

ACTION OF INHIBITORS AT THE MYONEURAL JUNCTION*

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On the basis of experiments performed more than 40 years ago, Langley (1) proposed a mechanism for the stimulating action of nicotine on muscle and for the antagonism of curare. He postulated that "receptive substances" are present in the neural region of the muscle, that the combination of these "radicles" with nicotine provides an adequate stimulus for contraction, and that curare competes with nicotine for the receptive substances. This concept was further developed quantitatively by A. J. Clark and his coworkers (2, 3), who used acetylcholine (Ach) as a stimulating agent and studied the inhibiting actions of atropine and of curare. Unfortunately this work seems to have been overlooked by electrophysiologists concerned with the mode of action of the "curares."

It is possible to apply to this problem one of the simplest of the mathematical formulations in common use in the field of enzyme chemistry. The theory assumes that the first step in an enzyme-mediated reaction is the formation of a complex between enzyme and substrate. On this basis a relationship between reaction velocity and substrate concentration was derived by Michaelis and Menten (4). Several other authors have extended the theory (5, 6). However, the type of equation used is applicable in certain situations which are believed to involve the formation of a complex between a small molecule and a large one, usually protein, but which may involve no reaction beyond this stage. For example, the oxygenation of myoglobin is such a function of the partial pressure of oxygen (7).

The following experiments offer quantitative support for the hypothesis that Ach stimulates striated muscle by some process involving the formation at the myoneural junction of an intermediary complex of the enzyme-substrate type.

Methods

The rectus abdominis muscle of the frog was dissected out and suspended for measurement of isotonic contraction. The muscle was washed in frog Ringer's solution buffered with sodium phosphate (10^{-3} M) and sodium bicarbonate (8.6×10^{-3} M) and

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so adjusted that the pH was maintained at 7.0 when a mixture of 95 per cent oxygen-5 per cent carbon dioxide was bubbled through the bath. The experiments were carried out at room temperature (usually about 23°C.). The volume of the bath was 6.0 cc.

The muscle was exposed to tetraethyl pyrophosphate (TEPP)¹ for 30 minutes (40 $\mu\text{g./cc.}$) to destroy the cholinesterase. It was then washed for 15 minutes and tested with Ach until the contractions were reproducible.²

For stimulation of the muscle Ach bromide was added from a solution containing 1 $\mu\text{g.}$ (as free base) per cc. A 1 minute contraction was recorded. Since the readings represented rates rather than equilibrium positions the muscle was allowed to begin contracting before the reading was begun. The solution was changed after the measurement and the muscle allowed to rest between readings. It was found that a 5 minute rest period sufficed for contractions less than 50 per cent of the maximal. As the response was increased the period between readings had to be increased to insure complete relaxation. When the rest periods were too short the responses near maximal were less than predicted by theory, with the result that the dose-response curve (Fig. 1) broke sharply and flattened out near the top.

The character of some of these experiments is such that the muscles were intermittently active for long periods of time. The condition was checked by making control readings and it has been possible to reproduce results over a period of 12 hours.

The contractions were measured by means of a simple optical system. A small concave spherical mirror (radius of curvature 1 meter) mounted on the isotonic lever focused the reflected image of a light source on a scale placed at a distance of 2 meters from the mirror. The use of this apparatus has afforded a degree of reproducibility not usually obtained with a kymograph. Details of the equipment will be described elsewhere.

Three "curares" were used in these experiments. Dihydro- β -erythroidine hydrobromide³ was used in much of the work for the reason that it is available in crystalline form. The results obtained with this drug have been duplicated using intocostarin, a partially purified but chemically heterogeneous mixture of alkaloids, and metubine, the dimethyl ether-diiodide of *d*-tubocurarine.

The inhibitor was incubated with the muscle for 30 minutes prior to addition of the Ach. Where a series of readings was taken at a single inhibitor concentration (Figs.

¹ Made available through the courtesy of the Central Research Department of the Monsanto Chemical Company, Dayton, Ohio.

² Some reversal of the cholinesterase inhibition produced by TEPP has been reported (8) when the muscle is washed after a short period in the inhibitor. This results in a decreased response to Ach, which is especially evident when the response is highly magnified. The inhibition can be maintained by keeping a small quantity of TEPP in the solution throughout the experiment. This was not done in the experiments reported here, and some error may have resulted, although in most cases magnification was low and such an error was probably negligible.

³ Kindly supplied by the Medical Division of Merck and Company, Inc., Rahway, New Jersey.

2, 3, and 5) the inhibitor was present throughout the sequence; *i.e.*, during and between the successive measurements.

Theory

The equation which describes the rate of an enzyme reaction as a function of the substrate concentration is that of a rectangular hyperbola (4). An analogous equation has been used to express the rate of contraction of a muscle as a function of the concentration of Ach applied to it (9). As originally proposed, this equation did not take account of the fact that a correction must be applied in order that the equation describe the experimental situation. For the purpose of emphasizing the underlying assumptions the equation will be derived here and the need for the correction demonstrated. Relationships which are corollaries of the fundamental assumptions will not be derived although they will be used throughout the paper. Their development, however, has appeared in several reviews (10-12).

It is assumed that there occurs the formation of a complex (ES) between a receptor or enzyme (E) and its substrate (S). The complex formation may be followed by a reaction, or, as in the case of oxymyoglobin, there may be no subsequent reaction. Thus



where the dots indicate that further steps may occur. According to the law of mass action,

$$K_s = \frac{(E)(S)}{(ES)} \quad (1)$$

where K_s may be treated as the dissociation constant of the complex (ES) and is known as the Michaelis constant. The total receptor concentration (E_t) may be written

$$(E_t) = (E) + (ES) \quad (2)$$

and, substituting for (E) in (1),

$$K_s = \frac{(E_t) - (ES)}{(ES)} \cdot (S) = \left[\frac{(E_t)}{(ES)} - 1 \right] (S) \quad (3)$$

As long as the formation of (ES) is not the rate-limiting step, the velocity of a subsequent reaction will be proportional to the amount of (ES) formed; *i.e.*, $c = k(ES)$. The maximal velocity C_m will be approached as E_t is saturated with S ; hence $C_m = k(E_t)$. Then

$$\frac{(E_t)}{(ES)} = \frac{C_m}{c}$$

and, substituting in (3) and solving for c ,

$$c = \frac{C_m(S)}{K_s + (S)} \quad (4)$$

Inspection of equation (4) indicates that c should be a hyperbolic function of (S) , which prediction is realized for the systems cited. As (S) increases indefinitely, K_s becomes negligible and c approaches C_m as a limit. That is, as the receptors are saturated by a very large substrate concentration the velocity of the dependent reaction approaches a maximum.

This simple situation may be altered by including an inhibitor. Inhibitors are usually divided into several classes depending on their modes of action, and the quantitative relationships make possible an analysis of the type.

An inhibitor may combine with the same receptor as does the substrate, or it may combine at a different site on the large molecule E . In the latter case combination with the complex ES is also possible. Thus two reactions are to be considered:



The kinetics are described by the equation

$$c = \frac{C_m(S)}{K_s \left[1 + \frac{(I)}{K_i} \right] + (S) \left[1 + \frac{(I)}{K_{esi}} \right]} \quad (5)$$

where I is the concentration of inhibitor, K_i is the dissociation constant of the complex EI , and K_{esi} is the corresponding constant of the complex ESI .

Several limiting cases of this equation are of practical importance. Thus when no inhibitor is present (I is zero) the equation reduces to (4). When the inhibitor combines only with E (reaction (C) does not occur), K_{esi} becomes infinite, and $(I)/K_{esi}$ is zero; hence equation (5) reduces to

$$c = \frac{C_m(S)}{K_s \left[1 + \frac{(I)}{K_i} \right] + (S)} \quad (6)$$

This is a case of competitive inhibition, so-called because the two molecules, substrate and inhibitor, are competing for the free receptors. Occupation of the site by one excludes the other.

When the inhibitor acts by combining with groups other than those which react with substrate, and the rate of combination is unchanged whether the latter are free or combined with substrate, then $K_i = K_{esi}$. If the rate of combination is modified in the presence of S , $K_i \neq K_{esi}$ and equation (5) must be used as written. In either case the inhibition is not competitive.

These equations hold for a system in which the response begins at very small substrate concentrations; *i.e.*, the curve extrapolates to the origin. As is well known, the dose-response curve for many biological systems shows a definite threshold concentration of substrate below which no response occurs. This is

apparent on inspection of Fig. 1. The origin, in this case, is below the abscissa, displaced by a factor $\frac{C_m(S_0)}{K_s + (S_0)}$ where (S_0) is the quantity of Ach referred to as the threshold concentration. Denoting this "factor" by the letter a , equation (4) becomes

$$c + a = \frac{C_m(S)}{K_s + (S)} \quad (7)$$

The same correction applies to equations (5) and (6).

The equations contain three theoretical constants which are of considerable importance in characterizing the system. The best method for evaluation of

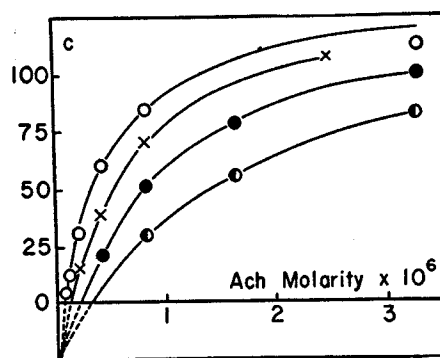


FIG. 1. Ach dose-response curve and effects of dihydro- β -erythroidine. Ordinate represents rate of contraction c (height of contraction in 1 minute, on an arbitrary scale). The dotted segments are the extrapolations used in evaluating the constant a . —○— no inhibitor; —×— dihydro- β -erythroidine 0.7×10^{-8} M; —●— 1.9×10^{-8} M; —●— 4.7×10^{-8} M.

these constants was developed by Lineweaver and Burke (13). If the reciprocal of equation (7) is taken,

$$\frac{1}{c + a} = \frac{K_s}{C_m} \cdot \frac{1}{(S)} + \frac{1}{C_m} \quad (8)$$

This is the equation of a straight line obtained when $\frac{1}{c + a}$ is plotted against $\frac{1}{(S)}$. The intercept is $\frac{1}{C_m}$ and the slope is equal to $\frac{K_s}{C_m}$ so that, by evaluation of the slope and intercept, a simple calculation gives the value of K_s .

Taking the reciprocal of equation (6), and including the correction a ,

$$\frac{1}{c + a} = \frac{K_s \left[1 + \frac{(I)}{K_i} \right]}{C_m} \cdot \frac{1}{(S)} + \frac{1}{C_m} \quad (9)$$

Equation (9) shows that if the data are gathered at progressively increasing concentrations of inhibitor (competitive in this case) a family of straight lines results. At zero inhibitor (9) reduces to (8). As the inhibitor concentration is increased the slope of the line obtained increases, but all the lines must extrapolate to the same intercept, $\frac{1}{C_m}$. The common intercept gives, as will be seen, a unique criterion for determining whether or not a given inhibitor is competitive.

If the reciprocal plot is made from data described by equation (5), the family of lines obtained allows some insight into possible mechanisms of inhibition. In this case

$$\frac{1}{c+a} = \frac{K_i \left[1 + \frac{(I)}{K_i} \right]}{C_m} \cdot \frac{1}{(S)} + \frac{1}{C_m} \cdot \left[1 + \frac{(I)}{K_{esi}} \right] \quad (10)$$

Some of the alternative types of non-competitive inhibition were discussed above. However, the following points should be stressed here. Equation (10) also describes a family of straight lines. In the absence of inhibitor it reduces to (8). If the combination is unaffected by the presence of substrate the slope should increase by the factor $\left[1 + \frac{(I)}{K_i} \right]$ (as for the competitive case), but here the intercept increases at the same rate. Thus, while with a competitive inhibitor the lines obtained at various concentrations have a common intercept, with a non-competitive inhibitor the intercepts increase as (I) increases. Where the combination of inhibitor with (E) is affected by the presence of (S) and $K_i \neq K_{esi}$, interpretation is more difficult. Graphically this will be shown by different rates of change of slope and intercept—the rates depending on the magnitudes of the constants K_i and K_{esi} .

If in the case of the frog rectus an inhibitor does not act at the end-plate, but rather at some other point (such as the contractile mechanism), K_i becomes infinite and the slope of the curve is unaffected by the presence of inhibitor. Any increase in slope therefore indicates inhibition at the end-plate, even when the results are complicated by other effects.

EXPERIMENTAL

(a) *Competitive Inhibitors*.—Certain of the crystalline "curares" were tested over a fairly wide concentration range and were found to exhibit an action consistent with the theory. The usual dose-response curve is plotted in Fig. 1. The value c is the reading (on an arbitrary scale) which represents the rate of shortening of the muscle (*i.e.* the height of contraction in a given short interval of time). The necessity for inclusion of the correction factor, a , can be seen. This value has been determined graphically by extrapolation. It should be noted that the steep approach of the curve to the ordinate makes the choice not very

exact. The data for several concentrations of dihydro- β -erythroidine are plotted as reciprocals in Fig. 2. The lines have a common intercept, as required by equation (9). The same proof of competition has been obtained with *d*-tubocurarine, intocostarin, and metubine.

The constant K_s can be evaluated from the slope (K_s/C_m) of the line in the absence of inhibitor, since C_m is known from the intercept ($1/C_m$). With these data (K_s and C_m), K_i can be calculated from the slope $K_s \left[1 + \frac{(I)}{K_i} \right] / C_m$ of one of the lines obtained in the presence of the inhibitor.

The values of the constants obtained with two of the "curares" are given in Table I. Since these constants are a measure of the affinity of the receptor for

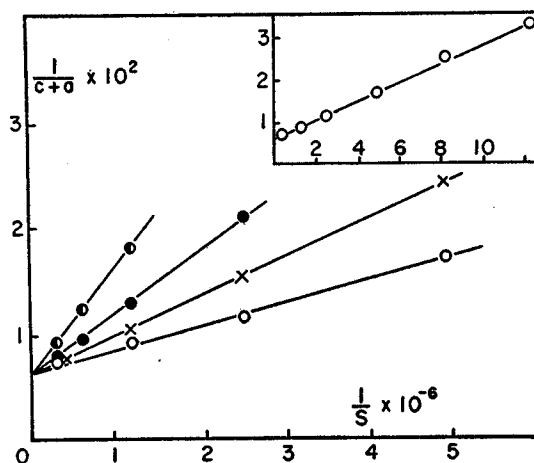


FIG. 2. Data from Fig. 1 plotted as reciprocals. Symbols as in Fig. 1. The inset shows the entire curve in the absence of inhibitor.

an inhibitor, the values obtained may be used to represent the "potency" of the drug.

(b) *Non-Competitive Inhibitors*.—As has been pointed out, non-competitive inhibitors may fall into several classes depending on the relative magnitudes of K_i and K_{si} . The action of atropine on the myoneural junction is possibly non-competitive. It was noticed a number of years ago that the quantitative data on atropine inhibition differ from those gathered after curarization. The data were made more consistent with theory by assuming that each receptor combined with 1.5 molecules of the drug, but that the basic mechanism was competition (14).

A further study shows, however, that the effect of atropine is not one of simple competitive inhibition. The reciprocal plot (Fig. 3) shows that both the slope and the intercept increase as the inhibitor concentration increases. In

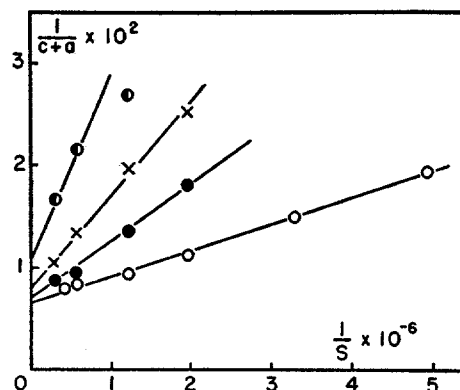


FIG. 3. Reciprocal plot showing the effects of atropine. —○— without atropine; —●— atropine 1.5×10^{-4} M; —×— 3.0×10^{-4} M; —●— 4.0×10^{-4} M.

TABLE I
Dissociation Constants for the Receptor-Substrate and Receptor-Inhibitor Complexes

Experiment	Inhibitor	K_s	K_i
1	Dihydro- β -erythroidine	3.4×10^{-7} M	3.2×10^{-8} M
2		3.8×10^{-7} M	3.2×10^{-8} M
3*		3.5×10^{-7} M	1.3×10^{-8} M
4		3.0×10^{-7} M	1.4×10^{-8} M
5	Atropine	8.9×10^{-7} M	1.1×10^{-4} M
6		9.6×10^{-7} M	1.5×10^{-4} M
7*†		3.0×10^{-7} M	1.1×10^{-4} M
8*	Eserine	2.5×10^{-7} M	1.0×10^{-4} M
9		2.9×10^{-7} M	1.6×10^{-4} M
10		2.2×10^{-7} M	1.1×10^{-4} M
11		2.2×10^{-7} M	1.5×10^{-4} M
12		3.2×10^{-7} M	1.3×10^{-4} M
13		3.3×10^{-7} M	2.0×10^{-4} M

* The curves from which these values were calculated appear in this paper.

† This experiment was run in the presence of eserine, which is itself a competitive inhibitor. The K_s calculated from the data in this figure was 4.0×10^{-7} M. This was corrected by dividing by $\left[1 + \frac{(I)}{K_i}\right]$, where (I) is the concentration of eserine used and K_i is the average of the above values for eserine.

this case the intercept increases more slowly than does the slope, indicating that $K_i < K_{esi}$. The value for K_i , included in Table I, indicates that the affinity of the receptors for atropine is of the order of a thousand times less than the affinity for dihydro- β -erythroidine.

No value has been assigned to K_{esi} , for the following reason: The complex nature of the system makes possible a number of alternative mechanisms for inhibition. One of these, which has been demonstrated for inhibition of nerve conduction by eserine, involves the penetration of the inhibitor into the fiber (15). The drug action, in that case, takes place not at a synapse (none was present in the stretch of nerve used) but within the fiber itself. It is possible that atropine is penetrating into the muscle fiber and exerting some action there. This would diminish the response of the muscle to Ach. The analysis used, however, would give a finite value for K_{esi} , implying that a locus on ES is reacting with atropine.

Thus, while some inhibition at the myoneural junction is evident, the data permit no conclusion as to whether this action is non-competitive or competitive since the results may be complicated by additional effects on other parts of the system.

(c) *Multiple Inhibitors*.—It has been claimed (16) that since certain inhibitors deviate from the above classification there must be a fallacy in the theory. An attempt was made to develop another equation to fit the data. However, the equation used was not very satisfactory even on the introduction of an arbitrary power. In dealing with the system used in this type of work it is requisite to bear in mind its complexities. The events purported to occur at the end-plate are measured in terms of the contractile response of the entire muscle. This response is the result of a sequence of activities involving at least four unique, but connected systems: end-plate, conductile, contractile (*i.e.* actomyosin), and energetic. Interference (by an inhibitor) in any one of the first three will result in an immediate variation in the response of the muscle. It was for such a reason that no value was assigned for K_{esi} -atropine, since the site of the inhibition could not be assigned to the end-plate alone with any certainty. Even at the end-plate the known events are mediated by two separate functional entities: the receptors which form the initial complex, and cholinesterase which presumably breaks down Ach after it has subserved its function. It is possible that a compound which affects one of these also has some activity toward the other, and indeed, the following experiment will illustrate this point.

The activity of eserine is very generally described in terms of its capacity as a cholinesterase inhibitor. It has, however, been demonstrated that eserine may either potentiate or depress the postganglionic response to preganglionic stimulation, depending on the concentration used (17). The same double activity can be demonstrated on the *in situ* muscle described above. The muscle in this experiment was not pretreated with TEPP and the cholinesterase at the end-plates was active. In Fig. 4 the muscle was stimulated by a constant quantity of Ach (eserine alone having no effect). The response was measured at different concentrations of eserine salicylate and plotted as a function of this variable. The sharp increase in response at low concentrations of eserine is well

known, as is the further increase on prolonging the time of exposure to eserine before stimulating (greater slope in curve B than in A). This potentiation of the response to Ach is the result of the inactivation of cholinesterase (18).

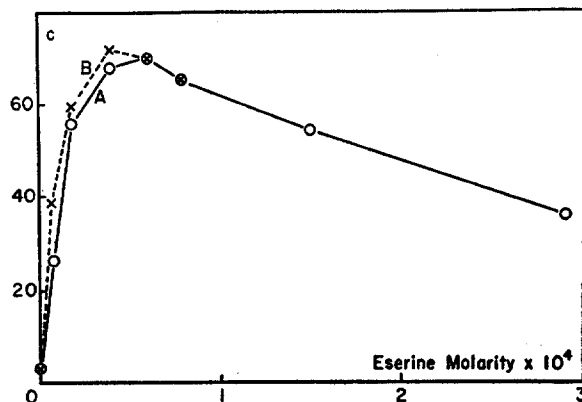


FIG. 4. Effects of eserine on response of frog rectus to $0.4 \mu\text{g. Ach}$. Ordinates represent rates of contraction in arbitrary units. —○— incubated for 11 minutes in eserine before adding Ach; —×— incubated 25 minutes. No TEPP was used in this experiment.

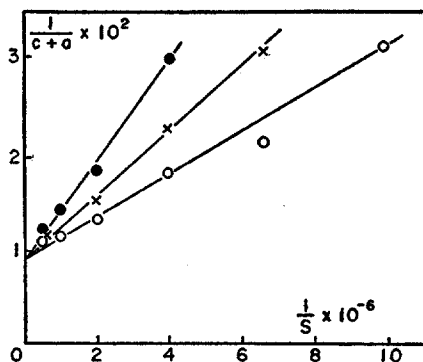


FIG. 5. Reciprocal plot showing the curare-like action of eserine on a TEPP-treated muscle. —○— no eserine; —×— eserine $0.5 \times 10^{-4} \text{ M}$; —●— $1.0 \times 10^{-4} \text{ M}$.

The striking fact, however, is that on further increasing the concentration of eserine the response of the muscle to Ach decreases and (not shown) is nearly abolished at $0.7-1.0 \times 10^{-3} \text{ M}$. The inhibition was completely reversible on washing the muscle (*cf.* reference 8). Fig. 5 defines the mechanism of the inhibition. Here the cholinesterase was inactivated by TEPP prior to exposure to eserine. In this case eserine had only an inhibitory effect and it can be seen that the inhibition is purely competitive.

The inference may be drawn, then, that a single compound may act at more than one locus in the Ach mechanism, and that the actions may be partially compensatory in terms of the measured response.

In the case of the controversy cited (16, 19) the inhibitor flaxedil (triiodide of tri(β -trimethylammonium methoxy)-1,2,3-benzene) did not follow the usual competitive relationship, but became relatively less potent as its concentration increased. It is possible that the compound was combining with two members of the Ach system. It may thus be analogous to eserine in exerting a dual effect, though acting as a curare at lower concentrations and as an anticholinesterase as the concentration was raised. Thus, if flaxedil has an anticholinesterase activity the muscle would become more sensitive to Ach as the concentration of flaxedil was raised and the curarization would be less than predicted. These experiments should be repeated after cholinesterase has been eliminated by preincubation with TEPP.

DISCUSSION

The experiments reported here demonstrate the mode of action of the "curares." They also give an indication of the difference between the action of a true "curare" and such a drug as atropine. Unfortunately, the action of atropine is too complex to be explained completely by the type of analysis used in this study.

Earlier treatment of the type of data presented here led to results which were extremely variable. As a result, no confidence was manifested in the values calculated for K_1 and K_2 (2). The variability may have been the result of several factors having nothing to do with the muscle *per se*. Use of the kymograph led to some variability in early experiments. The work reported here was undertaken with an optical recording device and the precision of measurement has been considerably improved. Another improvement is the use of the Lineweaver-Burke (reciprocal plot) analysis. A possible source of error in the older literature is the use of partially purified, but non-crystalline natural curares, the constancy of whose composition is highly questionable.

It has been noticed that while the constants calculated are generally reproducible, a value is occasionally encountered which is outside the normal range (Table I). These deviations have been noted several times in the course of a great many experiments and are possibly of some physiological significance, although no explanation can be given at this time. Incomplete inactivation of cholinesterase would give such a result, but this appears unlikely at the cholinesterase inhibitor concentrations used.

It is imperative in the calculation of K_2 to have a precise value for K_1 . The value of K_2 gives a measure of potency which has a theoretical significance, and such values could be used for comparing the potencies of new inhibitors. Using, say, *d*-tubocurarine as a standard curare, a series of compounds could be assayed on a single muscle and the values calculated for K_2 used for com-

parison with the reference and with each other. If this technique is used together with an *in vivo* method, such as the head drop, information may be gained both on the activity of the drug on muscle directly and on how the animal body deals with it (in terms of destruction etc.).

However, the physiological implications of the results obtained in this work are of more importance than the pharmacological applications. Combination of the results reported here with previously published studies on the effect of "curares" (20), Ach (21), and anticholinesterases (22) on the electrophysiology of muscle makes possible the synthesis of a coherent, if still incomplete picture of some of the events occurring at the neuromuscular junction.

Close arterial injection of Ach results in the repetitive firing of the fibers of the muscle, and the repetitive response of a single fiber has been recorded by means of a microelectrode buried in the muscle mass (21). Similar experiments have been reported on the synapses in a sympathetic ganglion. Perfusion of the ganglion with Ach solutions results in the repetitive discharge of the post-ganglionic elements (23, 24). The magnitude of the response increases with increasing Ach concentration.

More direct evidence on events at the end-plate has been reported in a beautiful series of experiments (25, 26). A single fiber was dissected out of the muscle (or a small group of fibers used) and placed in contact with recording electrodes. The direct application of Ach to the end-plate set off a train of propagated electrical responses, while application of much higher concentrations of Ach to any other site had no effect. It has been shown that the initial event in the electrical response of the muscle to a nervous impulse is the formation of a local "end-plate potential," and that the latter has to attain a critical magnitude before the propagation of the spike occurs. Data have also been presented (20) to show that the direct effect of curare is to diminish the size of the end-plate potential. These data were not discussed at length, but the curve obtained by plotting the magnitude of the end-plate potential against the concentration of curare is of the form described by Equation 6 (assuming S , the transmitter, to be proportional to the stimulus, which was held constant).

It should be pointed out that the real variable dependent on Ach is probably the end-plate potential. Apparently the frequency at which a fiber fires is a function of the magnitude of this quantity. It is evidently an empirical fact that the rate of shortening in the frog rectus is a linear function of the average frequency at which the fibers are firing; hence the dose-response relationships can be studied by the contraction of the entire muscle.

With respect to the data presented here for eserine it might be mentioned that the time course for eserization (*i.e.* the sensitizing phase) agrees with recently published data (27) on the time for development of a maximal end-plate potential in the presence of prostigmine.

Up to this point a fairly coherent picture of the initial events at the myoneural junction may be constructed. There are, however, a number of experimental findings which find no place in the scheme. Magnesium paralysis and the roles of potassium, calcium, and adrenalin are still obscure. It is also pertinent that other junctions respond in a more complex fashion than do those of the rectus. Much of the electrical work cited above was done on the sartorius of the frog and, as was pointed out, seems to confirm the predictions which might be made from the present experiments on the rectus. Yet the response of the sartorius to immersion in Ach differs from that of the rectus in that it is not maintained; *i.e.*, the former relaxes after a short tetanus although the drug is still present in the bath. The rectus maintains the tetanus as long as the Ach is present. It should be stressed that the contraction of the rectus is a true tetanic response and not a contracture (as it is often called). The electrical response to Ach is a series of spikes the frequency of which varies with the concentration of Ach applied (28). The propagated electrical response of the sartorius, on the other hand, is cut short soon after immersion in the solution (27), being in this respect analogous to the contractile response. Other junctions present responses differing in some degree from the simple response of the rectus to the drug. However, the pattern of responses to Ach; curare, and the anticholinesterases is so similar in the variety of peripheral synapses that there is little doubt that the same basic mechanism operates in all of them. The over-all response may then be modified by superimposition of secondary mechanisms subserving some specific function in one tissue and not in another (*e.g.* phasic rather than tonic contraction).

Two suggestions might be made in the interest of clarity in the literature. First, the term "curare" should be reserved for those junctional inhibitors whose action is competitive, and not applied to every compound which decreases a postjunctional response regardless of locus and mode of action. Second, the response of the rectus to Ach is a contraction, tetanic in nature, and not a contracture as defined by Gasser (29). The data relevant to this point will be submitted as part of a separate communication.

SUMMARY

1. A study is presented of the actions of certain inhibitors on the frog rectus abdominis muscle stimulated by acetylcholine.
2. A type of analysis has been developed which provides a reliable criterion for judging whether an inhibitor is competing with acetylcholine for receptors at the myoneural junction or whether acting by a different mechanism.
3. The "curares" are shown to act by competitive inhibition at the myoneural junction, confirming earlier work of others on the mode of action of curare.
4. Atropine acts as an inhibitor at the myoneural junction. The inhibition

may be non-competitive or it may be complicated by an additional effect at some point other than the myoneural junction.

5. A possible mechanism for anomalous inhibitor effects is the action of a single compound at more than one locus in the Ach mechanism. Eserine exerts such a dual effect at the end-plate.

6. Some of the available electrical and chemical data have been correlated to make possible a partial explanation of the role of Ach in transmission at the myoneural junction.

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