamethonium and has two iodine atoms per molecule as terminal iodine substituted ethyl groups attached to each quaternary nitrogen atom.

Labelling with C¹⁴ has the advantages that the process does not alter the structure of the tagged molecule. The label has a long half-life and because of its soft emission gives good resolution in radioautography. However, in the case of biological material, destruction of the sample is usually necessary in order to prepare the material in the form of a sufficiently thin film for counting.

The introduction of iodine into a molecule of neuromuscular blocking agent alters its structure and essentially produces a new compound which must be carefully tested before use to prove that it has the desired pharmacological actions. Moreover, since organically bound iodine compounds are not all stable, the product in question needs study from this standpoint. Nevertheless, the high specific activity which is readily obtainable and the hard gamma ray emitted are advantageous. The latter enables continuous counting throughout the course of a variety of experimental methods with consequent increase in accuracy and reduction of variance due to sampling of biological tissues. The whole procedure is also faster.

STRUCTURE ACTION RELATIONSHIPS

Monoquaternaries. Since the time of Crum, Brown and Fraser in 1869 it has been known that quaternary ammonium ions have a neuromuscular blocking action usually of low activity. Since then, there have been continuing efforts to define the structural requirements for high activity. There are many such requirements but since none of them can be studied in complete isolation, their individual contribution remains speculative.

Ing and Wright ⁴¹ pointed out that the activity of onium ions of the type R₃N⁺ was not only extraordinarily independent of the groups attached to the positively charged central atom (X) but also to some extent of the central atom itself. This atom could be nitrogen, phosphorus or sulfur, the common factor being the central localized ²⁵, ²⁸ positive charge.

More recently Beccari ²⁷ and Dwyer, Gyarfas, Wright, and Schulman ²⁸ have shown that certain large positively charged inorganic ions can also produce curare-like neuromuscular block. Here again the only common feature which all of these structures possess is a positive charge.

Di, Tri, and Tetraquaternaries. Since 1935 when King ³⁰ isolated crystalline d-tubocurarine and clarified its chemical constitution, it has been clear that high potency was associated with two quaternary nitrogen atoms in this compound. The synthesis of an extensive series of potent neuromuscular blocking diquaternaries by Bovet and his colleagues has confirmed the usefulness of this concept. It appears that in bifunctional compounds of this type, the interquaternary distance of ten carbon atoms is about optimal for neuromuscular blocking action.

Although tri and tetraquaternaries have been synthesized the further increase in potency has not been comparable to the difference between the mono and diquaternaries. In studying trisium ions of the NSN and NNN types an additional factor influencing response has been uncovered by Edwards et al. ²⁰, ³¹ Compounds of both series containing 5 and 6 carbon atoms between onium groups were antidepolarizing. Increasing the number of carbon atoms to 8 gave transitional compounds and when the methylene chain was extended to ten units, the action was depolarizing. Similar effects were noted in two tetraethylammonium compounds. ³²

It has not been possible, so far, to draw conclusions about the configuration of the receptors relative to one another from potency data on mono, di and higher quaternaries.

Molecular Bulk. All of the quaternary nitrogen containing drugs which depolarize the end-plate region of voluntary muscle are slim molecules. The antidepolarizing agents are bulky molecules and drugs like C-10 seem to be sensitive to even small increases in cross-sectional area. Such increases reduce depolarizing potency and favor antidepolarizing activity. On this basis Bovet et al. ³³ have classified these drugs "lepto-" and "pachycurares."

The chemical substitutions of C-10 which have this effect are of great variety and seem not to have any common chemical feature or possibility of bonding affinity for a receptor surface. In free solution, since decrease in diffusion velocity is related to the square root
of molecular weight, the reduction in this parameter would seem negligible. However, if the penetration of a membrane or any closely cross-linked structure were necessary for depolarizing activity quite a large effect could be expected. That the reduction in rate at which antidepolarizing agents reach the receptor area is not responsible for the absence of depolarizing action is shown by the fact that the rapid application of curare by micropipette to the end-plate region does not depolarize.12

There are exceptions to the rule that increase in bulk favors antidepolarizing action but from the evidence as a whole it seems to be a useful generalization.

The Nature of the Drug-Receptor Union

The number of known chemical bonds that can be invoked to explain the binding of a drug to its receptors is limited.24 The formation of stable covalent bonds by neuromuscular blocking agents like curare or C-10 is excluded because these substances are known to combine reversibly with their receptors; the weaker bonds which dissociate at body temperature are necessary to explain the reversibility of action.

The fact that a positive charge is necessary and that action is largely independent of the atom bearing the charge and of the attached groups, supports the idea that ionic bonds are the main forces involved.25 Van der Waals' forces will reinforce the binding because they occur between all atoms at close range, but since they are weak and active only over very short distances, they will only be significant with the larger molecules and then only when the drug receptor fit is close. In the latter, however, they may confer specificity on such sites when fit is good.

The concept of ionic bonds requires the presence of negative charges on the opposing receptor surface, and since hydrogen atoms on the surface of drug molecules can bear weak positive charges some contribution from hydrogen bonding is possible.

The concept of ionic bonding is important and has interesting implications. Some of these can be made clear by considering the properties of the familiar ion exchange resins in which ions are held by ionic bonds and can exchange for ions of like charge in the solution around them. When such exchange occurs the electrical neutrality of the ion exchanger is always maintained. This makes it obligatory that for each ion picked up an ion of the same charge is displaced. When the receptors at the neuromuscular junction bind curare, which has two positive charges, it is necessary either that the receptor structure cease to be electrically neutral or that ions of equal total charge be released to the surrounding solution. Since curare produces no detectable electrical disturbance,25, 36, 37 even when applied to the end-plate region directly by micropipette, an exchange process seems probable. Although the potassium ion has been suggested for the role of exchange ion, there is no direct experimental evidence for this.

Recently Waser and Liithi,19, 21 Waser, Liithi and Huber 20 and Waser 22 have been able to demonstrate localized binding of labelled depolarizing and antidepolarizing drugs at the mammalian motor end-plate using C14 labelled agents and autoradiography. Contact autoradiograms of the air dried diaphragms of white mice killed by an intravenous injection of a minimal lethal dose of labelled drug were prepared. These showed localization of activity at the end-plates which were estimated to bind ten million molecules of C-curarine. Since this number of drug molecules is insufficient to cover the area of the post junctional membrane, they suggest that only a few highly differentiated receptors are occupied by the blocking agent.

When the nerve to one half of the diaphragm was cut, the cholinesterase and the radioactivity due to bound labelled curarine decreased together and vanished completely after 60 days.22

The Receptor Substance

In 1953 Chagas, Bovet and Sollero 38 noted in the electric eel paralyzed with curare that the muscles recovered before activity returned to the electric organ. In preparations in which muscle recovery occurred in 6–8 hours, the electric organ required 24 to 96 hours. This observation led to the belief that a study of the binding of labelled antidepolarizing agents by the electric organ and by mammalian muscle would yield information on the site and nature of the receptors for curare.23, 39–43
The electric eel was injected with C\textsuperscript{14} labeled gallamine triethiodide in these experiments and 3 to 9 hours later the animals were killed and nonbound radioactivity washed out by perfusion with distilled water. The electric tissue was then ground with water. After centrifuging, the bulk of the radioactivity remained in the supernate in a nondialyzable form when dialyzed against distilled water, indicating that the labelled drug is bound to a nondiffusible macromolecular component. This complex can be dissociated to a considerable extent by salts, electrophoresis or denaturation with acetone or trichloroacetic acid. Although Chagas, Bovet and Sollers\textsuperscript{\textdegree} do not claim to have isolated the receptor for curare they assume it may be part of the macromolecular complex. As evidence for this view, they claim that curare antagonists diminish the complex, that the complex is pharmacologically inactive, and that there is a correlation between the complex formed and the state of curarization. They state that the evidence suggests that the receptor substance is a complex amino polysaccharide containing acetylglucosamines resembling some of the hyaluronic acids. Their most active preparation could fix about 10 per cent of its weight of quaternary ammonium salts.

The results are interesting and no doubt further work will answer many of the questions posed. It is probable that tissues contain substances bearing negative charges which can bind quaternary ammonium ions. If this is so, isolation experiments would be expected to yield at least two fractions, one containing non-specific binding sites and the other the true receptors. Cartilage and the collagen of connective tissue contains mucopolysaccharides which in turn contain the components mentioned above. It will be necessary to distinguish between these and the true receptors.

As mentioned earlier it has been suggested\textsuperscript{24, 25} that the receptor is a negatively charged site which normally binds potassium ions, and that when quaternary ammonium groups in acetylcholine, curare, decamethonium or other active molecules combine with these receptors, the potassium ions are displaced in an ion exchange reaction. It has recently been shown by Solomon, Lionetti and Curran\textsuperscript{45} that human blood contains a substance which can selectively bind potassium ions. This substance discriminates between potassium and sodium ions, binding the former preferentially. The complex it forms with the potassium ion is soluble in lipids and dissociates to some extent in water. They have also demonstrated that phosphatidyl serine has these properties. Whittaker,\textsuperscript{45} commenting on the work of Solomon \textit{et al.}, suggests the advisability of looking for acetylcholine and presumably curare receptors among the phosphatides.

**MODE OF ACTION OF CURARE AND THE ANTIDEPOLARIZING AGENTS**

In the interval between the time when the nerve impulse reaches the neuromuscular junction and the passage of the wave of excitation over the muscle fiber, acetylcholine is liberated from the nerve terminal, diffuses to its site of action, and produces depolarization of the post-junctional muscle membrane. Curare does not impede the liberation of acetylcholine but it prevents its action.

One of the characteristics of curare is that its actions manifest themselves almost entirely by preventing some other action or effect. It has no detectable effect on the direct electrical properties of end-plate or muscle fiber.\textsuperscript{35, 56, 37} Moreover, it is ineffective when placed inside the fiber by micropipette.\textsuperscript{37} Partly as a consequence of these points there is little quantitative data available on the basic reactions upon which its action rests.

The reaction between curare and its receptors at the neuromuscular junction can be represented by the completely reversible process: \textsuperscript{46}

\[ \text{Curare + Receptors} \rightleftharpoons \text{Curare-Receptor Complex} \]

In this case the degree of block would depend on the number of receptors occupied. Alternatively block might be represented by the fundamentally distinct process:

\[ \text{Curare + Receptors} \rightarrow \text{New Products} \]

In this case the drug is destroyed at the site of action and as the products of destruction are eliminated, the receptors might be conceived to be regenerated. There is good evidence that the first reaction represents the process of
curarization while the second is untenable. It can be summarized as follows:

(a) Curare and many of the onium ions are stable compounds not ordinarily susceptible to oxidative or hydrolytic chemical reactivity.

(b) Under carefully controlled conditions, after adequate time for equilibration, constant concentrations of curare produce steady degrees of partial paralysis lasting for several hours. Moreover, the degree of block produced for a given curare concentration is independent of whether equilibrium is approached by diffusing curare into or out of the preparation.

(c) In isolated muscle the rate of diffusion of curare to or from its site of action determines the rate of paralysis and of recovery of the preparation, and these two rates of diffusion are the same. This would appear to be proof of the truly reversible nature of the reactions. Curare is not destroyed by muscle.

If such a picture is correct, the rapidity of action of curare should be increased by decrease in distance between point of application and site of action. This is indeed the case. Paralysis of an isolated strip of diaphragm by curare added to the bathing fluid takes minutes. When the drug is applied by micro-pipette very close to the end-plate action can be detected after the lapse of a fraction of a second.

Although many factors such as distribution, excretion, metabolism and state of the circulation influence the curare concentration in muscle capillaries, and although the potency of the drug may be altered by changes in electrolyte pattern and other variables, the rate of curarization and recovery for any given set of variables will be markedly affected by the rate at which the drug diffuses to and from its site of action.

Relationship Between Concentration of Curare and Effect Produced on a Muscle. Experiments in whole animals do not yield data of value in describing the relationship between curare concentration and effect produced because the curare concentration in the vicinity of muscle fibers is a complex, varying and unknown function of time. It is only from carefully controlled experiments on isolated tissues that such data can be obtained. Available data are chiefly confined to the influence of curare on degree of paralysis, on magnitude of end-plate potentials, and on the uptake of an I^131 labelled depolarizing agent.

Since the site of action of curare is freely accessible to the drug in isolated muscle after time for equilibration, the concentration of the drug around the receptors will be the same as that surrounding the muscle. Dose response curves relating degree of paralysis to concentration of drug around receptors can therefore be determined.

All isolated muscles so far examined by this technique show characteristic S-shaped dose-response curves; there are, however, considerable differences between the positions and slopes of these curves for different muscles in the same animal. The slope and position of any one curve is presumably related to differences in sensitivity of different fibers to curare.

Mode of Action of the Depolarizing Agents

The discovery of decamethonium led to the recognition and analysis of a type of neuromuscular block basically different from that produced by d-tubocurarine.

Decamethonium produces a depolarization of the end-plate region which is sufficiently profound and prolonged to discharge and depolarize the adjacent muscle membrane. It depolarizes the end-plate region in a manner which appears to be basically identical with the same action of acetylcholine, but since it is not hydrolyzed by cholinesterase, the action is prolonged.

The initial depolarization produced by C-10 causes an increased excitability of the end-plate region resulting in fasciculation, especially if the drug is administered rapidly, and to repetitive firing in response to single nerve shocks. Later, as the depolarization increases and extends, the excitability decreases causing progressively greater block.

Curare and drugs like it are antagonized by the anticholinesterases, potassium ions and a fall of temperature, while phase 1 block by the depolarizing agents is not so affected.

From the standpoint of mechanisms of drug receptor interaction, however, the difference between the sequence of events which occurs when a muscle is exposed to a constant dose
of curare and when it is exposed to a constant dose of depolarizer such as C-10, is of theoretical interest and clinical significance.

When a submaximal paralyzing dose of curare is added to an isolated preparation under carefully controlled conditions, the drug diffuses to its site of action and, after a period of increasing block, sets up a steady state of partial paralysis which lasts for many hours. This steady state is reached in about half an hour.

The behavior of C-10 is in sharp contrast to this. Acting on isolated muscle from rabbit, guinea pig, and fasciculi of human intercostal muscle, it produces two blocks with a period of recovery of neuromuscular transmission between them. The first block is the characteristic depolarization block of C-10 and is of rapid onset and short duration. It is followed by recovery of transmission and the disappearance of depolarization. The second block is not accompanied by depolarization, and resembles that produced by curare in that it is antagonized by anticholinesterases, potassium ions, and a fall in temperature, but it differs from that produced by curare in that it develops 5 to 10 times more slowly.

Decamethonium and the depolarizers in general diffuse faster than curare, but they set up steady states of partial paralysis more slowly than curare. These steady states are in phase II block and constitute a paradox because it does not seem possible that the same mechanism is involved. If both drugs were reacting with the same receptors the faster diffusing molecules should equilibrate sooner. It would seem as if the depolarizers, in phase II, are acting at a deeper level than curare. In this connection experiments with labelled C-10 are of interest.

Influence of Curare on Uptake and Release of an \( ^{131} \)I-Labelled Depolarizing Agent

This depolarizing agent has been referred to as iodocholinium. If its uptake was confined to the same receptors as curare the drug would be taken up in small amounts over a period of half an hour and, upon washing out, would leave the receptors in a similar period. In fact, its behavior is nothing like this.

Voluntary muscle from guinea pigs, rabbits and rats exposed to radioactive iodocholinium shows a steady uptake of the drug extending over a period of several hours. Uptake is greater than can be accounted for by the diffusion of drug into the intracellular spaces, and is markedly inhibited by just paralytic doses of \( d \)-tubocurarine. Uptake by other tissues is low and is not affected by curare.

When voluntary muscle loaded with radioactive depolarizer is treated with high doses of curare, no detectable amount of depolarizer is released or displaced and curare has no effect on the rate at which the radioactivity leaves the muscle.

The uptake of labelled drug is parallel to the development of phase II block and may be associated with its cause. Presumably the iodocholinium is entering the fiber; in any case it is beyond the power of added curare to displace it. The marked effect which curare has on its uptake is a new action of an antidepolarizing agent and indicates a more complex mode of action for this agent than was suspected.

Drug Receptor Interaction

In considering the interaction between neuromuscular blocking agents and their receptors, it is customary to divide those agents into depolarizing and antidepolarizing groups, and it is worthwhile to examine how far this division is mutually exclusive.

In the majority of preparations curare behaves as a pure antidepolarizing agent. It is only in the extreme case of the lizard that it depolarizes.

On denervated muscle, curare produces electrical activity, contracture and fibrillation, all of which can occur during depolarization. Similar phenomena are seen in response to curare on the denervated rat soleus.

Normally only the end-plate region is sensitive to the depolarization by acetylcholine. Recently, however, Axelsson and Thesleff have shown that after denervation the whole of the muscle fiber membrane of the femoral muscle of the cat is sensitive to the depolarizing action of acetylcholine.

After section of the phrenic nerve in the mouse, Waser has demonstrated a progressive degeneration at the end-plate. The amount of C\(^{14} \) labelled C-curarine l bound by the end-plate decreases with time and disap-
pears after 60 days. Acetylcholine esterase shows a closely parallel decrease. The phenomena produced by the action of curare on denervated muscle occur at a selected time during the period when the receptors for this drug and also the cholinesterase are disappearing from the end-plate region. Moreover, the sensitivity of the muscle fiber membrane as a whole is changing markedly.

Denervation sensitizes to depolarization in an unknown manner. Results obtained with such preparations require cautious analysis.

It is the depolarizing agents which overlap into the antidepolarizing class rather than vice versa, and all depolarizing agents examined, including acetylcholine itself, can show anti-depolarizing activity under suitable experimental conditions. Broadly speaking, there are four ways in which the curariform component in the mode of action of these drugs shows itself.

**Biphasic Action.** In 1951 it was clearly demonstrated that C-10 and carbachol on the rabbit lumbral had a biphasic or dual-blocking action. An initial block, phase I, of rapid onset and short duration, with the characteristics of a depolarization block, was followed by recovery. Then phase II, with the characteristics of an antidepolarization block, appeared and slowly increased over a period of several hours to a steady state.

Further investigation has shown that all depolarizing agents, including acetylcholine protected by an anticholinesterase act biphasically if given adequate time and that all muscles which these agents can depolarize including human muscle can respond with a biphasic paralysis.

Since phase I is followed by recovery of transmission presumably the depolarization associated with phase I has passed off, as does the reduction in height of response to direct stimulation. Thesleff showed that, in frog muscle, it does pass off in spite of the continued presence of depolarizing agent.

In man, Grant, Ruddell, Hodges, and Guerier and Huxley-Williams have shown that depolarizing agents sometimes produce unexpectedly long-lasting effects which can be antagonized by anticholinesterases. These long-lasting effects could be potentiated by antidepolarizing agents. Both of these observations suggest that the blocks in these cases were of phase II type.

**Species and Other Factors Influencing Sensitivity to Depolarizing Agents.** The sensitivity of different species to depolarizing agents varies greatly more than does the sensitivity to curare. Sensitivity to C-10, for example, decreases in the order hen, cat, man, dog, rabbit, monkey, hare, mouse, and rat, the latter being very resistant indeed. Considerable sensitivity difference is also seen in the responses of different muscles in the same animals.

The evidence presented by Churchill-Davidson and Richardson indicates that myasthenics are resistant to the depolarizing action of C-10. Resistance to the depolarizing action of C-10 is also produced by antidepolarizing agents and it is difficult to decide whether the disorder in myasthenia gravis is more like the phenomena associated with some sort of phase II block, or to the simpler block of a pure antidepolarizing agent. It seems probable that neither of these analogies will suffice.

Resistance to depolarizing agents is also seen in children and neonates.

**Interaction of Depolarizing Drugs at the End-Plate.** It has been generally assumed for some time that the receptors for curare and the depolarizers including acetylcholine, are the same, and that curare differs from the depolarizers in that while it combines with the receptors it cannot cause the transformation which results in increased membrane permeability and depolarization. Moreover, by occupying the receptors it prevents their transformation by the depolarizers and hence causes block. This model can be represented (Castillo and Katz):

$$S + R \rightleftharpoons SR \rightleftharpoons SR'$$

where S represents acetylcholine or one of the blocking agents, SR the intermediate compound formed by curare or the depolarizers, and SR' the state which results in depolarization. Castillo and Katz have extended this model by showing that the depolarizers have some antidepolarizing ability, depending on the rate of transformation of their intermediate receptor complex into the depolarizing form. The difference between depolarizing and antidepolarizing agents is viewed as being the
relative rates of the second step in the reaction with the receptors.

In whole animals, however, the distinction between the two classes of drug may not be on exactly the same basis. The basis of phase I block by decamethonium has been demonstrated to be depolarization, although in the experiments of Castillo and Katz it shows marked antidepolarizing action, while the antidepolarizing component of its action in whole animals seems to be more associated with phase II phenomena.

Induced End-Plate Resistance to Depolarization. This phenomena has been recognized by many, under a variety of circumstances, and described and interpreted in several different ways. It is not too clear whether the resistance to depolarization observed is a single entity manifesting itself in different ways or in fact one or more basically different processes resulting in the same final effect.

As phase I block, due to a depolarizer, passes off, transmission returns, indicating the development of resistance to the depolarizer still present. This is followed by the development of the antidepolarizing phase II block, and the resistance to depolarization continues to develop and eventually produces complete block.

Are the resistance to the depolarizing drug seen as a return of transmission and the developing antidepolarizing phase II block manifestations of the same or two different phenomena? Their separate identity has not been proven, but since doses of antidepolarizer, too small to have any effect alone, can have a marked blocking effect on depolarizers the early part of phase II would be expected to have a large effect on, and to antagonize, phase I. It therefore seems possible that these two manifestations of resistance to depolarization may not be due to the same basic mechanism.

One of the most interesting phenomena encountered in connection with the action of C-10 in isolated muscle is the influence of duration of action of the drug on the time taken for recovery of initial sensitivity. Although muscle recovers rapidly from C-10 when washed, the effects of the drug have not completely disappeared. The phase I action of a repeated dose of C-10 is depressed or absent, and although recovery of transmission occurs in 15 minutes, it may take three hours of washing to restore the original response to the drug. It seems as if the antidepolarizing phase II block might be due to the slow accumulation of C-10 somewhere in the muscle from which it can only be removed slowly by washing. The evidence for this view, though scanty, may be worth summarizing. Burns and Paton, commenting on the tachyphylaxis or resistance to depolarization, produced by C-10 in whole animals, suggest that entry of C-10 into the interior of the fiber may be an essential part of the depolarizing process, and that depolarization depends on the concentration difference of the drug across the membrane. They consider accumulation inside to have an antidepolarizing influence.

In this connection the fact that muscle can take up large quantities of labelled depolarizer and that this uptake can be blocked by curare is of possible significance. Moreover, in these experiments the development of phase II block parallels the uptake of labelled depolarizer closely.

The C-10 tachyphylaxis referred to above is presumably a manifestation of phase II block, and has been noted in human volunteers by Pelikan et al. and by Zaimis in monkeys, rabbits and hares.

Induced desensitization to acetylcholine has also been noted by Fatt, by Castillo and Katz, and by Katz and Thesleff. Castillo and Katz interpret the phenomenon by suggesting that acetylcholine degrades the receptor into an inactive and nonreactive form from which it recovers slowly when the acetylcholine is removed from the bath.

Antagonists

The Potassium Ion. Although Wilson and Wright demonstrated that the potassium ion is a potent antagonist of curare, it cannot be used clinically for this purpose because of undesirable side effects. Nevertheless, the phenomenon has not been fully explained and it is of basic interest and importance.

Walker and LaPorte showed that small increases in K+ concentration increased end-plate potentials and antagonized curare but that depression was easily produced by membrane depolarization. Temperature reduction to about 25 C. increases the ability of K+ to
antagonize curare on rat diaphragm.4 Although the effect of K+ on muscle membrane produces depolarization and resembles an applied cathodal current this phenomenon does not explain the complex effects of temperature changes on the antagonism.

Anticholinesterases. Increasing doses of curare diminish contraction until paralysis is complete, but an end-plate potential can still be recorded. Further increases in the dose of curare abolishes this also; beyond this point, increasing doses do not directly manifest themselves, but there is no evidence that receptor occupation does not continue to increase and there is evidence to suggest that it does.22

In the reduction of the uptake of I131 labelled depolarizer by curare the maximal effect is exerted by a dose of curare about 20 times that required to cause complete paralysis. The maximum antagonism to curare block that can be achieved with an anticholinesterase is not effective against doses of curare that are much greater than those that produce full paralysis. The curare antagonism available from anticholinesterases is maximal when the enzyme is completely blocked. Beyond this point, further effect of the anticholinesterase will depend on its other pharmacological actions, and these may even augment the action of curare.

Anticholinesterases differ in the reversibility of their actions, in potency, duration of action, and in distribution in the body, and in the spectrum of other pharmacological actions they display.

Reversibility. The alkyl phosphate anticholinesterases differ from all other anticholinesterases in that their action is irreversible, although low doses of some of them show reversibility for short times. In fact, they phosphorylate the enzyme by attaching an alkyl phosphate radical to it by a strong stable covalent bond. After this has occurred it appears that recovery takes place by regeneration of the enzyme rather than removal of the alkyl phosphate radical. It has been shown by Douglas and Paton that the actions of tetraethylpyrophosphate at the neuromuscular junction in the cat can be attributed solely to its inhibition of cholinesterase at the end-plate.

The irreversibility of these agents together with the selectivity of their action makes them useful experimentally.

Mode of Action. The anticholinesterases evidently act by prolonging the life of acetylcholine liberated by nerve impulses at the end-plate. This effect is indicated by the great prolongation of the curarized end-plate potential so produced.77, 78

Even if in the presence of curare, the liberated acetylcholine has not sufficient time to displace any curare by competition, in the presence of an anticholinesterase adequate time would presumably be available. Under these circumstances the number of receptors available to acetylcholine would be increased. This explanation would seem to be a useful concept and is highly probable, but it is not necessarily the complete explanation.

If the occupation of receptors by bulky curare molecules had any obstructing effect on the diffusion of liberated acetylcholine then the increase in concentration of the latter for a longer time would increase its concentration gradient and so its action.

Effects of Changes of Temperature on Neuromuscular Blocking Agents

The effects of temperature changes on the action of neuromuscular blocking agents are of considerable interest because they affect different chemical processes in characteristically different ways and hence can be used analytically to study mechanism. The method is rendered difficult by the fact that temperature coefficient determinations are only of value when they refer to single uncomplicated processes. So far, the only process which it has been possible to analyze in this manner is the diffusion of curare to and from its site of action in isolated muscle.

However, some studies on the effects of temperature on the equilibrium potency of curare, the antagonism by the potassium ion, the cholinesterase at the end-plate, the liberation of acetylcholine, the frequency and distribution of miniature end-plate potentials, and on the form of the end-plate potential itself have been made and are interesting and useful in that they indicate clearly the large amount of material yet to be analyzed and explained in this connection.
The effect of temperature change on the equilibrium potency of curare is complex. Decrease in temperature of partially paralyzed isolated rat diaphragm to 26 C. antagonizes the action of curare. Further reduction causes a progressive increase in potency till just below 10 C. when the preparation paralyses in the absence of curare. The phenomenon is seen in rabbit, guinea pig, and human muscle.

In the rat diaphragm at least under comparable conditions the resting muscle fiber membrane potential remains unchanged down to 5 C. Changes in resting potential, therefore, cannot be invoked to explain the above effects. Boyd and Martin, working with cat muscle, conclude that a fall of temperature from 27 C. to 22 C. decreases the amplitude of the end-plate potential when transmission was blocked by curare but increases it when transmission was blocked by magnesium. In commenting on these results, they suggest that a fall of temperature intensifies the action of curare. Species differences may have been involved. Measurements by Straughan of acetylcholine liberation by isolated rat diaphragm in response to nerve stimulation show that at 20 C. the release was 20 per cent of that at 37 C., and that at 30 C. it was 75 per cent of that at 37 C. It might perhaps be thought that the increase in potency of curare with fall in temperature might be associated with a decrease in activity of cholinesterase, but the phenomenon is enhanced by eserine.

The changes in curare potency do not seem to be associated with membrane potentials, end-plate potentials, acetylcholine liberation or activity of cholinesterase!

The effect of temperature reduction on the two phases of action of a depolarizer was examined by Jenden. The whole series of actions is slower. At 40 C. maximum phase I block occurred after 4 to 6 minutes and maximal recovery between phase I and II after 15 to 30 minutes. At 30 C. maximal phase I block took 18 minutes and maximal recovery one hour. These effects have been further studied by Zaimis and the observations extended to lower temperatures.

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