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THE INTERACTION BETWEEN EDROPHONIUM (TENSILON) AND ACETYLCHOLINE AT THE MOTOR END-PLATE

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The effect of edrophonium (3-hydroxy-phenyl-dimethylethylammonium chloride) on the motor end-plate and its interaction with acetylcholine and carbachol has been investigated. Use was made of intracellular recording of membrane potential and of ionophoretic micro-application of drugs from single and twin-pipettes.

Small doses of edrophonium potentiate the depolarizing effect of acetylcholine, but not that of carbachol. This action can be observed with doses of edrophonium which have no depolarizing effect by themselves. Large doses of edrophonium have some depolarizing action and, at the same time, inhibit depolarizations produced by carbachol. After treatment with neostigmine, edrophonium fails to potentiate the acetylcholine response. The observations are in agreement with the view that the principal action of edrophonium on the neuromuscular junction is that of a potent and rapidly acting anticholinesterase.

The effects of anticholinesterases on muscle are usually tested under conditions in which the inhibitor/enzyme reaction has approached or reached equilibrium. In recent experiments (Castillo and Katz, 1957c), a different method was used, brief localized doses of the drug being applied to an end-plate with the help of an ionophoretic micro-technique. Under these conditions, the observed potency of a drug depends on the kinetics, rather than the equilibrium constant, of the reaction. In such experiments it was found that substances like neostigmine, which are strong but slowly acting enzyme inhibitors, produced no potentiation of the acetylcholine response, while less powerful but more rapidly acting esterase inhibitors (choline, decamethonium) caused a marked increase in the acetylcholine effect.

Similar experiments will be described in which edrophonium (3-hydroxy-phenyl-dimethylethylammonium chloride) was allowed to interact with acetylcholine (ACh), by applying the substances from micropipettes placed at close range to an end-plate of the frog's sartorius muscle. The membrane potential of the muscle fibre was recorded with an intracellular electrode inserted within a few hundred microns of the point of drug action.

The effect of edrophonium on neuromuscular transmission in the frog has previously been studied by Nastuk and Alexander (1954), who concluded that the anticurare action of this substance and the modifications which it produced in the shape of the electric end-plate response could be attributed to its anti-esterase activity (see also Smith, Cohen, Pelikan, and Unna, 1952). The present experiments confirm this view and provide additional evidence for the high speed at which the reaction between edrophonium and ACh-esterase proceeds.

METHOD

The technique has been described in detail in previous papers (Castillo and Katz, 1955, 1957a, c; see also Katz and Thesleff, 1957). The experiments were made on isolated sartorius muscles of *R. temporaria* at about 20° C. The preparations were mounted in a bath of Ringer solution which contained the electrodes for the recording of membrane potentials and for the electrophoretic application of drugs. Single or twin-pipettes were used, containing edrophonium and ACh, or edrophonium and carbachol, in the twin barrels. Edrophonium (Tensilon) was obtained by courtesy of Roche Products. Individual drug pipettes had tip diameters of less than 1 μ and were filled with a concentrated solution (0.5 to 2.5 M). The discharge of the drug was regulated by "braking" or "releasing" voltages (making the interior of the pipette more negative, or positive, respectively), in the way described in the earlier papers (Castillo and Katz, 1955, 1957a). The current flowing through the drug pipettes was registered on the second beam of the oscilloscope.

RESULTS

Fig. 1 illustrates the potentiating action of edrophonium. The records show potential changes produced at the end-plate region of a muscle fibre when ACh and edrophonium were discharged from a twin pipette in the immediate neighbourhood. In this experiment ACh was released from one barrel by slightly reducing the steady "braking" current which passed through it. This gave rise to a steady depolarization causing an upward displacement of the baseline from *a* to *b*, and then to *c*. The bottom trace serves to register the current through the pipettes, but the changes in the ACh-pipette were so small (of the order of 10^{-9} A.) that no visible displacement in the three successive lines occurred. The brief deflexion which interrupts this trace arose from a pulse through the edrophonium pipette (about 1.4×10^{-8} A., duration 13 msec.). The discharge had practically no effect in record *a* when ACh efflux was prevented. In record *b*, when a small efflux of ACh was present producing a steady depolarization of about 0.5 mV, this potential change increased to 3 mV after the edrophonium pulse. In record *c*, a steady ACh potential of 4 mV was increased to 14 mV by the same pulse of edrophonium.

It appeared from these results that a momentary application of edrophonium which, by itself, produced no potential change (record *a*), caused a several-fold increase of the depolarizing effect of ACh. The time course of this potentiation was rapid and had practically subsided within less than one second.

The effect shown in Fig. 1 might be explained by a rapid and quickly reversible anti-esterase action of edrophonium. To test this assumption,

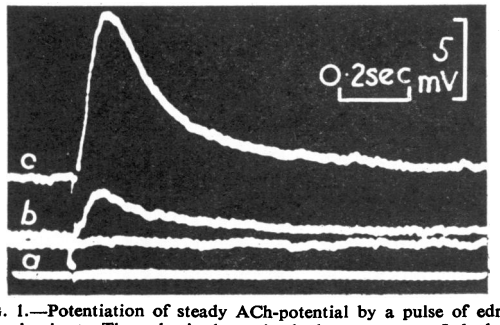

FIG. 1.—Potentiation of steady ACh-potential by a pulse of edrophonium. The pulse is shown in the bottom trace. It had no effect in (*a*) (when ACh-efflux was stopped by a small braking current), but produced a transient large increase of the steady depolarization in (*b*) and (*c*) (when a controlled small efflux from the ACh pipette occurred). See text for further details. The 5 mV scale refers to the membrane potentials (*a* to *c*). Calibration of the "current-monitor" (bottom trace): 5 mV scale $= 9.1 \times 10^{-8}$ A.

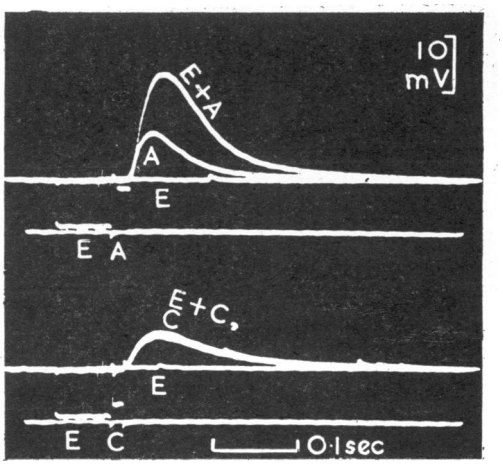

FIG. 2.—Upper part shows potentiation of a brief ACh-potential by a preceding edrophonium pulse. Lower part shows the barely noticeable effect produced by edrophonium on a carbachol potential. In each record, three traces were superimposed. E, edrophonium; A, acetylcholine; C, carbachol. See text for further details. Monitor calibration, 10 mV scale $= 6.7 \times 10^{-8}$ A.

two kinds of experiments were made: (*a*) effects of edrophonium were examined when ACh was replaced by a stable depolarizing substance (carbachol); (*b*) the interactions were studied before and after the muscle had been treated with neostigmine.

All the subsequent experiments were made with an assembly of three drug-pipettes, one—a twin pipette—containing edrophonium and carbachol in the two barrels, another separate pipette containing ACh and being placed nearby. This arrangement was chosen to obviate the possibility that any increase in the ACh effect could have been brought about by leakage between the twin barrels. It should be noted that with this set-up, because of the closer proximity of edrophonium and carbachol pipettes, any effect which edrophonium may have on the carbachol response would be more easily detected than interactions between edrophonium and ACh.

In Fig. 2, an example of the results is shown. In the upper part, three records are superimposed in which an edrophonium pulse (E), an ACh pulse (A), and both pulses together (E + A), were applied. The edrophonium pulse by itself produced a minute depolarization, barely rising above the base-line. The ACh dose alone produced the smaller of the two main deflexions. When the ACh pulse was preceded by the edrophonium pulse, the amplitude of the deflexion was more than doubled. In the lower part of the figure a similar series of three records is superimposed, but this time carbachol was used instead of ACh.

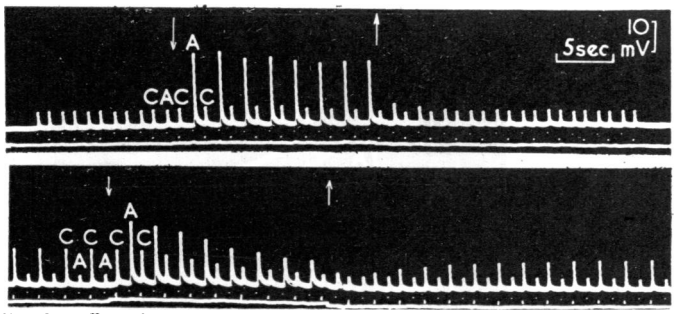

FIG. 3.—Effect of steady efflux of edrophonium (between arrows) on ACh (A) and carbachol (C) potentials. For full description, see text. Monitor calibration, 10 mV scale $=8\times10^{-8}$ A.

The combination of edrophonium + carbachol pulses produced only a very slightly increased effect above that due to the carbachol pulse alone.

That the edrophonium-potentiation is observed specifically with ACh, and not with carbachol, is brought out in a somewhat different fashion in Fig. 3. Here, alternate pulses of ACh and carbachol were applied to the end-plate, and the resulting brief depolarization recorded on slowly moving film. During the interval marked by arrows, the "brake" on the edrophonium barrel was reduced or reversed (signalled by a very small upward displacement of the bottom trace), and a steady efflux of this substance, therefore, occurred. In the upper part of Fig. 3, the pulses of ACh and carbachol had been adjusted initially so that the responses were of approximately the same amplitude. As soon as edrophonium began to be released, a large increase of the ACh-potentials occurred, while the carbachol potentials remained practically unaltered. After the end of the edrophonium application, the ACh-potentials declined within a few seconds to the level of the carbachol responses.

In the lower part of Fig. 3, different dosages were chosen. The pulses had been adjusted so that initially the carbachol potentials were about three times larger than the ACh potentials. The steady edrophonium dose was also increased, to a strength at which it produced a small, but noticeable, steady depolarization. The effect of edrophonium was two-fold. The ACh response was potentiated, its amplitude now exceeding the carbachol potential. The initial increase, however, was not maintained, but the responses gradually declined during the edrophonium period. This decline affected ACh and carbachol potentials to the same extent, and the ACh potentials remained larger than the carbachol until the efflux of edrophonium was stopped. It is clear, therefore, that the decline was not due to a gradual diminution of the specific ACh potentiation by the drug, but to a progressive "desensitization" of receptors which occurs whenever a depolarizing substance is applied for a prolonged period (see Katz and Thesleff, 1957, for a detailed study of this phenomenon).

It can be concluded from these results that small doses of edrophonium, which by themselves do not appreciably alter the membrane p.d., increase the depolarizing effect of ACh, but not that of carbachol. This supports the view that the effect is brought about by inhibition of ACh-esterase.

When the dose of edrophonium was increased, the potentiation became intensified as shown in Fig. 4 (left part). The method was the same as employed in Fig. 1: an edrophonium pulse was applied during a period of steady ACh depolarization. The bottom traces in each frame show an

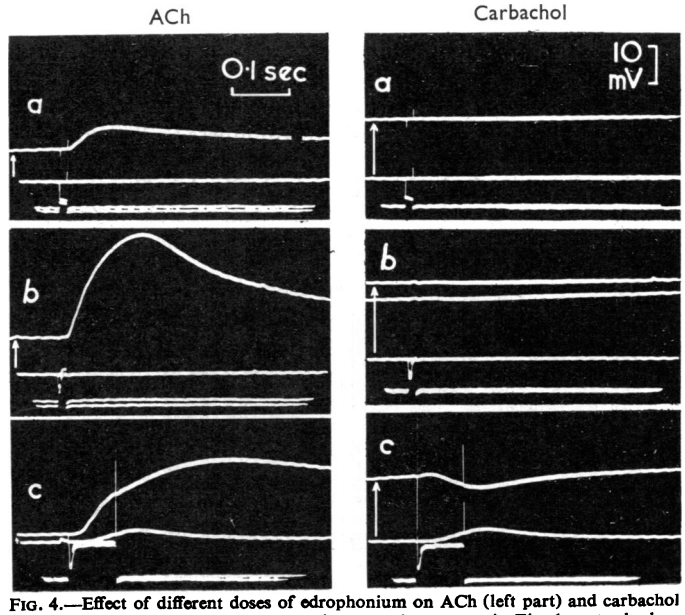

FIG. 4.—Effect of different doses of edrophonium on ACh (left part) and carbachol potentials (right part). The procedure was the same as in Fig. 1, a steady dose of ACh or carbachol being combined with a pulse of edrophonium. The depolarization due to ACh or carbachol is shown by the displacement of the trace in the direction of the arrow. The records in each horizontal row were obtained with the same edrophonium dose, whose strength was increased from *a* to *c*. Note that in *a* and *b*, the edrophonium by itself had no effect on the membrane potential, while it produced a small, transient depolarization in *c*. For further description, see text. Monitor calibration, 10 mV scale $=7.9\times10^{-8}$ A.

edrophonium pulse, whose coulomb strength was increased from *a* to *c*. In each frame, two records are shown, (1) when ACh efflux has been stopped by a large " braking " current, and (2) when ACh release was allowed to occur. In frame *c*, a very small steady dose of ACh was given, producing only 2 to 3 mV. depolarization ; this was a necessary precaution to avoid excitation and twitching. Potentiation increased, from a factor of 1.7 in *a*, to about 10 times in *c*. In addition, the effect was lengthened considerably.

It will be observed that in *a* and *b*, the edrophonium pulse by itself produced no potential change, while in *c* a transient depolarization of a few mV. was seen. This indicates that there is a substantial margin between the doses of edrophonium which interfere with ACh esterase, and those which combine effectively with the receptors (see also Nastuk and Alexander, 1954). In this respect, edrophonium may be classified as a " specific " anti-esterase, like neostigmine, but unlike decamethonium or choline which have a mixed action on esterase and receptors in the same dosage range (Castillo and Katz, 1957c).

The right-hand part of Fig. 4 shows, for comparison, interactions between edrophonium and carbachol at the same end-plate spot. Each horizontal row of records in Fig. 4 was obtained with identical edrophonium pulses. In the upper right frame, no effect is observed. In the middle frame, there is a barely noticeable trace of inhibition ; in the lower frame, the inhibitory effect of edrophonium on the carbachol-potential is well marked. We are dealing here with an example of competitive interference between two depolarizing drugs, of the kind described in detail by Castillo and Katz (1957c ; see also Ariëns, 1954 ; Stephenson, 1956).

Finally, the observations were repeated after the preparation had been treated for about 30 min. with neostigmine. With a concentration of 10^{-6} w/v (neostigmine methylsulphate/Ringer), the potentiating action of edrophonium was greatly reduced. With a two to four times larger dose of neostigmine, the potentiation of the ACh effect had vanished, and the interaction between edrophonium and ACh was now very similar to that observed with edrophonium and carbachol, that is, when relatively large doses were used, edrophonium now *inhibited* ACh as well as carbachol potentials. An example is shown in Fig. 5. The upper records show the depolarization produced by the large edrophonium pulse. The middle frames show an ACh and a carbachol potential, respectively ; the lower frames show the depression of both ACh and carbachol effects when they are preceded by an edrophonium pulse.

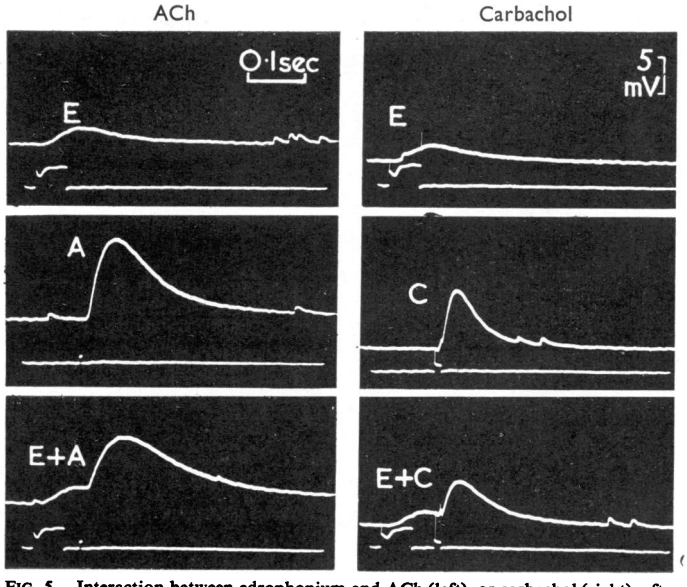

FIG. 5.—Interaction between edrophonium and ACh (left), or carbachol (right), after pre-treatment with neostigmine. The procedure was similar to that in Fig. 2, except that a large dose of edrophonium was used which depolarized by itself. E, edrophonium; A, ACh; C, carbachol. Note that the potentiation of the ACh effect (Fig. 4*c*, left) has now given place to inhibition, similar to that observed with carbachol. Monitor calibration, 5 mV scale=7.9×10^{-8} A.

Thus, initial treatment with a more slowly-acting, but powerful, anti-esterase abolishes the potentiation, and all that is left is the relatively weak depolarizing action of edrophonium and the associated inhibitory effect due to its competition with more powerful depolarizing agents.

DISCUSSION

The results fully support the conclusions reached by Nastuk and Alexander (1954) and by Smith *et. al.* (1952), namely that the principal action of edrophonium on neuromuscular transmission is that of an anticholinesterase. Application of this drug potentiates the effect of ACh, but not of its stable analogue, carbachol ; and the potentiation is not seen when the esterase activity has already been inhibited by neostigmine.

The time course of the potentiating effect is rapid. For example, in the experiment illustrated in Fig. 1, the effect of an edrophonium pulse rose

to a peak in 85 msec. and fell to one half in about 140 msec. which was only four times slower than a small depolarization produced at the same spot by a similar pulse of ACh from the adjacent pipette. In comparing the time courses of these two effects, a factor of two should be allowed for the enzymatic removal of ACh (see Castillo and Katz, 1957b), and some part of the remaining difference is probably due to slower diffusion rather than to the reaction kinetics. In any case, the bond between edrophonium and esterase must be rapidly reversible, with a time constant of dissociation of less than 0.1 sec. at 20° C. This is very much faster than the dissociation rate of the neostigmine- or eserine-enzyme complex (see Easson and Stedman, 1936; Eccles, Katz, and Kuffler, 1942; Augustinsson and Nachmansohn, 1949; Goldstein, 1951), which have time constants of the order of several minutes and exert their effects too slowly to be usefully investigated with the present method.

Some comment is needed on the relatively small interaction between edrophonium and carbachol. The reduction of a carbachol depolarization by a large pulse of edrophonium can be explained on the asumption that both drugs combine with receptor molecules, but that the edrophonium receptor complex has less "depolarizing efficacy" (see Stephenson, 1956) than the carbachol receptor compound.

With weaker doses of edrophonium, however, a small increase of the carbachol potential was often observed (Fig. 2). This was seen usually when the edrophonium application by itself produced a just noticeable depolarizing effect. It is probable that this small, positive, interaction arises from the fact that two *weak* doses of any depolarizing drug produce a more than additive effect, their dose/response relation having an S-shaped, rather than a linear, start (Katz and Thesleff, 1957).

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