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THE EFFECT OF ATROPINE ON THE FROG SARTORIUS NEUROMUSCULAR JUNCTION

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SUMMARY

1. The effect of atropine sulphate and of (+)-tubocurarine chloride (TC) on the amplitude and time course of end-plate potentials (e.p.p.s) and miniature end-plate potentials (m.e.p.p.s) was studied in the sartorius muscle of the frog.

2. Atropine sulphate reduces the amplitude of intracellularly recorded e.p.p.s and m.e.p.p.s, in concentrations 100 times higher than TC (6×10^{-5} M for 50% reduction of amplitude compared with 6×10^{-7} M for TC).

3. Atropine sulphate causes a marked shortening of both e.p.p.s and m.e.p.p.s: when the amplitude of e.p.p.s or m.e.p.p.s is reduced by 50%, their rise-time and half-decay time are both shortened by 40%. The corresponding shortening produced by TC is 15%.

4. E.p.p.s prolonged by prostigmine 10^{-6} g/ml. undergo a larger shortening (30%) in TC, while atropine-induced shortening related to the corresponding drop of amplitude is the same whether prostigmine is used or not.

5. On repeated applications after recovery of amplitude and time course, TC loses its shortening effect on e.p.p.s while the atropine shortening effect remains unchanged.

6. Atropine sulphate shortens the rise-time but not the falling phase of brief depolarizations produced by electrophoretic applications of acetylcholine (ACh) to the muscle fibre surface in the end-plate region. It also reduces their amplitude, in the same way that it reduces the amplitude of e.p.p.s.

7. Atropine sulphate in concentrations which markedly reduce the amplitude and time course of e.p.p.s has no effect on their quantum content.

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8. Atropine sulphate at a concentration of 10^{-4} M does not change the amplitude and time course of an electrotonic potential produced by a rectangular current pulse passed through the end-plate region of a muscle fibre.

9. It is suggested that either enhanced removal of ACh or a spatial gradient of effectiveness of the blocking drugs, or both these mechanisms, participate in shortening the e.p.p. by atropine sulphate and TC.

INTRODUCTION

In previous work (Beránek, 1965; Beránek & Vyskočil, 1967) it was shown that atropine blocks neuromuscular transmission in the rat diaphragm by reducing the e.p.p.s. As with curare the reduction of e.p.p.s could be accounted for by post-synaptic action of the drug, but the concentrations needed to produce the same effect were shown to be 2000 times higher for atropine. In similar experiments with the frog sartorius muscles we noticed that apart from the reduction of the amplitude of the e.p.p.s, atropine causes a marked shortening of the duration of the e.p.p.s. It is known that curare shortens the e.p.p.s of frog sartorius muscles when they have been prolonged by the action of eserine (Eccles, Katz & Kuffler, 1942) but the sensitivity to atropine was so much greater, even in muscles untreated with anticholinesterase, that we decided to study this effect in more detail and to compare the action of the two drugs. Before this work was finished the shortening of the e.p.p. by atropine was observed independently by M. Kordaš (personal communication).

METHODS

The experiments were performed on neuromuscular preparations from the sartorius muscle of *Rana temporaria*. The muscles with their nerves were stretched over the convex bottom of a Perspex recording chamber and perfused with a solution of the required composition at room temperature (20–23° C). Standard 3M-KCl-filled glass capillary electrodes of 10–30 MΩ resistance were used for intracellular recording of membrane potentials of individual muscle fibres, while the nerve was stimulated through a pair of platinum electrodes. The amplitude, half-rise and half-decay times of e.p.p.s and m.e.p.p.s were obtained by averaging the values for 180–200 potentials recorded photographically. KCl-filled glass capillary micro-electrodes were also used for passing polarizing current through the end-plate region of the muscle fibres to study electrotonic effects. Similar micropipettes filled with 1 g/ml. acetylcholine chloride were employed to produce short depolarizations of the muscle fibre membrane by electrophoretic ejections of ACh from the micropipette tips which were placed on the sensitive parts of the membrane (Nastuk, 1953; del Castillo & Katz, 1954). Details of the perfusion technique are given in Beránek & Vyskočil (1967).

The perfusing fluid had the following basic composition (in mM): Na^+ 115; K^+ 2·5; Ca^{2+} 1·8; NaH_2PO_4 0·85; Na_2HPO_4 21·1. In order to study the e.p.p.s undisturbed by action potentials and twitches, we reduced the quantum content of the e.p.p.s below the spike triggering level by adding 10–16 mM-Mg^{2+}, substituting $MgCl_2$ for an isosmotic amount of

NaCl. The blocking drugs, atropine sulphate (Burroughs-Wellcome) and (+)-tubocurarine chloride (Borroughs-Wellcome) were added to the solutions. Prostigmine (neostigmine methylsulphate, Roche) in a concentration of 10^{-6} g/ml. was used as an anticholinesterase, and acetylcholine chloride (Roche) was used for the iontophoretic pipettes.

RESULTS

The action of atropine and curare on the amplitude of e.p.p.s. Both atropine sulphate and TC displayed their full actions within 10–15 min, but a residual depression of e.p.p. and m.e.p.p. amplitude (5–15%) persisted even after 60 min of recovery from atropine sulphate, while the recovery of potentials from TC was complete. No resting potential changes were observed.

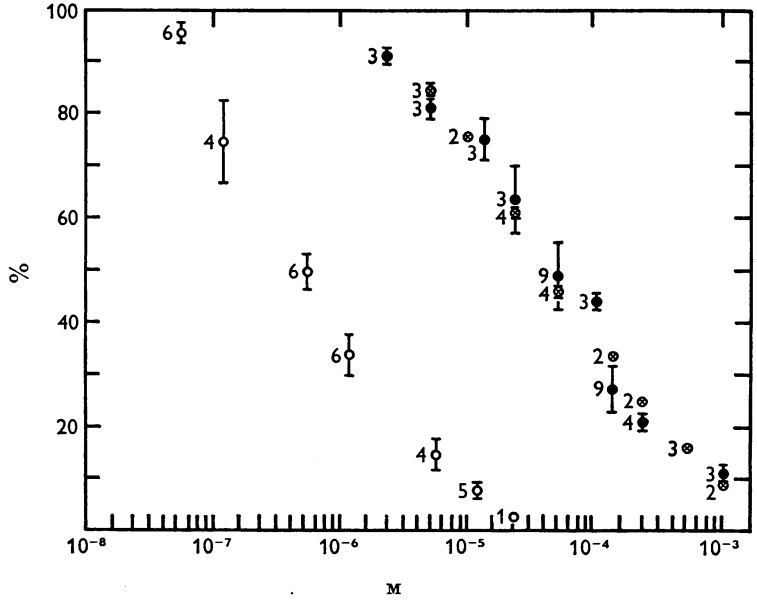

Fig. 1. The blocking action of TC (○) and atropine sulphate (●) on the amplitude of intracellularly recorded e.p.p.s and the blocking action of atropine sulphate on ACh potentials at the end-plate region (⊗) of frog sartorius muscle fibres. Ordinate: amplitude expressed as percentage of control value. The latter was the mean amplitude of e.p.p. or ACh-potential measured before applying and after washing out the blocking drug. Abscissa: molar concentration of blocking drugs. Vertical bars: ±S.D. Numbers refer to the number of measurements, each performed on a different preparation. On one preparation up to four different concentrations were tested in one experiment.

The dose–response curves for both the drugs are represented in Fig. 1. As in the rat diaphragm (Beránek, 1965; Beránek & Vyskočil, 1967) much higher concentrations of atropine are needed to produce an effect comparable to that produced by curare. The dose–response curve was the

same whether prostigmine was present or not. The curare/atropine coefficient (i.e. dose ratio for equal effects (Itina, 1959)), however, is higher for the frog sartorius (0·01 compared with 0·0005 for rat diaphragm). The difference is accounted for by the lower effectiveness of TC in frog muscle, the potency of atropine sulphate being the same in both preparations.

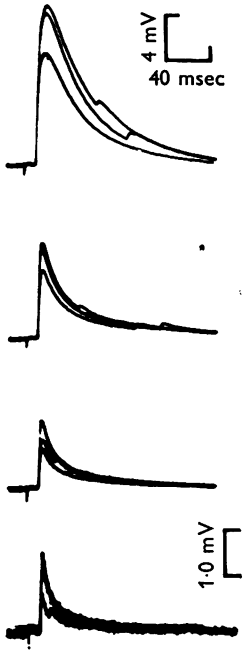

Fig. 2

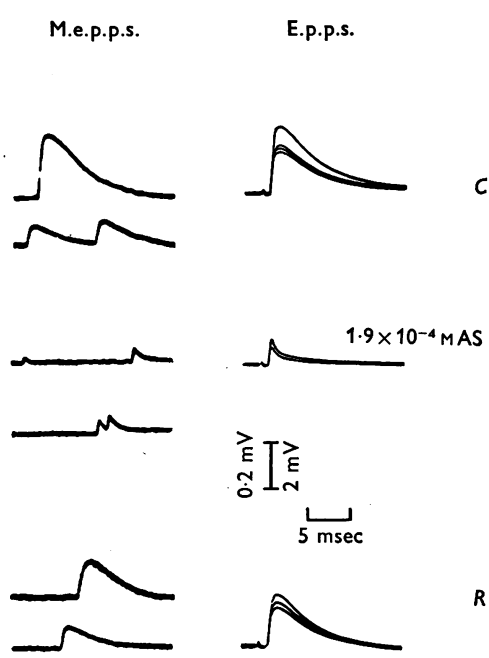

Fig. 3

Fig. 2. From top to bottom: drop of e.p.p. amplitude with concomitant shortening of the falling phase produced by increasing concentration of atropine. In this experiment a drop of atropine sulphate solution ($2·9 \times 10^{-3}$ M) was allowed to fall on the preparation near the micro-electrode and e.p.p.s were recorded at 25 sec intervals as the drug diffused over the end-plate region. The nerve was stimulated once every 2 sec. Note the simultaneous shortening of superimposed e.p.p.s. Prostigmine, 10^{-6} g/ml., present.

Fig. 3. The action of $1·9 \times 10^{-4}$ M atropine sulphate (AS) on m.e.p.p.s (left) and e.p.p.s (right) recorded from the same fibre. *C*, control; *R*, recovery. Prostigmine not present in this experiment.

Changes in the time course of e.p.p.s and m.e.p.p.s caused by atropine sulphate and TC. Figures 2–5 show that with increasing concentration of atropine sulphate a concurrent reduction of the amplitude and a shortening of both the rising and falling phases of the e.p.p.s occurs. When the amplitude of e.p.p.s is reduced by 50%, their half-rise time and half-decay time are both shortened by approximately 40%. From the same figures

it can be seen that similar reversible changes appear in the m.e.p.p.s recorded through the same electrode (Table 1, Fig. 8).

TABLE 1. Results of experiments in which atropine sulphate in a concentration of 6×10^{-5} M was applied. The values are given as percentages of the amplitudes and times before application of the drug. The numbers in parentheses give the numbers of experiments performed each on a different fibre. ± = S.D. Prostigmine 10^{-6} g/ml. present

	During application of atropine sulphate	After recovery
E.p.p. amplitude	(8) 53·1 ± 6·2	(7) 91·9 ± 5·2
M.e.p.p. amplitude	(6) 50·2 ± 4·3	(6) 85·9 ± 7·2
E.p.p. half-decay time	(8) 61·0 ± 5·3	(7) 87·6 ± 3·5
E.p.p. half-rise time	(2) 56·2	(2) 89·2
M.e.p.p. half-decay time	(6) 61·4 ± 2·2	(6) 87·5 ± 3·9
M.e.p.p. half-rise time	(2) 62·0	(2) 90·5
Quantum content ($\overline{V}/v$)	(6) 102·9 ± 13·2	(6) 105·6 ± 6·9

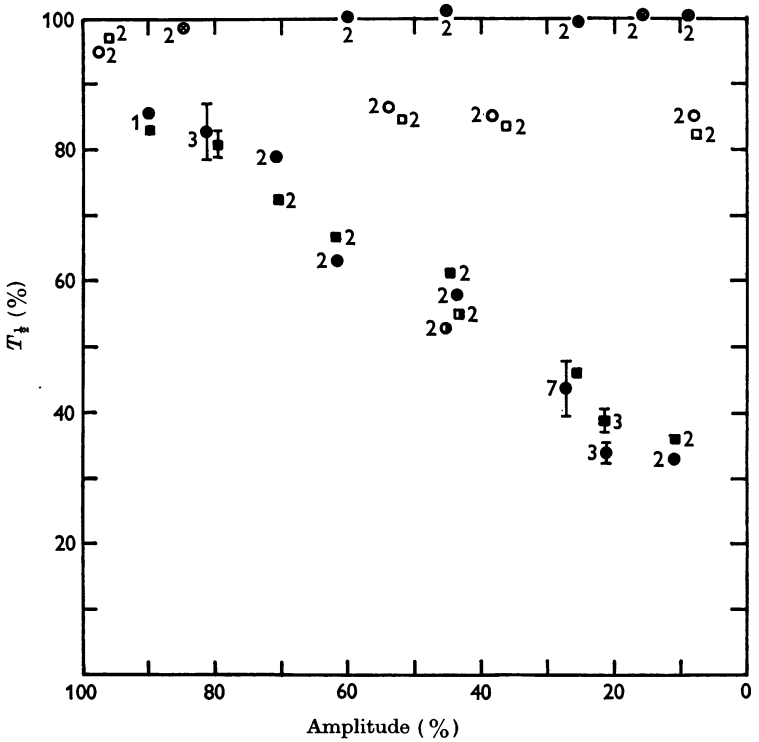

Fig. 4. The relation of the decrease in amplitude to the half-rise time of e.p.p.s (■) and m.e.p.p.s (◨) and to the half-decay time of e.p.p.s (●), m.e.p.p.s (◑) and ACh potentials (⊗) in atropine sulphate compared with half-rise times of e.p.p.s (□) and half-decay times of e.p.p.s (○) in TC. Ordinate: half-rise and half-decay time of potentials expressed as percentages of the value measured before application of drug. Abscissa: amplitude of e.p.p.s expressed as percentage of control value. Prostigmine not present.

TC causes a much smaller shortening (15% at 50% of amplitude), but its effect is markedly enhanced when the potentials are prolonged by prostigmine, whereas the atropine-induced shortening of e.p.p.s, when related to the reduction of their amplitude, is the same whether prostigmine is present, or not (Figs. 4, 5). The difference between curare and atropine is more prominent at higher concentrations where the effect of TC in shortening e.p.p.s tends to reach a plateau, while the atropine sulphate-induced shortening stays proportional to the drop of amplitude (Figs. 4, 5).

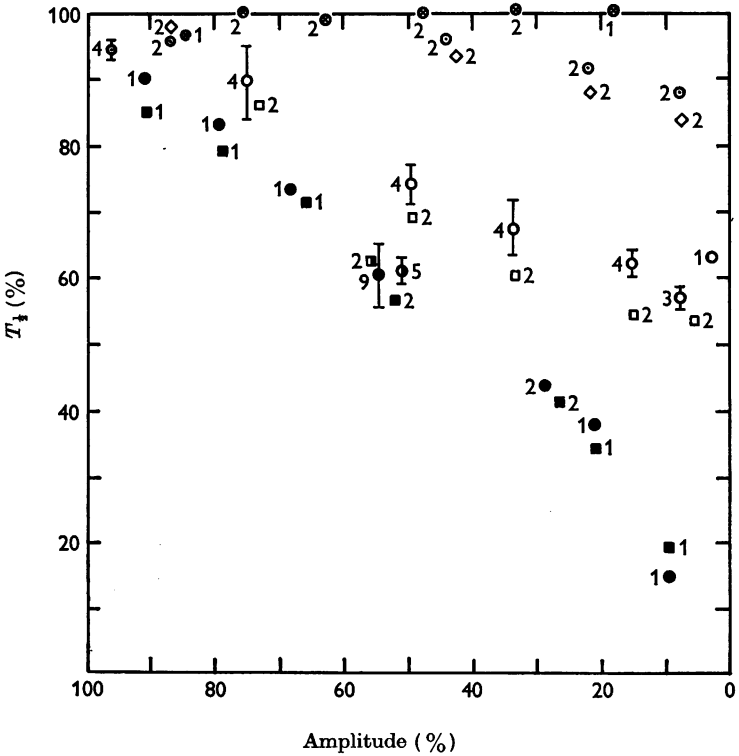

Fig. 5. Measurements similar to those in Fig. 4 but modified by the presence of prostigmine 10^{-6} g/ml. (⊙) refers to half-decay times and (◇) to half-rise times of e.p.p.s in experiments with TC repeated after recovery from the first TC application. Other symbols as in Fig. 4.

Another difference in the action of these two drugs appeared when the experiments were repeated after 60 min of recovery, when both the amplitude of the potentials and their time course returned to almost initial values. On repeated application of TC shortening of e.p.p.s was almost absent, while the action of atropine was unchanged (Fig. 5).

The effect of atropine on the time course of acetylcholine potentials. An ACh-filled micropipette was placed on a spot of maximum sensitivity in the end-plate region and depolarizations were produced by ejecting small amounts of acetylcholine iontophoretically on to the outer surface of the membrane. The rising phase of these potentials lasted 6–10 msec. The

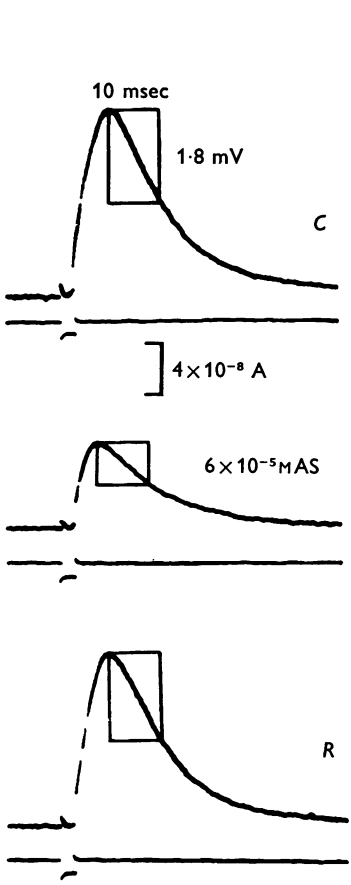

Fig. 6

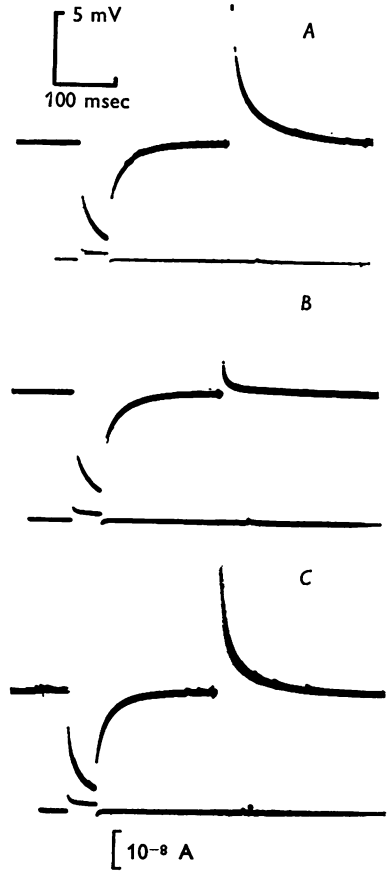

Fig. 7

Fig. 6. The effect of atropine sulphate (AS) on ACh potentials produced by electrophoretic applications of ACh in the end-plate region of a frog sartorius muscle fibre. The upper trace in each pair records the potential change of the membrane through an intracellular electrode, the lower trace monitors the ACh current. *C*, control: *R*, recovery. Prostigmine 10^{-6} g/ml. present.

Fig. 7. Upper traces: intracellularly recorded membrane potential changes of a muscle fibre during passage of a rectangular current pulse through the membrane followed by an e.p.p. elicited by nerve stimulation. Lower traces: time course of polarizing current. Two records are superimposed in each trace. *A*, control; *B*, 10^{-4} M atrophine sulphate; *C*, recovery.

reduction in the amplitude of these depolarizations by atropine sulphate was the same as that of e.p.p.s. Their rising phase was shortened, but their half-decay time remained unchanged (Figs. 4, 5, 6).

The electrotonic potentials of the muscle fibre membrane in the presence of atropine. An intracellular electrode was inserted in the end-plate zone, and a second electrode introduced within 20 μ of the first was used to pass rectangular pulses of polarizing current of 50 msec duration through the membrane. Neither the amplitude nor the time course of the resulting electrotonic potentials were changed by atropine sulphate at a concentration of 10^{-4} M (Fig. 7).

The effect of atropine on the quantum content of e.p.p.s. The quantum content (m) of e.p.p.s was calculated from intracellular recordings as

$$m = \frac{\bar{V}}{\bar{v}} = \frac{\text{mean amplitude of response}}{\text{mean amplitude of spont. potentials}}$$

(del Castillo & Katz, 1954). The results of experiments in which quantum content was measured after application of 6×10^{-5} M atropine sulphate are given in Table 1 and presented diagrammatically in Fig. 8 together with corresponding values of e.p.p. amplitudes and e.p.p. half-decay times. It can be seen that no appreciable change in m results from application of the drug.

DISCUSSION

The experiments show that the potency of TC in reducing e.p.p.s is lower in the frog sartorius muscle than in the rat diaphragm, while atropine sulphate is equally effective in both. Similarly, ACh depolarizations of denervated rat diaphragm muscle fibres are less sensitive than normal e.p.p.s to the action of TC (Beránek, 1965; Magazanik, 1966; Beránek & Vyskočil, 1967), but there is no difference, after denervation, in the sensitivity of ACh potentials to atropine.

The shortening of e.p.p.s produced by blocking drugs could be explained in several different ways.

(1) The drug could act on transmitter release. The data from the present work, however, make its effect on the release improbable. Such a change would be likely to interfere with the quantum content of e.p.p.s but no such effect has been observed. The quantum content remained the same while the time course of individual m.e.p.p.s was changed in the same manner as that of whole e.p.p.s. One might argue that the release of transmitter molecules within individual quanta could be changed, but the minimum size of released transmitter packets has been shown to be a rigid value, constant over a wide range of different conditions (Katz, 1962, 1966).

(2) A shortening of both the rising and falling phases of e.p.p.s and m.e.p.p.s would occur if the *effective* size of each quantum were reduced by

quick removal of the normally released transmitter from the synaptic cleft. Atropine might cause such a removal by acting as cholinesterase activator or by directly displaying cholinesterase activity. In the former case, however, it would be difficult to explain the shortening in prostigmine, unless activation of some prostigmine-resistant cholinesterase is considered. Alternative mechanisms by which ACh would quickly be removed may include, e.g. resorption of the transmitter by nerve endings caused by atropine or an increased removal of acetylcholine by facilitated diffusion. All these mechanisms are, as yet, purely hypothetical and require further experimental studies.

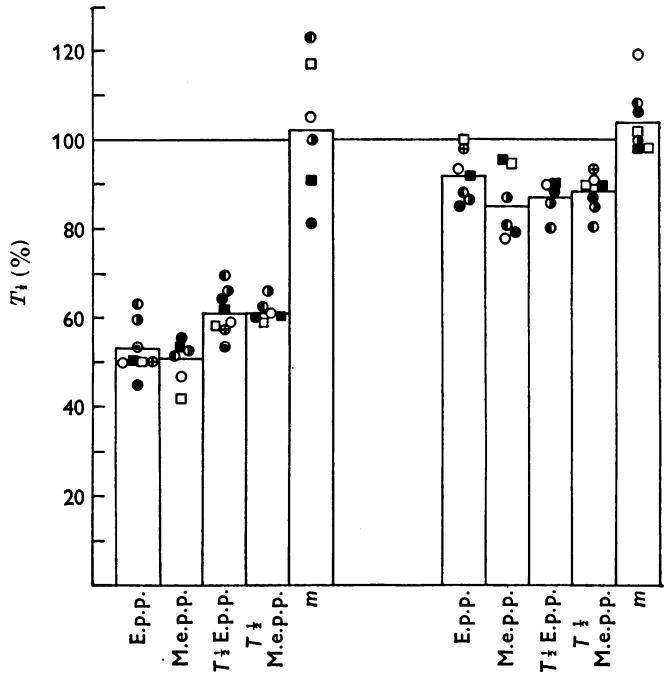

Fig. 8. Effect of 6×10^{-5} M atropine sulphate on quantum content, amplitude and half-decay time of e.p.p.s. 1st column, reduction of e.p.p. amplitudes; 2nd column, reduction of m.e.p.p.s; 3rd column, shortening of the falling phase of e.p.p.s; 4th column, shortening of the falling phase of m.e.p.p.s; 5th column, quantum content of e.p.p.s. Left-hand group of columns: values during application of atropine sulphate expressed as percentage of controls. Right-hand group of columns: after washing out the drug and giving time for recovery. Each symbol refers to one muscle fibre. Prostigmine 10^{-6} g/ml. present.

(3) As the decay of the later (approximately exponential) part of e.p.p.s depends on the RC constant of the membrane (Fatt & Katz, 1951; Katz, 1966), a shortening would be expected if atropine sulphate reduced

this membrane time constant. That this does not occur was shown in our experiments in which the electrotonic response to a rectangular polarizing pulse was unchanged by a high concentration of atropine sulphate. Besides, a closer inspection of the e.p.p.s shortened by atropine sulphate (Fig. 3) reveals that repolarization is accelerated predominantly during the early part of the falling phase.

(4) The possibility should also be considered that the ionic permeability change produced by the transmitter action in the post-synaptic membrane is modified by atropine sulphate, but no experimental evidence is available as yet to discuss the point.

(5) None of our results invalidates the original suggestion by Eccles *et al.* (1942) to account for e.p.p. shortening which curare produces in eserinized frog muscles. In their experiments, compound e.p.p.s prolonged by the action of eserine were shortened to practically their original length on application of curare. Curare, in their opinion, prevented the surface spread of ACh by blocking its combination with receptors, thus simultaneously enhancing the chance of free ACh being removed by any residual eserine-resistant enzyme or any other mechanism, e.g. uptake by nerve endings. A similar idea could explain the shortening of e.p.p.s and m.e.p.p.s by blocking agents at junctions with fully active cholinesterase. A shortening would occur if the receptor blockage by the drug was least effective at the points of quantal release and much more effective at more distant points. Such a spatial gradient could be due to difference in the affinity of the receptor molecules for the particular blocking drug or simply due to a gradient of the local density of receptors. The studies of the local distribution of sensitivity in the end-plate region suggest that its gradient may be very steep at focal points (Miledi, 1960, 1962). This idea would also explain why ACh potentials react differently from e.p.p.s to the application of atropine sulphate, since ACh applied from outside to the end-plate would be likely to spread along a different gradient. Whether complementary gradients of efficacy of the two blocking drugs within the end-plate region of the frog sartorius muscle in fact exist and whether the gradient for atropine is much steeper than for curare (thereby accounting for the more marked shortening produced by the former) are matters for further investigation. It is quite possible that more than one of the above-mentioned mechanisms participates in the shortening of e.p.p.s by blocking drugs.

We have no explanation for the reduction of the shortening effect of TC on repeated applications.

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