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THE EFFECT OF PROCAINE ON NEUROMUSCULAR TRANSMISSION

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SUMMARY

1. The mechanism of procaine action on post-synaptic receptors for acetylcholine was studied by recording the end-plate current at membrane potentials ranging from about +30 to about −140 mV.

2. It has been found that at resting membrane potential of about −60 to −80 mV the end-plate current has a fast initial and a slow late component. During hyperpolarization of the muscle fibre the amplitude of the slow component is depressed and its half-time lengthened. When the membrane potential is inverted the difference in the time course of both components is much less pronounced or absent.

3. It is suggested that procaine modifies the receptor response induced by acetylcholine, and that this modification is dependent on membrane potential.

INTRODUCTION

It has generally been accepted that the end-plate potential (e.p.p.) is due to a transient increase in conductance of the end-plate muscle fibre, induced by the transmitter (Eccles, Katz & Kuffler, 1941, 1942; Katz, 1942, 1948; Kuffler, 1942*a*, *b*; Fatt & Katz, 1951). By using a voltage–clamp technique the time course of this conductance change can be studied by recording the end-plate current (e.p.c., Takeuchi & Takeuchi, 1959). There are several drugs which either shorten or lengthen the time course of the e.p.c. (e.g. curare, atropine, or anticholinesterases). In the presence of the local anaesthetics procaine, xylocaine and its analogues the time course of the e.p.p. and of the externally recorded e.p.c. shows a fast initial and a slow late component (Furukawa, 1957; Maeno, 1966; Steinbach, 1967, 1968*a*, *b*; Hirst & Wood, 1969). A similar effect of procaine on the time course of miniature e.p.c. was also reported by Gage & Armstrong (1968).

At present, there are two different views on the mechanism of procaine action. According to the first, procaine combines secondarily with the

receptor–mediator complex. The resulting three-component complex has a greater stability and a smaller 'shunting' ability than the normal receptor–mediator complex. Therefore, the resulting e.p.c. shows a fast initial and a slow late component (Steinbach, 1967, 1968*a*, *b*). According to the other view, there are separate conductance channels for Na^+ (ΔG_{Na}) and K^+ (ΔG_K) in the muscle fibre end-plate membrane, which increase on transmitter action. In the absence of procaine the time courses of ΔG_{Na} and ΔG_K are slightly different. In presence of procaine, however, the time course of ΔG_{Na} is profoundly changed showing a fast initial and a slow late component (Maeno, 1966; Gage & Armstrong, 1968).

It is implicit in either view that the contribution of Na^+ and K^+ ion movements to the total e.p.c. is dependent on the membrane potential (E_m) and the equilibrium potentials for Na^+ (E_{Na}) and K^+ (E_K). If the time course of the e.p.c. is determined by the time courses of ΔG_K and ΔG_{Na}, and if the time course of the latter is affected by procaine (Maeno, 1966; Gage & Armstrong, 1968), the effect of this drug should be most pronounced when the e.p.c. is mainly due to Na^+ movements, i.e. when $E_m = E_K$.

In order to decide between these views, the time course of the e.p.c. during application of procaine was recorded over a wide range of membrane potentials.

METHODS

All experiments were carried out at room temperature (20–22° C) on sciatic nerve–sartorius muscle preparations of the frog (*Rana esculenta*).

The Ringer solution had the following composition: NaCl 116 mM, KCl 2 mM, $CaCl_2$ 1·8 mM, Tris 4 mM, HCl 3·3 mM, pH 7·4. Procaine was added to a concentration of $1{\cdot}8–3{\cdot}6 \times 10^{-4}$ M. In some experiments procaine concentration was increased to $7{\cdot}2 \times 10^{-4}$ M. In this experimental condition the e.p.c. amplitude was very small; therefore the anticholinesterase prostigmine was added to a concentration of $3{\cdot}10^{-6}$ M.

To record the e.p.c., a modified voltage–clamp technique similar to that of Takeuchi & Takeuchi (1959) was used as described previously (Kordaš, 1968).

In this study it was necessary to record the e.p.c. over a wide range of membrane potentials. To avoid contraction during depolarization of the muscle fibre, experiments were performed on glycerol-treated muscles (Howell & Jenden, 1967; Gage & Eisenberg, 1969*a*; Howell, 1969; see also Kordaš, 1969*a*). A few experiments were also made on muscles with an intact transverse tubular system.

RESULTS

The e.p.p. and e.p.c. in presence of procaine. The e.p.p. and e.p.c. were recorded in muscle fibres with intact and disrupted transverse tubular system. At membrane potentials of about -60 to -80 mV the e.p.c. had always a fast initial and a slow late component. The rise time of the e.p.c.

was 0·4–0·6 msec, the half-decline time of the fast and slow components 0·3–0·5 and 5–20 msec, respectively. The time course of the e.p.p., however, was somewhat different in the two types of preparation. As already reported, in intact muscle fibres the 'hump' on the falling phase of the e.p.p. is rather pronounced (Fig. 1*A*; Furukawa, 1957; Maeno, 1966; Steinbach, 1968*a*, *b*; Hirst & Wood, 1969). In muscle fibres with disrupted transverse

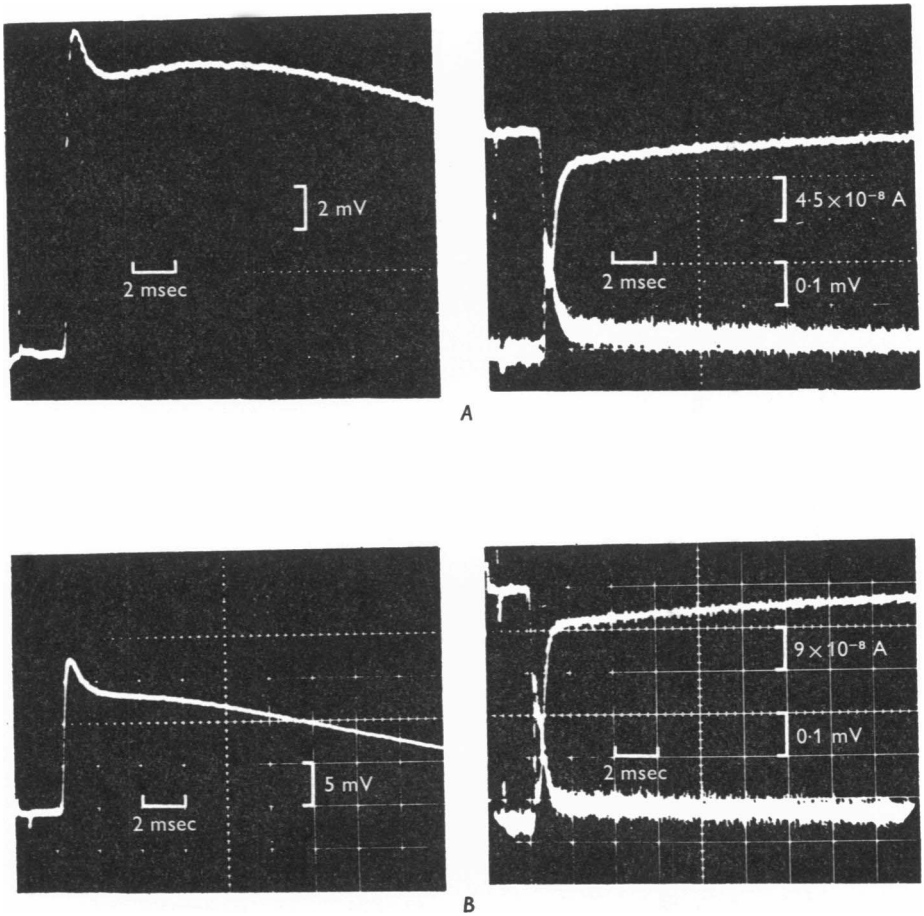

Fig. 1. End-plate potential and end-plate current in presence of procaine. Left hand pictures: the traces are intracellular recordings of membrane potential change (inside-positive deflexion upwards) during neuromuscular transmission. Right hand pictures: lower traces show the reduced membrane potential change (inside-positive deflexion upwards) during the flow of the 'voltage-clamping' current, recorded on the upper beam (inward current downward). *A*: muscle with intact transverse tubular system. Membrane potential 77 mV. *B*: muscle with disrupted transverse tubular system. Membrane potential 68 mV.

tubular system, the hump is in general less pronounced (Fig. 1*B*), probably because in these fibres the membrane capacitance is reduced (Steinbach, 1967; Gage & Eisenberg, 1969*b*). However, it should be stressed that not only the membrane capacitance, but also the relative sizes of the fast and slow component of the e.p.c. determine the amplitude and time course of the 'hump'.

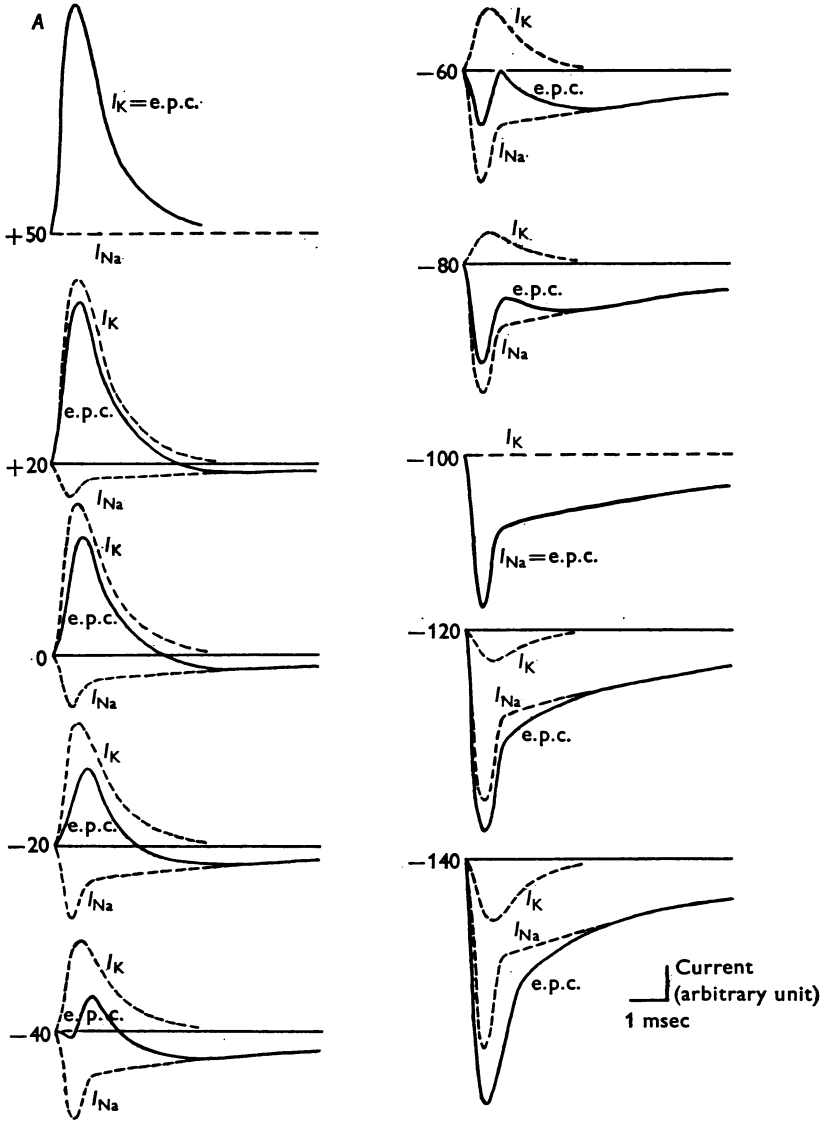

Fig. 2A. For legend see facing page.

The effect of procaine on the time course of the e.p.c. recorded at different membrane potentials. Maeno (1966) and Gage & Armstrong (1968) suggested that in presence of procaine ΔG_{Na} is depressed and its time course profoundly changed, showing a fast initial and a slow late component. Contrary to ΔG_{Na}, ΔG_K is believed to be unaffected by procaine (Maeno, 1966).

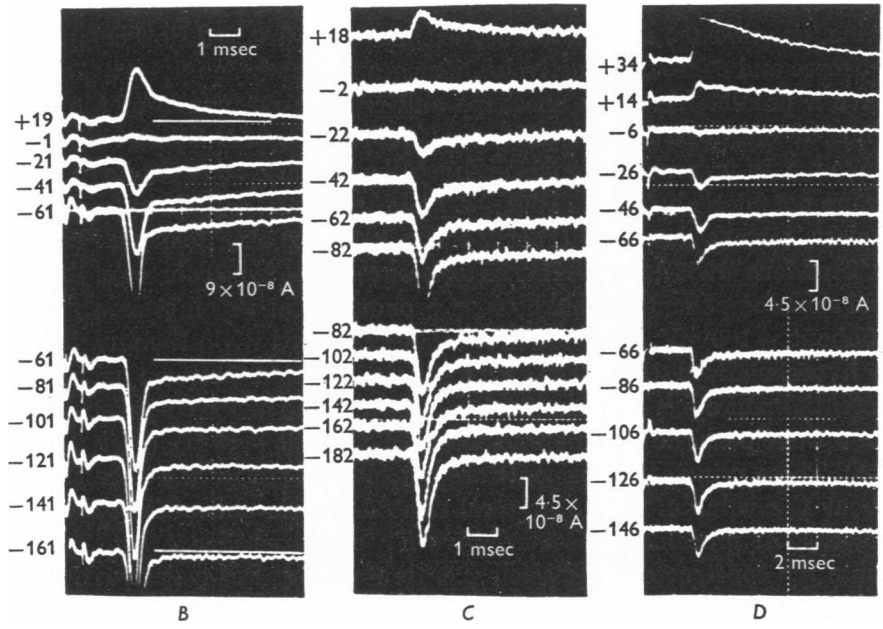

Fig. 2. The effect of membrane potential on shape of the e.p.c. as predicted according to the hypothesis of Maeno (1966) and Gage & Armstrong (1968) (*A*), and as found in two different muscle fibres in presence of $1{\cdot}8 \times 10^{-4}$ M procaine (*B*, *C*) and in presence of $7{\cdot}2 \times 10^{-4}$ M procaine and 3×10^{-6} M prostigmine (*D*). Membrane potential indicated by numbers beside records and tracings. Note that during severe hyperpolarization the slow component of the e.p.c. runs almost parallel to the base line, and that the two components of the e.p.c. are much less pronounced or absent when membrane potential is inverted. Curves in *A* constructed on following assumptions: rise and half-decline time of ΔG_K are 0·7 and 0·8 msec respectively; rise time of ΔG_{Na} is 0·5 msec, half-decline time of the fast and slow component 0·5 and 5 msec, respectively; $\Delta G_{Na}/\Delta G_K = \frac{2}{3}$; maximum amplitudes of Na^+ and K^+ current (I_{Na} and I_K) drawn proportional to $E_m - E_{Na}$ and $E_m - E_K$, respectively.

It is known that the e.p.c. is due to Na^+ and K^+, but probably not to Cl^- ion movements (Takeuchi & Takeuchi, 1960). Thus, if $E_m = E_K$, the e.p.c. is mainly due to Na^+ movements. Similarly, if $E_m = E_{Na}$, the e.p.c. is mainly due to K^+ movements. According to the view of Maeno (1966)

and Gage & Armstrong (1968), when $E_m = E_K$, the time course of the e.p.c. should be very similar to the time course of ΔG_{Na}. If the latter only is altered by procaine, its effect should be most pronounced when $E_m = E_K$, and absent when $E_m = E_{Na}$. If it is assumed that E_K and E_{Na} are -100 and $+50$ mV, respectively, that the rise and half-decline time of ΔG_K are 0·7 and 0·8 msec, and that the time course of ΔG_{Na} is very similar to the time course of the e.p.c. at the resting potential and in presence of pro caine (rise time 0·5 msec, half decline time of the fast and slow component 0·5 msec and 5 msec, respectively), changes in membrane potential should have a predictable effect on shape and relative amplitude of the e.p.c. Thus, according to the view of Maeno (1966) and Gage & Armstrong (1968), the e.p.c. in presence of procaine should be diphasic or triphasic at membrane potentials between about -80 and $+20$ mV, and should not show a clear 'reversal potential (Fatt & Katz, 1951; see also Bennett, Freeman & Thaddeus (1966); Fig. 2*A*). The difference in the time courses of the fast and slow components should be most pronounced at a membrane potential of about -100 mV, and should be less pronounced when the muscle fibre is hyperpolarized (Fig. 3*A*). Finally, the relation between membrane potential and e.p.c. amplitudes should have three linear components and show three peaks at a membrane potential of about -40 mV (Figs. 2*A* and 4*A*). These principal statements are not invalidated by the uncertainties about the value of E_K in glycerol-treated muscle and of $\Delta G_K/\Delta G_{Na}$ in presence of procaine. A less negative E_K and a smaller $\Delta G_K/\Delta G_{Na}$ will cause the constructed triphasic e.p.c. to appear at less negative levels of membrane potential.

The predicted changes in the time course and amplitude of the e.p.c. do not agree well with experimental findings. It appears that the e.p.c. has a true reversal potential between about -5 and $+10$ mV (Fig. 2*B*, *C*, *D*). At a membrane potential of about -60 to -80 mV, the falling phase of the e.p.c. has a fast and a slow component with half-times of about 0·5 and 5–20 msec, respectively. During hyperpolarization the half-decline time of the slow component is very much lengthened. On the other hand, when the membrane potential is inverted, the two components of the e.p.c. are much less distinct, and the e.p.c. appears as a single-component, monophasic event (Figs. 2*B*, 3*B*; see also Gage & Armstrong, 1968). A similar effect of procaine was also observed in muscle fibres with an intact transverse tubular system. In this experimental condition the e.p.c. was mainly recorded at the resting membrane potential and during hyperpolarization of the muscle fibre (Kordaš, 1969*b*).

The effect of procaine on maximum amplitude of the e.p.c. recorded at different membrane potentials. When the muscle fibre is hyperpolarized, the amplitude of the e.p.c. increases. The membrane potential – e.p.c. ampli-

tude relation has been reported to be linear (Takeuchi & Takeuchi, 1959, 1960) or, if recorded over a wide range of membrane potentials, slightly curved (Kordaš, 1969*a*). In the presence of $1{\cdot}8 \times 10^{-4}$ M procaine this non-linearity is more pronounced (Fig. 4*B*). It is known that procaine and some related drugs have curare-like properties (Furukawa, 1957; del Castillo & Katz, 1957; Steinbach, 1968*a*, *b*). The non-linearity of the membrane potential – e.p.c. amplitude relation may be explained by assuming that,

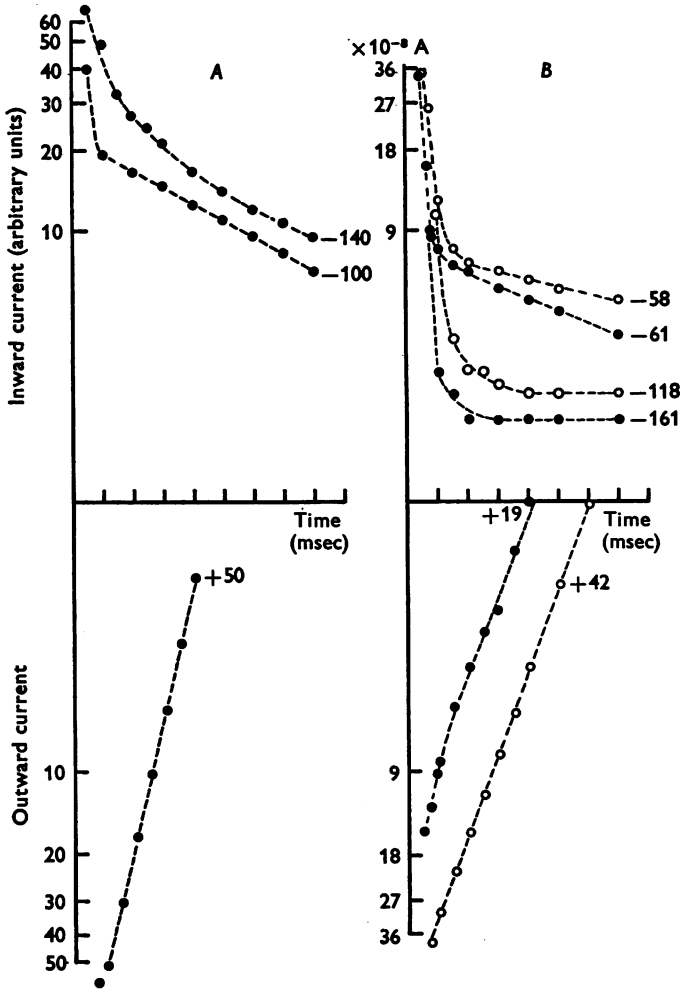

Fig. 3. The effect of membrane potential on the time course of the falling phase of the e.p.c. as predicted (*A*), and as found in two different muscle fibres (*B*). To show the exponential decay of the two components, the amplitude of the e.p.c. is shown on logarithmic scale. Note that the time course of the slow component is profoundly affected by membrane potential.

during hyperpolarization, procaine becomes more effectively attached to the receptor. Thus, it could be that, with sufficient procaine concentration, the increase in the curare-like property of the drug would just balance the usual effect of hyperpolarization on e.p.c. amplitude. To test this hypothesis, various procaine concentrations were used. At $3{\cdot}6 \times 10^{-4}$ M, the

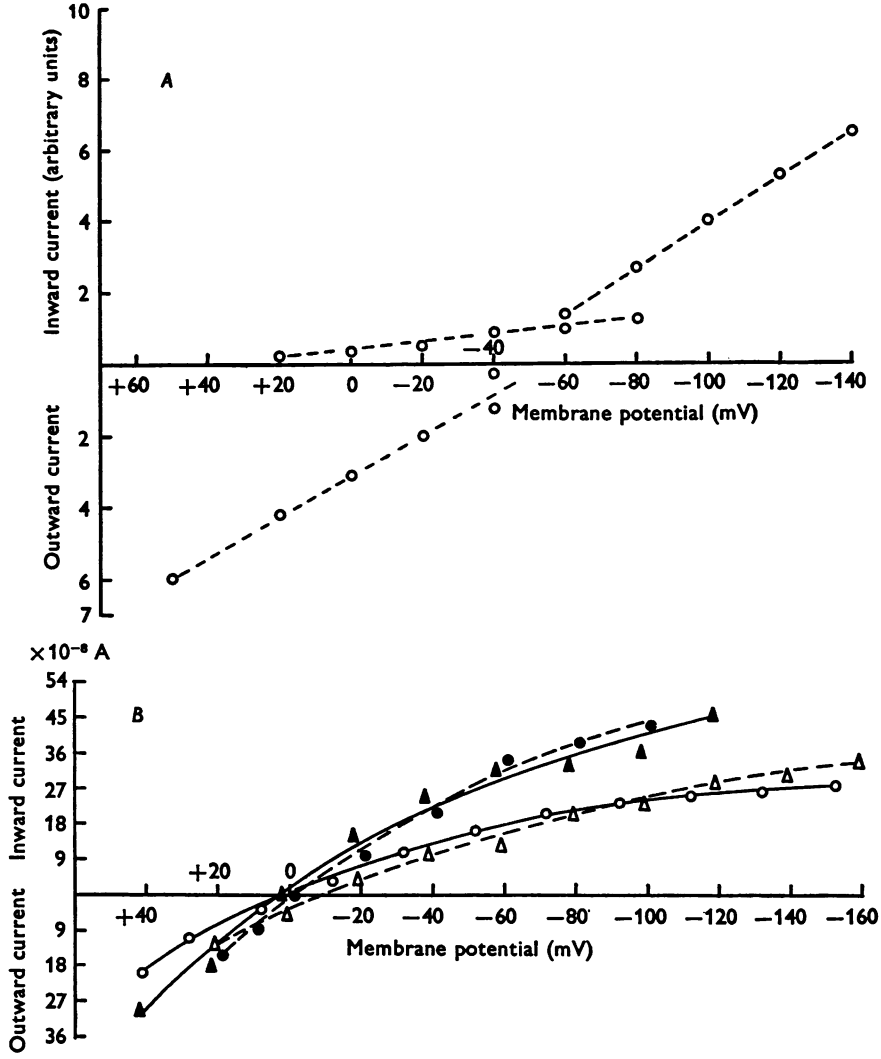

Fig. 4 A and B. For legend see facing page.

non-linearity was not much more pronounced than with $1{\cdot}8 \times 10^{-4}$ M. At a procaine concentration of $7{\cdot}2 \times 10^{-4}$ M the small e.p.c. amplitude made measurements difficult. To counteract this prostigmine was added

(3×10^{-6} M, final concentration). In this condition the relation between the e.p.c. amplitude and membrane potential was highly non-linear, and there was no increase of e.p.c. amplitude during hyperpolarization (Fig. 4*C*). In these experiments the effect of membrane potential on the time course of the e.p.c. was very similar to what was observed at lower concentrations of procaine and in the absence of anticholinesterase (Fig. 2*D*).

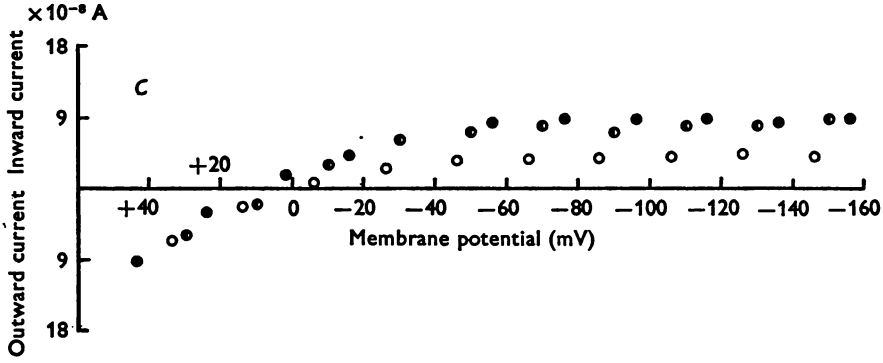

Fig. 4. The effect of membrane potential on maximum amplitude of the e.p.c. as predicted (*A*), and as found in different muscle fibres in presence of $1{\cdot}8 \times 10^{-4}$ M procaine (*B*), and in presence of $7{\cdot}2 \times 10^{-4}$ M procaine and 3×10^{-6} M prostigmine (*C*). In *A* the relation was constructed by using data from Fig. 2*A*. Note that the three components of the constructed e.p.c., indicated by the dashed line, are linearly related to the membrane potential. In *B* and *C* each set of symbols means a set of measurements in an individual muscle fibre.

In glycerol-treated muscle, after the e.p.c. had been recorded over a wide range of membrane potentials, the level of the membrane potential usually falls by 10–15 mV, when it is finally 'unclamped'. The reason for this is not clear. However, the set of measurements can be repeated with similar results several times, though each time starting from a lower resting potential and smaller e.p.c. amplitude.

The effect of procaine on the initial rate of rise of the e.p.c. To assess the curare-like property of procaine, the initial rate of rise of the e.p.c. was studied before and after adding procaine to the bathing fluid. It has been reported by Steinbach (1968*a*) that xylocaine reduces the amplitude and shortens the rise time of the e.p.p., but does not greatly change the initial rate of rise of the e.p.p. In the present experiments similar observations were made, except that the rate of rise was also reduced significantly. Thus, in a typical experiment procaine $1{\cdot}8 \times 10^{-4}$ M depressed the amplitude and the rise time of the e.p.c. by about 60 and 50%, respectively, while the initial rate of rise of the e.p.c. was reduced from $7{\cdot}4 \times 10^{-7}$ A/

msec to $3{\cdot}1 \times 10^{-7}$ A/msec. The reason for this slight discrepancy with Steinbach's (1968*a*) results may be different experimental conditions, e.g. the absence of curare in the present experiments.

DISCUSSION

The present results show that in the presence of procaine the e.p.c. shows a fast and a slow component, both of which are dependent on membrane potential. When intracellular negativity is increased, the amplitude of the fast component increases non-linearly, whereas the amplitude of the slow component is depressed and its half-decline time lengthened. The present results cannot be explained on the assumption that procaine affects simply the time course of ΔG_{Na}.

It has been reported that procaine behaves as a curare-like agent (Furukawa, 1957; del Castillo & Katz, 1957). Further, it has been suggested that, during neuromuscular transmission, procaine combines with the receptor-mediator complex. This three-component complex has a low 'membrane-shunting' activity, but considerable stability (Steinbach, 1968*a*, *b*). The present results can be reconciled with this hypothesis if the effects of procaine vary with the membrane potential, that is if during hyperpolarization of the muscle fibre the curare-like fixation of procaine is potentiated, especially during the later part of the e.p.c. The resulting increased stability of the procaine-receptor-mediator complex could explain the change in time course as well as size of the e.p.c. when the membrane potential is altered.

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REFERENCES

BENNETT, M. V. L., FREEMAN, A. R. & THADDEUS, P. (1966). 'Reversal' of postsynaptic potentials in non-isopotential systems. 10th Annual Meeting Biophysical Society, 122.

DEL CASTILLO, J. & KATZ, B. (1957). A study of curare action with an electrical micro-method. *Proc. R. Soc.* B **146**, 339–356.

ECCLES, J. C., KATZ, B. & KUFFLER, S. W. (1941). Nature of the 'endplate potential' in curarized muscle. *J. Neurophysiol.* **4**, 362–387.

ECCLES, J. C., KATZ, B. & KUFFLER, S. W. (1942). Effect of eserine on neuromuscular transmission. *J. Neurophysiol.* **5**, 211–230.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol.* **115**, 320–370.

FURUKAWA, T. (1957). Properties of the procaine end-plate potential. *Jap. J. Physiol.* **7**, 199–212.

GAGE, P. W. & ARMSTRONG, C. M. (1968). Miniature end-plate currents in voltage clamped muscle fibres. *Nature, Lond.* **218**, 363–365.

GAGE, P. W. & EISENBERG, R. S. (1969*a*). Action potentials, afterpotentials, and excitation–contraction coupling in frog sartorius fibres without transverse tubules. *J. gen. Physiol.* **53**, 298–310.

GAGE, P. W. & EISENBERG, R. S. (1969*b*). Capacitance of the surface and transverse tubular membrane of frog sartorius muscle fibres. *J. gen. Physiol.* **53**, 265–278.

HIRST, G. D. S. & WOOD, D. R. (1969). The action of procaine on transmission at the mammalian neuromuscular junction. *Br. J. Pharmac. Chemother.* **35**, 353–354*P*.

HOWELL, J. N. (1969). A lesion of the transverse tubules of skeletal muscle. *J. Physiol.* **201**, 515–533.

HOWELL, J. N. & JENDEN, D. J. (1967). T-tubules of skeletal muscle: morphological alterations which interrupt excitation–contraction coupling. *Fedn Proc.* **26**, 553.

KATZ, B. (1942). Impedance changes in frog's muscle associated with electrotonic and 'endplate' potentials. *J. Neurophysiol.* **5**, 169–184.

KATZ, B. (1948). The electrical properties of the muscle fibre membrane. *Proc. R. Soc.* B **135**, 506–534.

KORDAŠ, M. (1968). The effect of atropine and curarine on the time course of the end-plate potential in frog sartorius muscle. *Int. J. Neuropharmac.* **7**, 523–530.

KORDAŠ, M. (1969*a*). The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. *J. Physiol.* **204**, 493–502.

KORDAŠ, M. (1969*b*). Properties of the procaine end-plate current. *Jugoslav. physiol. pharmacol. acta* **5**, 359–361.

KUFFLER, S. W. (1942*a*). Electric potential changes at an isolated nerve–muscle junction. *J. Neurophysiol.* **5**, 18–26.

KUFFLER, S. W. (1942*b*). Further study in an isolated nerve–muscle fibre preparation. *J. Neurophysiol.* **5**, 309–322.

MAENO, TAKASHI (1966). Analysis of sodium and potassium conductances in the procaine end-plate potential. *J. Physiol.* **183**, 592–606.

STEINBACH, A. B. (1967). Unusual end-plate potentials which reflect the complexity of muscle structure. *Nature, Lond.* **216**, 1331–1333.

STEINBACH, A. B. (1968*a*). Alteration by xylocaine (lidocaine) and its derivatives of the time course of the end-plate potential. *J. gen. Physiol.* **52**, 144–161.

STEINBACH, A. B. (1968*b*). A kinetic model for the action of xylocaine on receptors for acetylcholine. *J. gen. Physiol.* **52**, 162–180.

TAKEUCHI, A. & TAKEUCHI, N. (1959). Active phase of frog's end-plate potential. *J. Neurophysiol.* **22**, 395–411.

TAKEUCHI, A. & TAKEUCHI, N. (1960). On the permeability of end-plate membrane during the action of transmitter. *J. Physiol.* **154**, 52–67.