Further Studies of the Effect of Calcium on the Time Course of Action of Carbamylcholine at the Neuromuscular Junction

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ABSTRACT The rate at which the postjunctional membrane of muscle fibers becomes desensitized to the action of carbamylcholine is increased after the muscle has been soaked in solutions containing increased concentrations of calcium. Some further aspects of this effect of calcium were investigated by measuring changes in the input resistance of single fibers of the frog sartorius during local perfusion of the neuromuscular junction with $2.73 \times 10^{-3}$ M carbamylcholine in isolated muscles immersed in 165 mM potassium acetate. It was found that (a) sudden changes in the local concentration of calcium brought about by perfusing fibers with carbamylcholine solutions containing 20 mM calcium, 40 mM oxalate, or 40 mM EDTA were followed within 20 sec by marked changes in the rate of desensitization; (b) prior to 13 sec after the introduction of carbamylcholine, however, no effect on the input resistance could be detected even though the muscle had been presoaked in 10 mM calcium; (c) the ability of high concentrations of calcium to bring about rapid desensitization disappears when a lower concentration of carbamylcholine ($0.137 \times 10^{-3}$ M) is applied to the muscle fiber. These findings suggest that calcium present in the extracellular fluid can act directly on the postjunctional membrane to promote the desensitization process and that an increased permeability of the membrane to calcium brought about by the presence of carbamylcholine is a factor which contributes to this action.

INTRODUCTION The application of acetylcholine to isolated skeletal muscle causes an immediate and rapid depolarization of the postjunctional membrane of the muscle fibers. If the muscle remains in contact with this agent for more than a few seconds, however, repolarization of the postjunctional membrane begins
to occur at a slow rate even though the concentration of acetylcholine is main-
tained at a constant level. This gradual disappearance of the depolarizing
action of acetylcholine is one aspect of a general decrease in chemosensitivity
which occurs when cholinceptive tissues are exposed to this agent for long
periods. The phenomenon has been referred to by different authors as
“desensitization” (1), “inactivation” (2), or “fade” (3), and it may also be
related to the “second phase block” produced by decamethonium at the
neuromuscular junction (2, 4) and nicotine in autonomic ganglia (5). Since
desensitization occurs during the action of several different cholinergic com-
pounds (6) and in different tissues (3, 7–10), it may be due to some general
and fundamental feature of their action at cholinceptive sites. Several
mechanisms based on this assumption have been proposed (2–4, 10).

Desensitization in frog skeletal muscle has been studied in this laboratory
by seeing what are the important factors which influence its rate. Two factors
which are known to cause an increase in the rate of desensitization are
(a) an increase in the concentration of acetylcholine applied to the muscle (2) and
(b) an increase in the concentration of calcium present in the extracellular
fluid (11). In the present investigation the action of calcium on the rate of
desensitization was examined in greater detail in order to further specify
its mode of action and its relationship to the desensitization process.

METHODS

Because it is resistant to hydrolysis by tissue cholinesterase, carbamylcholine was used
throughout these experiments as a test agent for producing desensitization in muscle
fibers. In order to prevent twitches and contractures during drug application and to
allow constant recording with intracellular capillary electrodes, muscle preparations
were immersed in solutions containing 165 mM potassium acetate, a procedure which
causes the fibers to be almost completely depolarized and therefore electrically in-
exitable. Since the application of carbamylcholine to the postjunctional membrane
under these conditions produces little or no change in the membrane potential, it
was necessary to use some measurement other than membrane potential for the de-
tection of drug action. Therefore, the input or “effective” resistance of the fiber was
chosen for this purpose.

Muscle Preparation

Whole isolated sartorius muscles of the frog (*Rana pipiens*) mounted for direct micro-
scopic observation were used throughout these experiments. Prior to use, the animals
were stored at room temperature.

Measurement of Input Resistance

The input resistance of single muscle fibers on the exposed inner surface of the prepa-
ration was measured by the “electrotonic pulse” method. Two glass capillary elec-
trodes filled with 3 M KCl were inserted into a muscle fiber within 50 μ of each other
in the region of the neuromuscular junction under microscopic observation at a magnification of 150 times. One electrode was used to deliver repeated pulses of current from a square wave stimulator. The amplitude of this "test" current was always between 9 and $11 \times 10^{-8}$ amp and the duration of each pulse was 300 msec. The deflections of membrane potential produced by the test current were recorded by the other electrode, amplified, and then displayed on an oscilloscope. Records of the oscilloscope trace were obtained on moving 35 mm film by means of an oscilloscope recording camera. Since the total resistance of the current delivery circuit was always much greater than the input resistance of the muscle fiber membrane, the current delivered to the membrane was virtually independent of changes in the input resistance produced by carbamylcholine. Therefore, the amplitudes of the pulsatile changes in membrane potential caused by the injected test currents (such as appear in Fig. 1) are a direct measure of the input resistance. No corrections were made for the variations in internal resistance of individual fibers.

**Drug Application**

Solutions of carbamylcholine were applied to the junctional region of surface muscle fibers by means of a special perfusion pipette. This pipette, which measured approximately 30–50 μ at the tip, was connected to a length of glass tubing and both were filled with a solution identical in composition to the fluid bathing the muscle except for the addition of carbamylcholine and in some cases a change in the concentration of calcium. At the time of drug application this pipette, initially above and outside the bath fluid, was rapidly brought into position about 0.1 mm above the impaled fiber. When in contact with the bath fluid, the solution within the pipette was expelled at a constant rate by gravity. It was determined in control experiments that this method of local perfusion did not alter the measurement of input resistance to any significant degree (11). It is not known how closely the concentration of carbamylcholine produced at the surface of the fiber approximates that present in the perfusion pipette. Nevertheless, a certain degree of reproducibility in electrical response was attained by using the same pipette in many experiments and maintaining as nearly as possible the same geometrical relation of the pipette tip and the impalement site. Another variable which was difficult to control was the rate of onset of drug action. In most cases the time taken for the input resistance to decline by one-half of the maximum decrease of the onset phase varied from 0.5 to 1.0 sec although extreme values of 0.2 and 1.5 sec were also encountered.

**Solutions**

Muscle preparations were dissected and initially mounted in normal Ringer fluid which contained the following amounts of dissolved salts in mM/liter: NaCl, 120, KCl, 2.5, CaCl₂, 1.8, THAM (tris[hydroxymethyl]aminomethane), 1.0. The pH was adjusted to approximately 7.3 by the addition of HCl. Prior to an experiment, the isolated muscle was equilibrated for 30–60 min in a bath medium containing the following concentrations in mM/liter of dissolved solids: potassium acetate, 165, calcium acetate, 1.8, sodium acetate, 3.0, sucrose, 55, THAM, 1.0. The pH was adjusted to approximately 7.3 by the addition of HCl. In experiments in which increased
amounts of calcium were added to the bath or perfusion fluids, corresponding adjustments were made in the concentration of sucrose so as to maintain the same osmolarity. Solutions of carbamylcholine chloride were made up from the freshly weighed salt on the same day of each experiment. It was determined in control experiments that the amount of chloride in the most concentrated solution of carbamylcholine used ($2.73 \times 10^{-3} \text{M}$) did not affect the input resistance of the fiber when applied to the impalement site. Furthermore, this concentration of carbamylcholine did not produce any mechanical response that could be seen under microscopic observation at a magnification of 150 times. All experiments were performed at room temperature which varied on different days from 22 to 25°C.

Statistical Analysis

Student's $t$ test was used in comparing means of groups of data. A valid difference between two means was assumed to exist if the calculated $P$ value was less than 0.05. In some cases the correlation coefficient ($r$) was used to establish a relationship between variables. The 95% confidence interval for $r$ was found by consulting Table A7 on page 175 of reference 12.

RESULTS

A. Effect on the Rate of Desensitization of Rapid Changes in the Local Concentration of Calcium in the Extracellular Fluid

It was shown in an earlier study (11) that the rate at which muscle fibers become insensitive to the action of carbamylcholine is greatly increased when the muscle is presoaked in a solution containing five times the normal amount of calcium. In the further investigation of this action of calcium, one important question concerns its time of onset. If, for example, the accumulation of a large amount of calcium in the intracellular phase were necessary for this effect, changes in the time course of drug action would occur gradually over a period of many minutes, whereas a very rapid onset might indicate a simple action of the calcium ion at the cell surface. Experiments were undertaken, therefore, to find out how soon the rate of desensitization reaches a maximum after a sudden and rapid increase is made in the concentration of calcium in the extracellular fluid.

In order to serve as a basis for later comparison, the records in Fig. 1a and b show first the time course of carbamylcholine action in muscle fibers that have been equilibrated for long periods in solutions containing 1.8 mM calcium (Fig. 1a) and 20 mM calcium (Fig. 1b). Since the bath medium in these experiments also contains 165 mM potassium acetate, the resting potential of the postjunctional membrane (denoted by the thick continuous line in the record) is always close to zero mv. The series of elongated dots below the resting potential level are momentary deflections of the membrane potential produced by repeated pulses of test current. Because this current is delivered to the fiber from a "constant current" source, the amplitude of
these deflections is a measure of the input resistance of the muscle fiber. When, as shown in Fig. 1 a, perfusion of the postjunctional region with carbamylcholine is begun (heavy vertical line), there is at first a rapid decrease in the input resistance. As the perfusion continues, however, the input resistance gradually returns toward control values at a slow rate. Since the latter period of increasing membrane resistance occurs in the continued presence of carbamylcholine, it signifies a gradual waning of the drug effect and there-

![Figure 1](image)

**Figure 1.** The time course of changes in input resistance of single muscle fibers during perfusion with 2.73 × 10⁻⁴ m carbamylcholine under various conditions. The bath and perfusion fluids both contained 165 mM potassium acetate. The muscle preparations were soaked in the bath solution for at least 60 min prior to perfusion. The upper and lower limits of the calibration marks to the left of each record indicate respectively 0 and −20 mV potential with respect to the bath medium. The series of dots are momentary deflections of the membrane potential produced by pulses of test current delivered to the fiber every 2 sec. The blank areas in the dot sequences of each record indicate periods of 60 sec during which the recording camera was stopped. Perfusion was begun at the moment indicated by the heavy vertical lines and continued at a constant rate thereafter.

fore the onset of the desensitization process. In this and subsequent experiments, the “time of half-desensitization” is used as a measure of the rate of desensitization. This is calculated as the time taken for the input resistance to increase toward control values by an amount equal to one-half the total change in resistance during the phase of desensitization. In Fig. 1 a, where the membrane resistance increases rather slowly, the time of half-desensitization is about 44 sec, whereas in Fig. 1 b it is only 11 sec, indicating a much more rapid rate of desensitization. The mean times of half-desensitization from several experiments in 1.8 and 20 mM calcium are shown in the first two horizontal rows of Table I. These measurements confirm the observation (11) that soaking muscle fibers in high calcium solutions produces an increase in
the rate of desensitization and they show further that the same action of calcium is evident in fibers depolarized by solutions of potassium acetate.

An attempt was now made to find out how fast the time of half-desensitization approaches a low value when the concentration of calcium in the bath fluid is suddenly increased from 1.8 to 20 mM. Initial experiments in which the bath medium was simply exchanged with one containing 20 mM calcium showed that the rate of desensitization had already reached a maximum within 2-3 min after exposure to the high concentration of calcium. Therefore, in the next experiment (Fig. 1 c) the conditions were changed so as to reduce as far as possible the time of contact of the muscle fiber with the high calcium solution. In order to accomplish this, the muscle was soaked first in a bath medium containing 1.8 mM calcium, and then at the time of drug application perfusion of a fiber was begun with a solution which contained 20 mM calcium in addition to the usual amount of carbamylcholine. It is assumed that at the onset of perfusion under these conditions there is a rapid rise in the local concentration of calcium at the fiber surface which coincides very nearly with the delivery of carbamylcholine itself. The result in Fig. 1 c shows that, despite this short exposure to the high calcium solution, the membrane resistance again increases at a rapid rate within 20 sec after the beginning of the perfusion. The mean time of half-desensitization from several such experiments shown in the third row of Table I is not different at the 5% level of probability from the value in the second row ($P > 0.05$) obtained from experiments in which the bath and perfusion fluids both contained 20 mM calcium. In control experiments perfusion with a solution containing 20 mM calcium in the absence of carbamylcholine did not affect the input

| Bath fluid | Perfusion fluid (2.73 × 10⁻⁴ M carbamylcholine) | Mean time of half-desensitization |
|------------|-----------------------------------------------|----------------------------------|
| 1. 1.8 × 10⁻³ M Ca | 1.8 × 10⁻⁺ M Ca | 47.7* sec ± 4.2† (15)§ |
| 2. 20 × 10⁻⁴ M Ca | 20 × 10⁻⁺ M Ca | 9.8 ± 0.7 (10) |
| 3. 1.8 × 10⁻³ M Ca | 20 × 10⁻⁺ M Ca | 12.8 ± 1.4 (9) |
| 4. 20 × 10⁻⁴ M Ca | 40 × 10⁻⁺ M oxalate | 43.9 ± 9.4 (7) |
| 5. 20 × 10⁻³ M Ca | 40 × 10⁻⁺ M EDTA | 40.8 ± 3.2 (5) |

* One value for the time of half-desensitization was more than 9 SD from the mean and was not included in this calculation.
† The value of 1 SEM.
§ Figures in parentheses are no. of fibers tested.
resistance of the fibers. These findings demonstrate that, when carbamylcholine is present, the full accelerating effect of calcium on the rate of desensitization appears within a few seconds of introduction of the ion into the extracellular medium.

Further experiments were done in order to find whether a rapid decrease in the extracellular concentration of calcium will also cause a rapid disappearance of its effect. In the example shown in Fig. 1 d the muscle was first soaked in a solution containing 20 mM calcium and then perfusion of a single fiber was carried out with a solution of carbamylcholine which contained in addition 40 mM potassium oxalate. It is assumed that the sudden delivery of oxalate by this method causes a rapid decrease in the local concentration of ionized calcium at nearly the same time as the onset of the action of carbamylcholine. The result in Fig. 1 d shows that, even in the early moments of the desensitization phase, the rate of increase of resistance is much slower than in Fig. 1 b where a high concentration of calcium was present in both the bath and perfusion fluids. The mean time of half-desensitization from several experiments of this kind appears in the fourth row of Table I and this value is different at the 5% level of probability from that in the second row which was obtained from experiments in which 20 mM calcium was present in both the bath and perfusion fluids. Similar experiments were performed in which the perfusion fluid contained 40 mM EDTA, and these also showed prolonged phases of desensitization (fifth row of Table I). When, therefore, the concentration of free ionized calcium in the extracellular fluid is rapidly decreased from a high value, there is a corresponding immediate slowing of the desensitization process.

B. The Effect of Calcium in the Early Moments of Carbamylcholine Action

Although calcium applied in the external medium can act rapidly to promote the desensitization process, it is not clear from the previous results whether or not this effect is also present in the very early moments of carbamylcholine action. This was tested in a separate group of experiments in which a higher frequency of test pulses and a more rapid film speed were used in order to measure the early events of drug action more accurately. Fig. 2 presents composite time courses of drug action from several experiments performed in 1.8 mM calcium (open circles) and 10 mM calcium (closed circles). Each point was obtained by first superimposing the records from many experiments so that the initial moments of onset of drug action coincided (zero time) and then calculating a mean value of input resistance at subsequent intervals of 1 sec. The muscles used in these experiments were presoaked in the respective solutions for 30–60 min prior to testing, and the perfusion fluid always contained the same concentration of calcium as that of the general bath medium.
The two sets of data were first examined to find at what stages a true difference due to the increased calcium concentration can be detected. This was done by using Student's t test to compare the mean input resistance in each of the 1 sec intervals. The intervals of time in which $P$ values smaller than 0.05 were obtained are indicated by the horizontal line in the upper right section of Fig. 2. The analysis shows that at the 5% level of probability the increased calcium concentration does not have any effect on the input resistance until about 13 sec after the onset of drug action.

From these calculations it also appears that the action of calcium occurs somewhat later than the moment of maximum drug action when the membrane resistance is at its lowest value. In order to verify this the time of minimum resistance was determined for each experiment by plotting the time rate of change of resistance ($\dot{R}$) in each 1 sec interval and estimating when this value was zero. The mean time of minimum resistance obtained in this way from both sets of data is also shown in the upper portion of Fig. 2. It can be seen that within an interval containing 2 SEM the time of minimum resistance occurs sooner than the onset of action of calcium.
Although calcium does not seem to have much effect on the input resistance in the early moments of perfusion with $2.73 \times 10^{-3}$ M carbamylcholine, it may be that some effect is present but not evident at this high concentration of the drug. Therefore, further experiments were done using one-tenth and one-twentieth of the concentration of carbamylcholine in the perfusion fluid, and the mean values of minimum input resistance obtained under these conditions are summarized in Table II. No difference between the results with 1.8 and 10 mM calcium could be demonstrated at any of the concentrations of carbamylcholine tested ($P > 0.05$).

In summary, these experiments show that the influence of calcium on the time course of action of carbamylcholine appears only after the drug has been present for about 13 sec, and that prior to this, when the action of carbamylcholine is maximal, calcium does not produce any significant degree of desensitization as measured by changes in the input resistance.

### Table II
THE EFFECT OF CALCIUM ON THE MAXIMUM CHANGE IN INPUT RESISTANCE PRODUCED BY VARIOUS CONCENTRATIONS OF CARBAMYLCOLINE

| Concentration of carbamylcholine in the perfusion fluid | Mean maximum fractional decrease in input resistance* | 1.8 mM Ca | 10 mM Ca |
|--------------------------------------------------------|-----------------------------------------------------|-----------|----------|
| $2.73 \times 10^{-3}$ M                                | 0.753 ± 0.016†                                      | 0.736 ± 0.018 |
| $0.273 \times 10^{-3}$ M                               | 0.442 ± 0.092                                      | 0.431 ± 0.024 |
| $0.137 \times 10^{-3}$ M                               | 0.329 ± 0.056                                      | 0.282 ± 0.028 |

* Calculated as the maximum change in input resistance attained in the initial moments of drug perfusion divided by the control resistance immediately prior to perfusion.
† Value of 1 SEM.

C. Dependence of the Action of Calcium on the Concentration of Carbamylcholine in the Perfusion Fluid

Since the main effect of calcium appears only some time after the moment of maximal drug action, it may be that activation of the postjunctional membrane by carbamylcholine limits or controls in some way the action of calcium. In experiments designed to examine further this possibility, the time of half-desensitization was measured during perfusion with high ($2.73 \times 10^{-3}$ M) and low ($0.14 \times 10^{-3}$ M) concentrations of carbamylcholine while the concentration of calcium in the bath and perfusion fluids was varied between 1.8 and 10 mM. The results are summarized in Fig. 3. The lower curve (closed circles) shows that when fibers are perfused with $2.73 \times 10^{-3}$ M carbamylcholine an increase in the calcium concentration from 1.8 to 10 mM causes a fourfold decrease in the time of half-desensitization. The
correlation coefficient calculated for these data is $-0.61$ and the 95% confidence interval of this value lies between $-0.32$ and $-0.80$. On the other hand, when the perfusion fluid contains only $0.14 \times 10^{-3} \text{ M}$ carbamylcholine (open circles) little or no change occurs over the same range of calcium concentrations. The correlation coefficient in this case is $-0.073$ and the 95% confidence interval from $+0.37$ to $-0.5$. The ability of calcium to bring about a rapid rate of desensitization therefore depends on the presence of a sufficient concentration of carbamylcholine acting on the postjunctional membrane.

![Figure 3](image-url)

**Figure 3.** The effect of calcium on the time of half-desensitization during perfusion with different concentrations of carbamylcholine. The closed circles denote results obtained during perfusions with $2.73 \times 10^{-3} \text{ M}$ carbamylcholine and the open circles with $0.137 \times 10^{-3} \text{ M}$ carbamylcholine. Each point represents the mean time of half-desensitization in 6 to 15 different fibers from several different muscle preparations. In no case was the same fiber perfused with more than one concentration of carbamylcholine. The vertical lines represent 2 SEM.

**DISCUSSION**

As shown in this and a previous communication (11), there is a marked increase in the rate of desensitization to applied carbamylcholine when isolated muscle fibers are soaked in solutions containing increased amounts of calcium ion. In the first group of experiments some indication was sought whether calcium introduced rapidly into the extracellular fluid acted immediately to produce this result or whether a very slow process was involved, such as the accumulation in the intracellular compartment of a high concentration of calcium. If the latter were true, changes in the rate of desensitization would be expected to take place gradually over a period of several minutes or perhaps hours (13) because of the very low membrane permeability of this ion. The results in Fig. 1 c and Table I, row 3, show, on the contrary,
that the effect of calcium is apparent within 20 sec of its rapid introduction
by means of a perfusion pipette. Furthermore, even after long exposure to a
high concentration of calcium, the desensitizing action of the ion can be
rapidly removed by perfusion with oxalate or EDTA (Fig. 1 d; Table I,
rows 4 and 5). Since these agents probably penetrate cell membranes slowly
(14), it is likely that in this short time they are reacting primarily with cal-
ccium present in the extracellular fluid rather than some intracellular fraction
of calcium. The rapidity with which these sudden changes in the local extra-
cellular concentration of calcium are followed by changes in the time course
of carbamylcholine action suggests that calcium in the extracellular fluid
can act directly on the chemosensitive surface membrane and that a large
accumulation or depletion of the ion in the intracellular phase is not an
essential step.

Although these results demonstrate a fairly rapid action of calcium, further
measurements were made in order to determine more accurately when
during the action of carbamylcholine the desensitizing effect of calcium is
first evident (Fig. 2). This was done in particular to examine the possibility
that the calcium ion can bring about a desensitized state within a very
short time of the activation of membrane receptors by carbamylcholine. An
onset time of only a few milliseconds, for example, would probably be the
case if calcium acted only by simple adsorption to a site on the outer surface
of the postjunctional membrane since the rates of such reactions are usually
limited only by free diffusion in the fluid near the reactive site. An example
of this type of reaction is perhaps the antagonism of the depolarizing action
of acetylcholine by d-tubocurarine, which can occur with apparently little
or no delay in experiments in which both drugs are applied by microelec-
trophoresis and diffusion time is only a few milliseconds (15). The method of
microperfusion used in the present study, however, would not provide an
accurate measure of such a rapid time course of action since, even in the
most favorable cases, full activation of the postjunctional membrane by
applied carbamylcholine was not achieved until at least one or more seconds
after the onset of perfusion. Nevertheless, a desensitizing action of calcium
which is faster than this would be evident in these experiments as an increase
in the minimum input resistance in the initial moments of drug application,
since in the 1 or 2 sec required to reach full postjunctional activation some
degree of desensitization would already have been complete. When the early
events of drug perfusion were measured carefully, however, it was found
(Fig. 2) that an increased concentration of calcium (10 mM) which produces
an almost maximal increase in the rate of desensitization has no demonstra-
tionaleffect on the minimum input resistance in the initial moments or at times
thereafter until about 13 sec following the onset of perfusion. Moreover, this
lack of effect on the initial input resistance was found to be the case over a
wide range of concentrations of carbamylcholine (Table I). Therefore, on the basis of these results, it appears that the desensitizing action of calcium, although rapid, does not occur immediately upon the activation of the membrane receptors by carbamylcholine (as might happen if the ion acted by adsorption to an exposed surface membrane site) but only after a delay of several seconds.

There may be several possible explanations for this delay. The calcium ion, for example, could be the initiating factor in a slow change or series of changes in membrane structure which ultimately lead to a desensitized state but which take several seconds to reach completion. A mechanism of this type, however, would be difficult to explore further at present because of general lack of detailed knowledge of membrane structure. A simpler hypothesis is that the site at which the calcium ion acts, although perhaps part of the membrane structure, nevertheless, is not immediately available to ions of the extracellular medium and that the time needed for the calcium ion to penetrate to this site results in the observed delay in its effect. One implication of this proposal which is susceptible to further test is that the membrane permeability of calcium is a rate-limiting factor in its effect on the desensitization process. On this basis some relation would be expected between the ability of calcium to bring about rapid desensitization and the degree of activation of the postjunctional membrane, a factor which Takeuchi (16) has shown can increase the membrane permeability of calcium. Such a relationship is demonstrated by the results in Fig. 3 which show that the action of calcium in accelerating the desensitization process disappears when the concentration of carbamylcholine in the perfusion fluid (and hence the degree of postjunctional activation) is reduced. Although it is possible that some other aspect of the action of carbamylcholine is responsible for this result, the simplest explanation at present seems to be that the more potent desensitizing effect of calcium seen with higher concentrations of carbamylcholine depends on the ability of the latter to increase the permeability of the membrane to calcium.

A final word is added concerning the apparent discrepancy between some of the results presented here and those of Nastuk and Liu (17). In the latter study calcium was shown to reduce the maximal response of the postjunctional membrane to carbamylcholine while in the present investigation such an effect was not observed. No explanation for this is offered at this time but two important differences in the experimental conditions should be noted: (a) Change in membrane potential was used by Nastuk and Liu as a measure of drug action, whereas in the present work change in input resistance was employed for this purpose. These two measurements may differ with respect to their sensitivity to drug action and also their susceptibility to the effects of the intracellular accumulation of ions. (b) Most of the concentrations of
carbamylcholine reported by Nastuk and Liu were below $10^{-4}$ M, while the lowest concentration used here was above this level. It is possible that a competitive interaction between carbamylcholine and calcium at the receptor site would be more evident at low concentrations of the drug.

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REFERENCES

1. Katz, B., and S. Thesleff. 1957. A study of the 'desensitization' produced by acetylcholine at the motor end-plate. J. Physiol. (London). 138:63.
2. Nastuk, W. L., A. A. Manthey, and A. J. Gissen. 1966. Activation and inactivation of postjunctional membrane receptors. Ann. N. Y. Acad. Sci. 137:999.
3. Paton, W. D. M. 1961. A theory of drug action based on the rate of drug-receptor combination. Proc. Roy. Soc. Ser. B. Biol. Sci. 154:21.
4. Taylor, D. B., and O. A. Nedergaard. 1965. Relation between structure and action of quaternary ammonium neuromuscular blocking agents. Physiol. Rev. 45:22.
5. Paton, W. D. M., and W. L. M. Perry. 1953. The relationship between depolarization and block in the cat's superior cervical ganglion. J. Physiol. (London). 119:43.
6. Thesleff, S. 1955. The mode of neuromuscular block caused by acetylcholine, nicotine, decamethonium, and succinylcholine. Acta Physiol. Scand. 34:218.
7. Curtis, D. R., and R. M. Eccles. 1938. The excitation of Renshaw cells by pharmacological agents applied electrophoretically. J. Physiol. (London). 141:435.
8. Furchgott, R. F., W. Sleator, and T. de Gubareff. 1960. Effects of acetylcholine and epinephrine on the contractile strength and action potential of electrically driven guinea pig atria. J. Pharmacol. Exp. Ther. 129:405.
9. Tauc, L., and J. Bruner. 1963. 'Desensitization' of cholinergic receptors by acetylcholine in molluscan central neurons. Nature (London). 198:25.
10. Bennett, M. V. L., and H. Grundfest. 1961. The electrophysiology of electric organs of marine electric fishes. III. The electroplaques of the stargazer, Astroscopus y-graecum. J. Gen. Physiol. 44:931.
11. Manthey, A. A. 1966. The effect of calcium on the desensitization of membrane receptors at the neuromuscular junction. J. Gen. Physiol. 49:963.
12. Dunn, O. J. 1964. Basic Statistics. John Wiley and Sons, Inc., New York.
13. Cosmos, E., and E. J. Harris. 1961. In vitro studies of the gain and exchange of calcium in frog skeletal muscle. J. Gen. Physiol. 44:1121.
14. Foreman, H., M. Vier, and M. Magie. 1953. The metabolism of C14-labeled ethylenediaminetetraacetic acid in the rat. J. Biol. Chem. 203:1045.
15. del Castillo, J., and B. Katz. 1957. A study of curare action with an electrical micromethod. Proc. Roy. Soc. Ser. B. Biol. Sci. 146:339.
16. Takeuchi, N. 1963. Effects of calcium on the conductance change of the end-plate membrane during the action of transmitter. J. Physiol. (London). 167:414.
17. Nastuk, W. L., and J. H. Liu. 1966. Muscle postjunctional membrane: Changes in chemosensitivity produced by calcium. Science (Washington). 154:266.