J. Physiol. (1972), **224**, *pp.* 333–348
With 11 *text-figures*
Printed in Great Britain

AN ATTEMPT AT AN ANALYSIS OF THE FACTORS DETERMINING THE TIME COURSE OF THE END-PLATE CURRENT

II. TEMPERATURE

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(*Received 10 January 1972*)

SUMMARY

1. An attempt was made to analyse further the factors which might determine the time course of the falling phase of the end-plate current.

2. The end-plate current was measured by 'clamping' the membrane potential during neuromuscular transmission. The falling phase of the end-plate current had an exponential and a non-exponential portion.

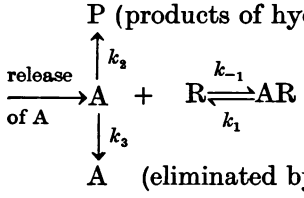
3. The exponential portion of the falling phase of the end-plate current was found to be strongly affected by changes in the membrane potential and by relatively small changes in temperature.

4. It is suggested that the two rate constants which determine the dissociation of the receptor-mediator complex are affected by both temperature and the membrane potential. Under certain experimental conditions these two rate constants alone seem to determine the time course of the exponential portion of the falling phase of the end-plate current.

INTRODUCTION

By clamping the membrane potential during neuromuscular transmission, the time course of the conductance change of the junctional membrane can be measured by recording the end-plate current (e.p.c., Takeuchi & Takeuchi, 1959). Although direct evidence is lacking, it will be assumed for simplicity (see e.g. Steinbach, 1968*a*, *b*; Werman, 1969) that the law of mass action can be applied to the reaction between the released transmitter (A) and receptors in the junctional membrane (R), and that the conductance increase (and so the e.p.c.) is linearly related to the concentration of receptor-mediator complex (RA). It was found (Kordaš, 1972) that the falling phase of the e.p.c. consists of a non-exponential and an exponential portion, the duration of both of which varies under different experimental

conditions. It is probable that during the e.p.c., the following reactions take place:



It is clear that, in the light of the above scheme, the rate of RA dissociation is determined by k_{-1}, k_1, k_2 and k_3 (see Kordaš, 1972 for details). If k_1/k_{-1} is small and $k_1 \ll k_2+k_3$, the time course of RA dissociation and, consequently, of the falling phase of the e.p.c. will be exponential. The finding of Takeuchi & Takeuchi (1959) that the Q_{10} of the falling phase of the e.p.c. is about 2 suggests that the underlying mechanism is a chemical process, possibly the reaction $RA \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} R+A$, where k_1 is essentially more depressed by a temperature decrease than is k_{-1}.

Suppose that at room temperature $k_1/k_{-1} = 10^{-1}$ mol.l^{-1} and $k_1 = (k_2+k_3)/20$, but that at a lower temperature $k_1/k_{-1} = 5\times10^{-2}$ mol.l^{-1} and $k_1 = (k_2+k_3)/40$ (see Kordaš, 1972, Figs. 8*B* and 9*B*). Under the latter experimental condition k_1 may be the rate limiting step for the dissociation of RA. If this is so, a decrease in k_2, such as was observed after cholinesterase inhibition, would not prolong the falling phase of the e.p.c. as it usually did at room temperature. On the other hand, it has been found that the falling phase of the e.p.c. is affected not only by changes in the temperature, but also by changes in the membrane potential (Kordaš, 1969): the more negative the membrane potential, the slower the decay of the e.p.c. In the light of the presented kinetic scheme it can be speculated that the more negative the membrane potential, the lower the value of k_1/k_{-1}, as a result of which the decay of the e.p.c. is slowed down.

A similar phenomenon was also observed in muscles treated with procaine and procaine-like drugs, which are believed to combine with RA and to increase its 'stability' (Steinbach, 1968*a*, *b*) which in turn gives rise to the 'slow' component of the e.p.c. The half-time of the latter has been found to increase on hyperpolarization (Kordaš, 1970); in the light of the proposed kinetic scheme the increase in the half-time may also be due to a decrease in the value of k_1/k_{-1}.

According to these speculations, the effect of the membrane potential on the time course of the e.p.c. should be intensified by a decrease in temperature.

In an attempt to test these speculations, two groups of experiments were

performed. In the first group, the effect of different anticholinesterases on the time course of the e.p.c. was studied at different temperatures. In the second group, the effect of the membrane potential on the time course of the e.p.c., and on the e.p.c. in the presence of procaine was studied at different temperatures.

METHODS

The experiments were carried out on preparations of sciatic nerve-sartorius muscle of the frog (*Rana esculenta*). E.p.c. was recorded by a technique similar to that of Takeuchi & Takeuchi (1959) as described previously (Kordaš, 1968).

In the first group of experiments the effect of anticholinesterases at different temperatures was studied. The muscle was curarized by 3×10^{-6} to 5×10^{-6} M tubocurarine (Nutritional Biochemical Corp., Cleveland; Mann Research Lab., New York) in Ringer solution of the following composition: NaCl 116 mM, KCl 2 mM, $CaCl_2$ 1·8 mM, Tris 4 mM, HCl 3·3 mM, pH 7·4. Junctional cholinesterase was progressively inhibited either by prostig nine (S. A. F. Hoffmann, La Roche & Co, Basel) in final concentrations from 10^{-6} to 10^{-5} M, or by methanesulphonylfluoride (MSF; Eastman Organic Chemicals, New York) in the final concentration of 10^{-3} M. Since MSF is an irreversible cholinesterase inhibitor (Alexander, Wilson & Kitz, 1963) it was expected that the degree of junctional cholinesterase inhibition was dependent on the time of the incubation of the muscle with the drug. In this group of experiments the time course of the e.p.c. in an end-plate was recorded at temperatures, ranging from 20 to 24° C once with junctional cholinesterase intact and then during progressive inhibition. Subsequently, the experiment was repeated in another muscle at temperatures ranging from 14 to 15° C. In this group of experiments also the effect of eserine (British Drug Houses, Poole), final concentration from 3×10^{-6} to 10^{-4} M, on neuromuscular transmission was studied.

The experiments of the second group were performed on glycerol-treated muscles in order to avoid contraction of the muscle fibre during severe depolarization (Howell & Jenden, 1967; Gage & Eisenberg, 1969*a*, *b*; Howell, 1969; see also Kordaš, 1969, 1970). The e.p.c. in an end-plate was recorded for a wide range of membrane potentials at temperatures ranging from 20 to 23° C. Following this, the temperature of the bath was lowered to the range of 15–17° C. At these temperatures the amplitude of the e.p.c. is significantly depressed and its time course significantly lengthened, but a reasonably accurate analysis of the e.p.c. is still possible. Subsequently the effect of the membrane potential on the time course of the e.p.c. was again measured in the same end-plate. In another series of experiments of this group procaine was added to the bath in final concentrations from 10^{-4} to 3×10^{-4} M and the effect of membrane potential on the e.p.c. at different temperatures was studied as described above.

An attempt was made to study the time course of the e.p.c. within a wider temperature range. However, at temperatures below about 15° C the e.p.c. was depressed to such an extent that no accurate measurement of its time course was possible. In the presence of procaine, the situation was even more unfavourable because the e.p.c., particularly its slow component, is small even at room temperature. Therefore, in these experiments an analysis of the time course of the e.p.c. was not feasible at temperatures below about 18° C.

RESULTS

The time course of MSF action on the e.p.c. As shown in Fig. 1 *A*, *B* and as judged by the changes in the time course and by the amplitude of the e.p.c., junctional cholinesterase inhibition depends on the time of incubation of the muscle with the drug. The reason for the late decrease of the e.p.c. amplitude (Fig. 1 *B*) is not clear, but may be related to the changes in R. It should be pointed out, however, that the recording of the e.p.c. over such a long period could be made only in one experiment. In almost all the

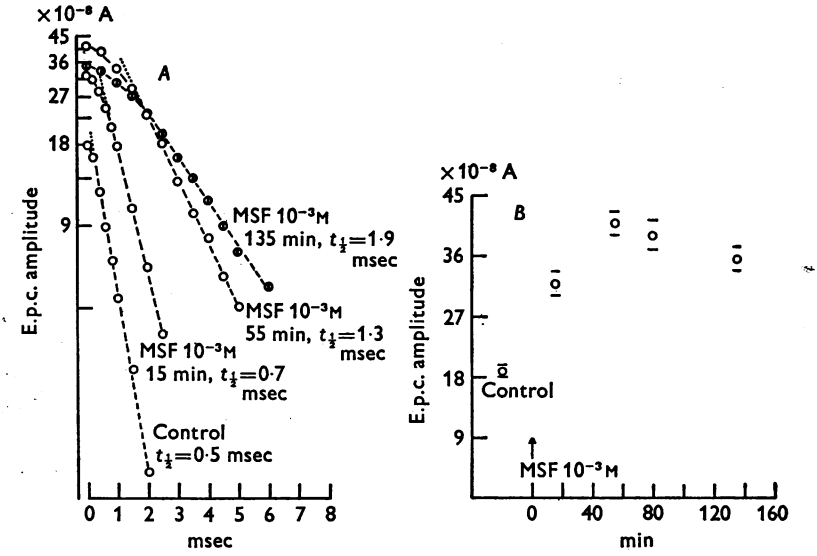


Fig. 1. The effect of MSF 10^{-3} M on the e.p.c. as a function of time. Note the lengthening of both the non-exponential and the exponential portion of the falling phase of the e.p.c. (*A*), and the increase of its amplitude (*B*; mean ± S.D.). All measurements from one end-plate.

other experiments the muscle began to contract on stimulation if the incubation time was longer than about 1 hr. It was not possible to overcome this difficulty by increasing the concentration of curarine because the control e.p.c., recorded during the early part of the experiment, became too small to be accurately measured. It was for these reasons that in the experiments described below the effect of MSF on the e.p.c. was studied at around the 15th and around the 60th minute of incubation.

The effect of MSF on the time course of the e.p.c. recorded at different temperatures is shown in Figs. 2 and 3. The time course of the e.p.c. is faster at about 24° C than at about 15° C. But at either temperature MSF increases

both the rise time and the amplitude of the e.p.c. by roughly the same factor, suggesting that at both temperatures cholinesterase inhibition is roughly equal. The exponential portion of the falling phase of the e.p.c.,

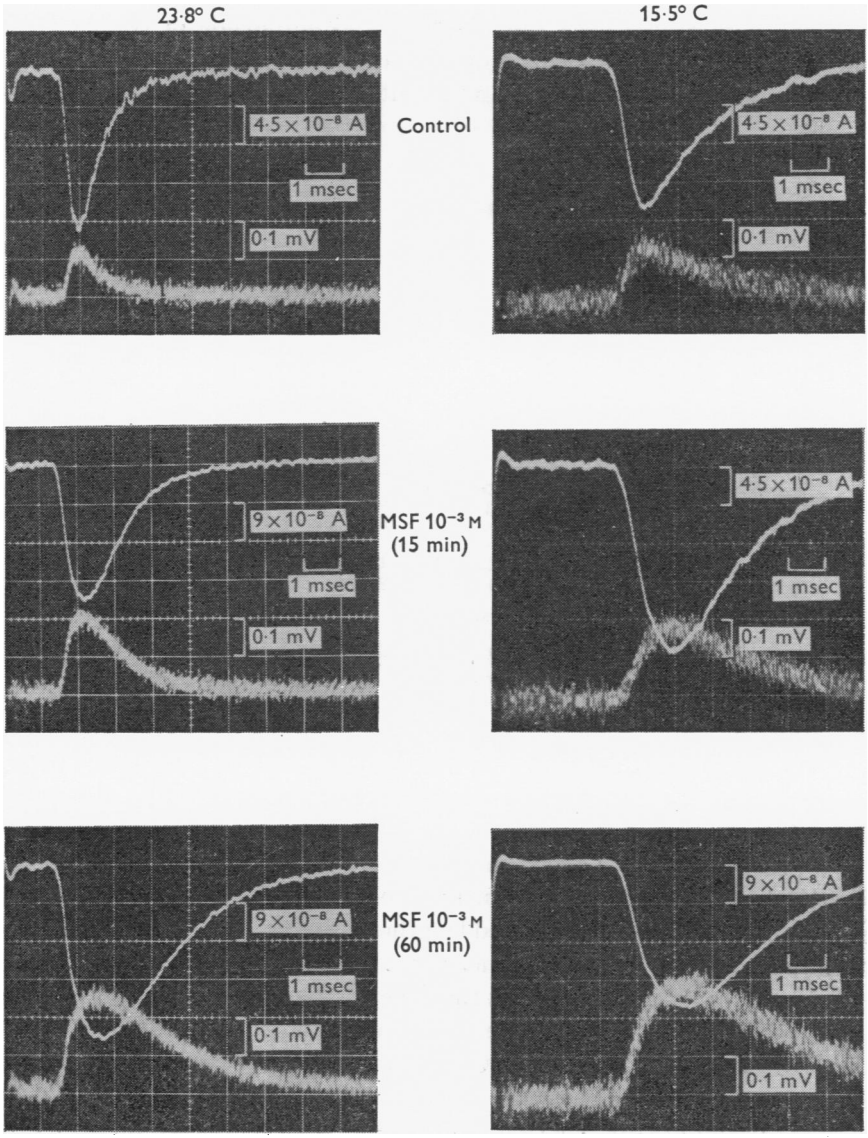


Fig. 2. The effect of MSF 10^{-3} M on the e.p.c. (upper beam, inward current downward), recorded at 23·8 and at 15·5° C. On the lower beam the reduced membrane potential during the flow of the clamping current, the e.p.c., is recorded. Calibrations as shown. Records from two different end-plates.

however, behaves differently: at about 24° C its half-time is lengthened, whereas at about 15° C its half-time is not affected by MSF action.

The effect of prostigmine on the time course of the e.p.c. recorded at different temperatures is shown in Fig. 4. It is clear that at either temperature prostigmine increases both the rise time and amplitude of the e.p.c. by roughly the same factor, suggesting that at both temperatures cholinesterase inhibition is roughly equal. At about 20° C prostigmine both at the

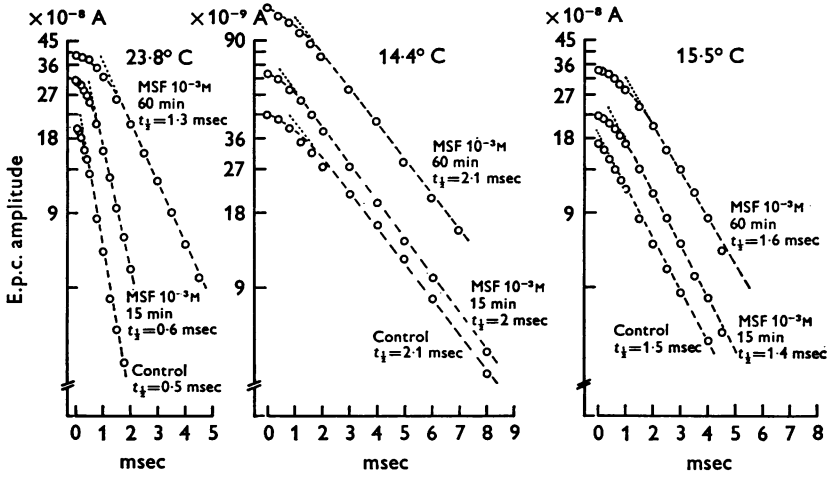


Fig. 3. The effect of MSF 10^{-3} M on the falling phase of the e.p.c. recorded at 23·8, 14·4 and 15·5° C. Note that at a decreased temperature there is no change in the half-time while the amplitude of the e.p.c. is strikingly increased.

concentration of 3×10^{-6} M and at 10^{-5} M effects a lengthening of the half-time of the exponential portion of the falling phase of the e.p.c. At about 15° C prostigmine at the concentration of 3×10^{-6} M affects a very small lengthening of the half-time. Contrary to this prostigmine, at the concentration of 10^{-5} M, effects a marked lengthening of the half-time, comparable to that effected by prostigmine at about 20° C.

Takeuchi (1958) reported that the effect of eserine on the amplitude and the time course of the end-plate potential was much more pronounced at about 20° C than it was at 15° C. His finding seems to disagree with the data presented here. The reason for this disagreement is not known. Eccles & MacFarlane (1949) reported that eserine was relatively ineffective in increasing the amplitude of the end-plate potential. In order to check this claim, eserine was tested in a few experiments. It was found that its effect was much weaker and much more variable than that of prostigmine or of MSF. Therefore, no further experiments with eserine were performed.

The time course of the falling phase of the e.p.c. in glycerol-treated muscle is similar to that recorded in curarized muscles and in muscles treated with sodium-deficient solution (Fig. 5; see also Kordaš, 1972). The falling phase has a short non-exponential portion, while the rest of the e.p.c. decays exponentially. After procaine is added to the bath, the falling phase of the

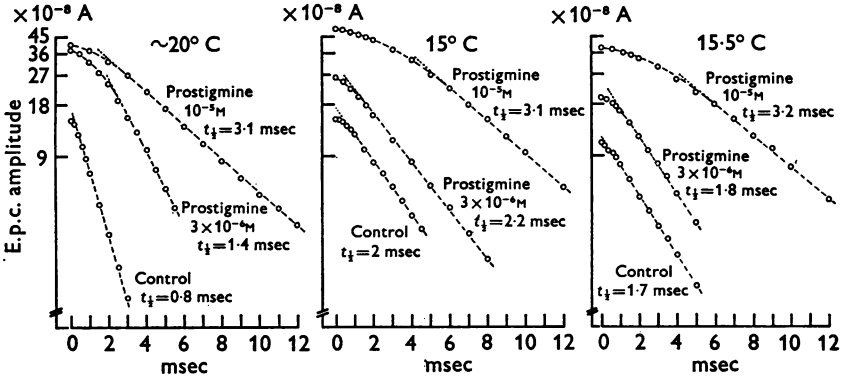


Fig. 4. The effect of prostigmine 3×10^{-6} M and 10^{-5} M on the falling phase of the e.p.c. recorded at about 20, at 15 and 15·5° C. Note that the effect of prostigmine is much more potent than the maximum effect of MSF (cf. Fig. 1).

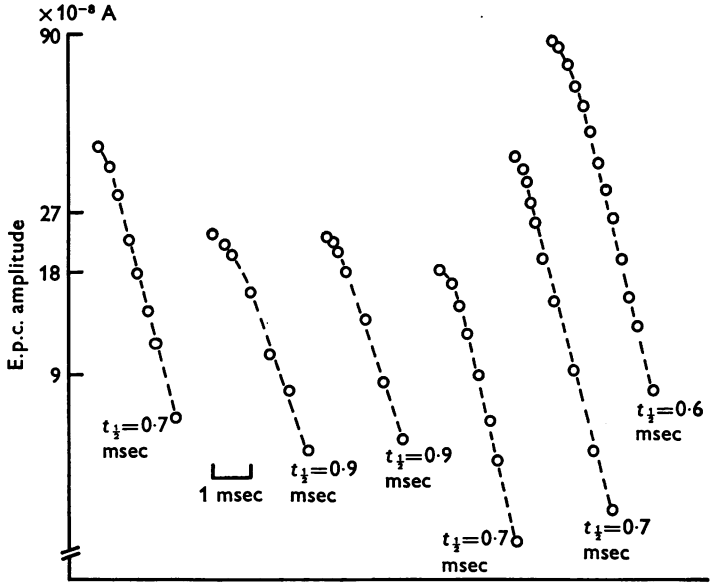


Fig. 5. The falling phase of the e.p.c. recorded in six different end-plates of glycerol-treated muscle.

e.p.c. shows an early fast and a late slow component, the latter decaying exponentially (Fig. 6). The results reported here are similar to those reported earlier (Maeno, 1966; Kordaš, 1969, 1970; Maeno, Edwards & Hashimura, 1971).

The effect of the membrane potential on the falling phase of the e.p.c., recorded at different temperatures. The half-time of the exponential portion is dependent on the membrane potential and also on the temperature of the

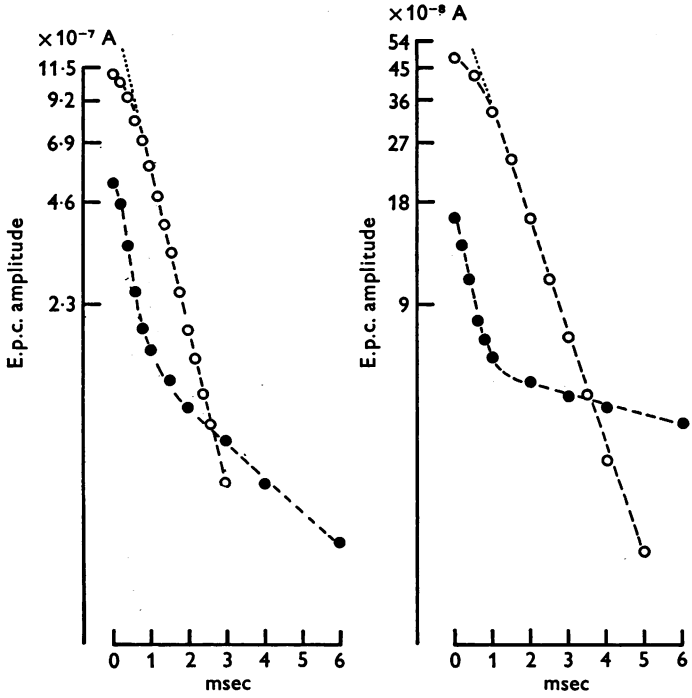


Fig. 6. The falling phase of the e.p.c. recorded in two different end-plates of glycerol-treated muscle before (open circles) and after (filled circles) treatment with procaine.

bath (Figs. 7, 8 and 9). If at 21·1° C the membrane potential is shifted from about +60 mV to about −140 mV, the half-time increases from 0·4 to 0·9 msec. At 16·6° C, however, a similar change in the membrane potential increases the half-time from 0·55 to 2·6 msec, and the amplitude of the e.p.c. is smaller and the rise time longer than at 21·1° C. The equilibrium potential of the e.p.c. does not seem to be affected by temperature (Fig. 8). The effect of the membrane potential on the maximum amplitude of the e.p.c. is slightly non-linear (cf. Kordaš, 1969; Maeno *et al.* 1971), at either temperature.

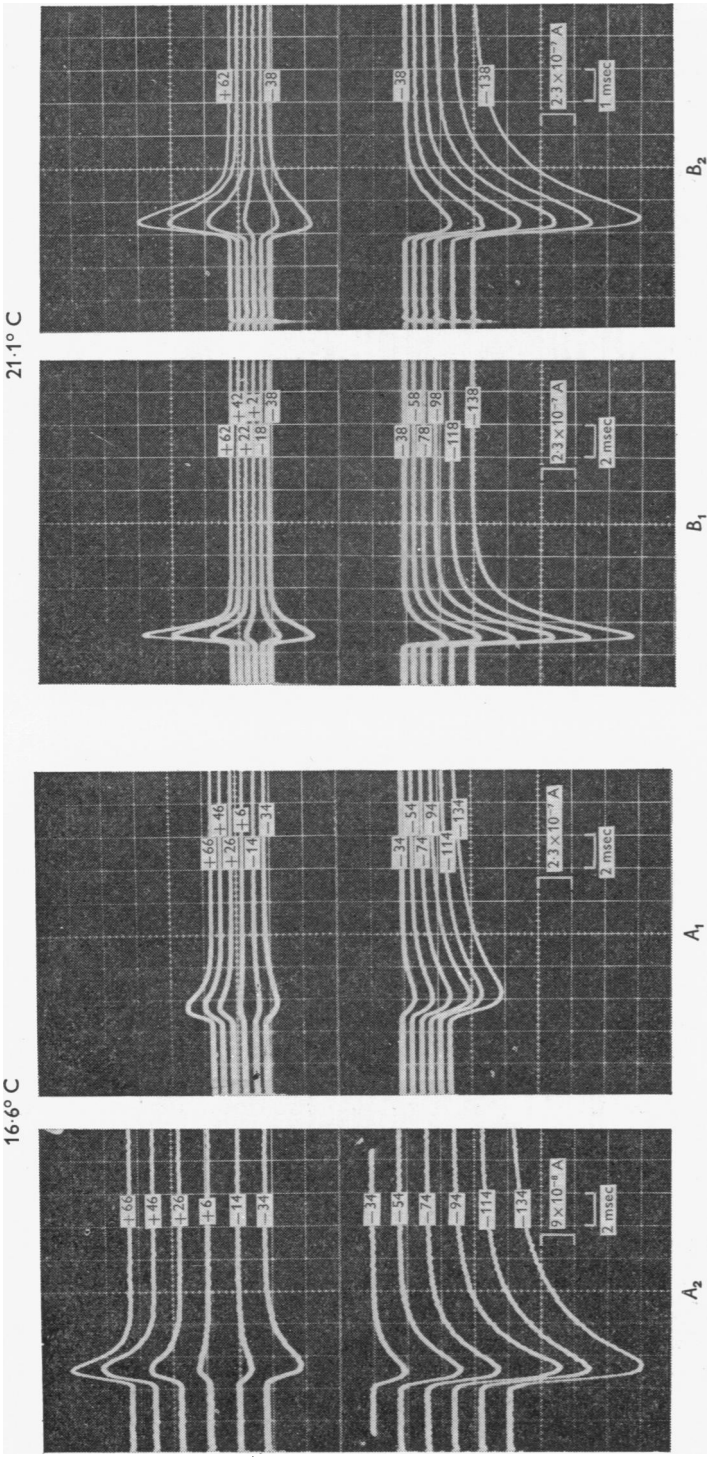


Fig. 7. The effect of the membrane potential on the e.p.c. recorded at an end-plate of glycerol-treated muscle at 16·6° C (A_1) and at 21·1° C (B_1). For the sake of clarity the e.p.c.s are shown also at a greater sensitivity (A_2) or faster time base (B_2). Numbers beside the traces indicate membrane potentials. Inward current recorded downward.

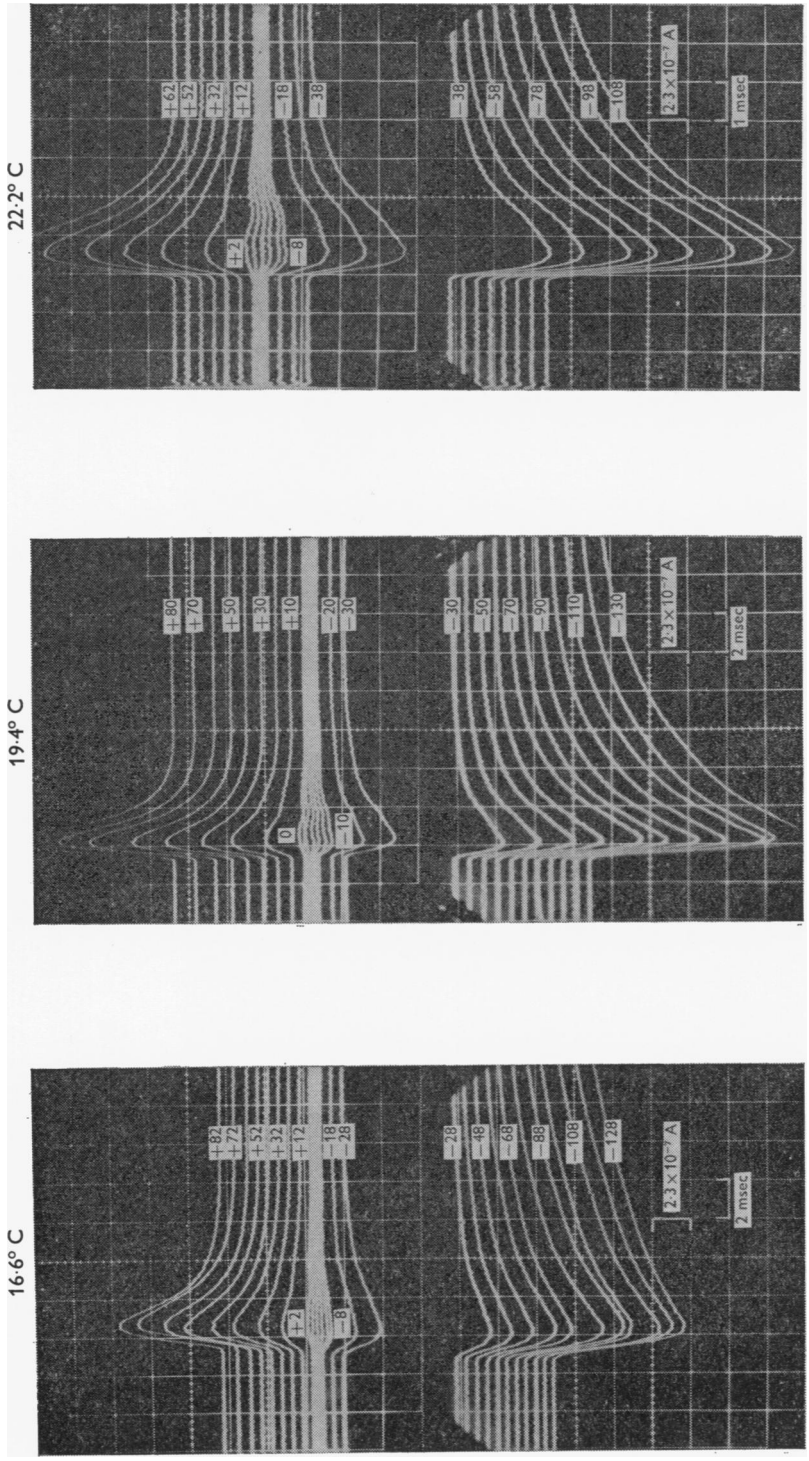


Fig. 8. The effect of the membrane potential on the e.p.c., recorded at an end-plate at 22·2, 19·4 and 16·6° C. Note that the equilibrium potential of the e.p.c. is not affected by a change in temperature. Numbers beside the traces indicate membrane potentials. Inward current recorded downward.

The effect of the membrane potential on the falling phase of the e.p.c., recorded at different temperatures in presence of procaine. As already reported (Kordaš, 1970), the time course of the e.p.c. in the presence of procaine is critically dependent on the membrane potential. When the

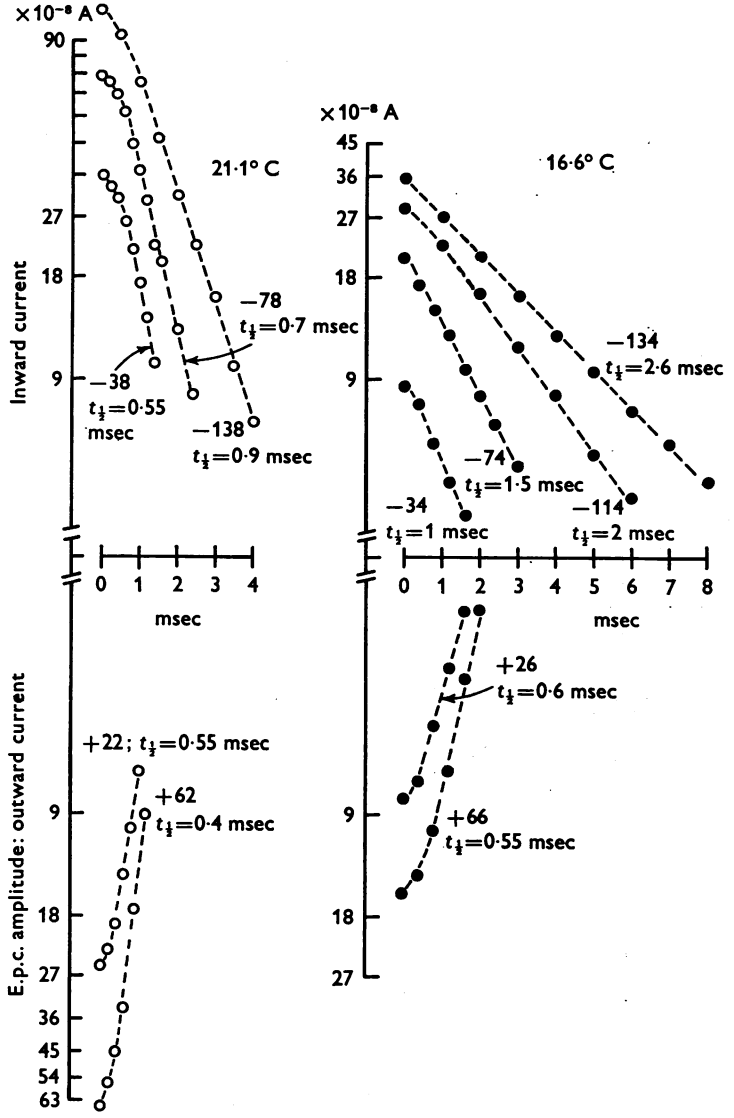


Fig. 9. The effect of the membrane potential on the falling phase of the e.p.c. recorded at 21·1 and 16·6° C. Numbers beside curves indicate membrane potentials and the half-times of the exponential portion of the falling phase of the e.p.c.

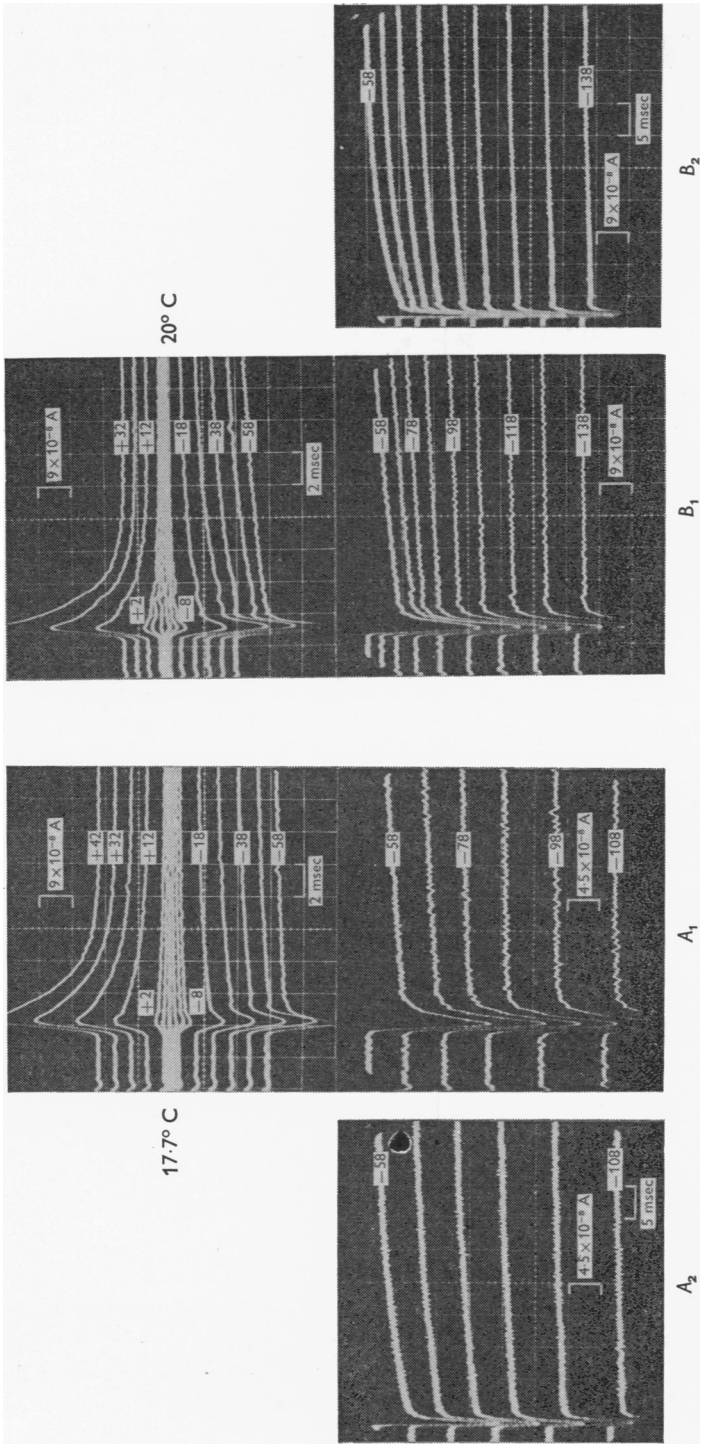


Fig. 10. The effect of the membrane potential on the e.p.c. in the presence of procaine at 17·7° C (A_1) and 20° C (B_1). For the sake of clarity, the e.p.c.s were also recorded at a slower time base (A_2 and B_2). Note that the equilibrium potential of the e.p.c. is not affected by a change in temperature. Numbers beside the traces indicate membrane potentials. Inward current recorded downward.

latter is electropositive, the e.p.c. decays exponentially. When the membrane potential is electronegative, the e.p.c. decays showing a fast and a slow component, the latter being exponential. If at 20·5° C the membrane

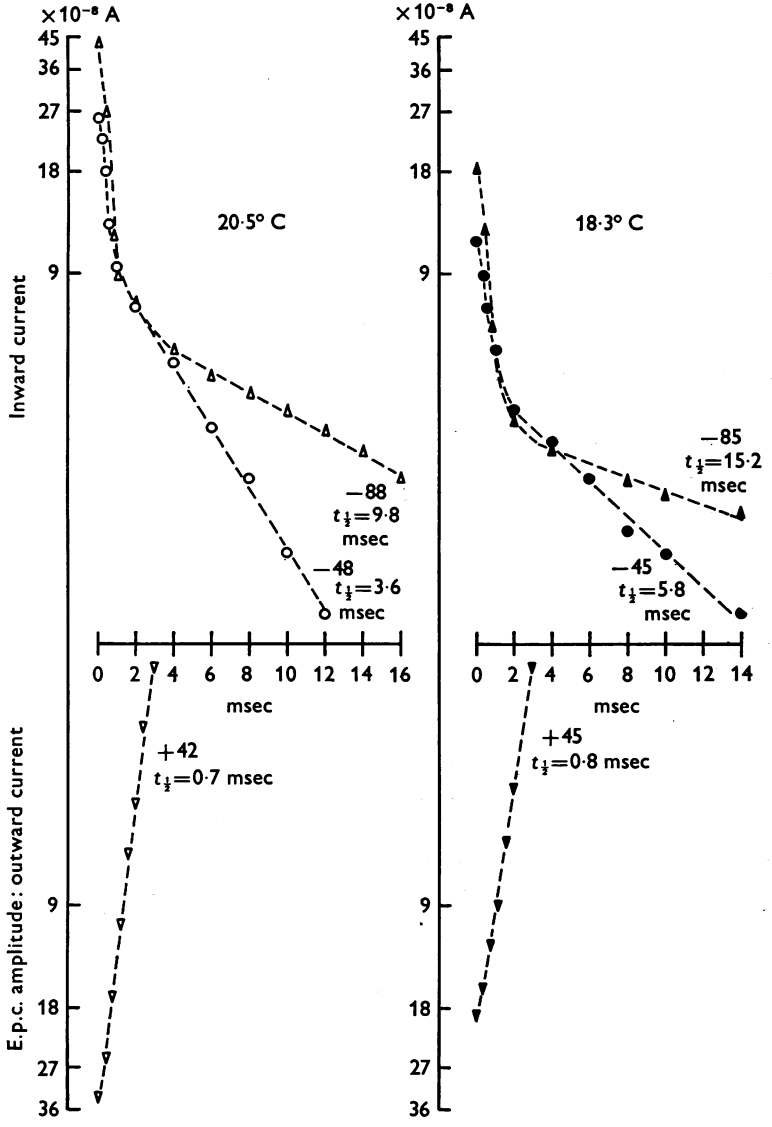


Fig. 11. The effect of the membrane potential on the falling phase of the e.p.c. in the presence of procaine recorded at 20·5 and at 18·3° C. Numbers beside the curves indicate membrane potentials and the half-times of the exponential portion of the falling phase of the e.p.c.

potential is shifted from about +40 to about −90 mV, the half-time of the exponential portion of the e.p.c. increases from 0·7 to 10 msec. At 18·3° C, however, a similar change in membrane potential increases the half-time from 0·8 to 15 msec (Figs. 10 and 11). The effect of the membrane potential on the maximum amplitude of the e.p.c. is markedly non-linear, as reported previously (Kordaš, 1970; Maeno *et al.* 1971). The equilibrium potential of the e.p.c. does not seem to be affected by temperature.

DISCUSSION

The results reported here confirm the earlier finding (Eccles, Katz & Kuffler, 1941, 1942; Takeuchi, 1958; Takeuchi & Takeuchi, 1959; see also Kordaš, 1972) that the time course of the e.p.c. depends, among other factors, on junctional cholinesterase activity and on the temperature of the bath. The falling phase of the e.p.c. was found to consist of a non-exponential and an exponential portion, the duration of both of which varies under different experimental conditions.

An attempt was made to explain the present results in the light of the view that there exist independent conductances for Na^+ and K^+ ions in the end-plate membrane (Maeno, 1966; Gage & Armstrong, 1968; Maeno *et al.* 1971) but it was found that there were several objections to this view. If the decay of the two conductances is exponential and the two half-times different (cf. Gage & Armstrong, 1968), the falling phase of the resulting e.p.c., the sum of the Na^+ – and K^+ – current, will not be exponential. Details regarding this problem were dealt with elsewhere (Kordaš, 1969, 1970).

The results can be discussed in relation to the mechanism of the exponential portion of the falling phase of the e.p.c. During this phase of the e.p.c., RA is assumed to dissociate exponentially. As has already been shown (Kordaš, 1972) this can happen only if k_1/k_{-1} is small and $k_1 \ll k_2 + k_3$. If k_1 is small enough, it alone will determine the rate of RA dissociation. Thus all other processes with much higher rate constants, and therefore important in determining the time course of the rising phase, can be neglected during the falling phase of the e.p.c.

The experiments with MSF seem to support this assumption. A decrease in the temperature of the bath may depress k_1 to such an extent that the depressed k_1 alone will determine the rate of decay of the e.p.c.

The experiments with MSF differ from those performed with prostigmine. It is known prostigmine in a relatively high concentration (10^{-5} M) has curare-like properties (Eccles & MacFarlane, 1949), that is, it may combine not only with the esteratic site of junctional cholinesterase but also with R. It is not known whether the prostigmine-receptor complex is

not activable, or activable only to a relatively small extent, by the released transmitter. If the latter possibility applies it is possible that the amplitude of the e.p.c. is depressed and that k_1 is decreased or k_{-1} increased, giving rise to a slow time course of the falling phase of the e.p.c.

The present results are compatible with the view that, if the temperature is decreased, the quantum content of the e.p.c. is decreased (Takeuchi, 1958; Hubbard, Jones & Landau, 1971). It is also interesting to discuss present results in the light of the view that the slow time course of the e.p.c. recorded at a decreased temperature is due to either a decreased activity of junctional cholinesterase or to a relatively asynchronous release of quanta (for review see Hubbard, Llinas & Quastel, 1969). It is possible that the increase in the rise time of the e.p.c. recorded at a decreased temperature (Figs. 7 and 8) may, in part at least, be due to an asynchronous release of quanta. However, it is unlikely that the latter mechanism is involved also in the lengthening of the half-time of the falling phase of the e.p.c.; if it were, the falling phase should have been non-exponential. Similarly, it is unlikely that in present experiments junctional cholinesterase was greatly affected by a change in temperature. If this mechanism were involved, anticholinesterases would not have affected the rise time and amplitude of the e.p.c. recorded at a decreased temperature.

The striking temperature dependence of the effect of the membrane potential on the falling phase of the e.p.c. suggests that the underlying mechanism is not a depressed diffusion of mediator out of the synaptic cleft (Takeuchi & Takeuchi, 1959), but probably a chemical one. Thus, it seems more likely that a decrease in temperature or an increase in the negativity of the membrane potential, or both, may depress the value of k_1/k_{-1}, giving rise to a slower decay of the normal e.p.c., or of the 'slow' component of the procaine-e.p.c.

Note added in proof

Data very similar to those described above have been obtained independently by K. I. Magleby & C. F. Stevens (*J. Physiol.* **223**, 151–171).

The author would like to thank Professor A. O. Župančič for his continuous interest in this study, and to Professor B. Katz for the criticism of the manuscript.

This work was supported by the Boris Kidrič Foundation, Ljubljana.

REFERENCES

ALEXANDER, J., WILSON, I. B. & KITZ, R. (1963). The reactivation of acetylcholinesterase after inhibition by methanesulphonic acid esters. *J. biol. Chem.* **238**, 741–744.

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.

ECCLES, J. C. & MACFARLANE, W. V. (1949). Actions of anti-cholinesterases on endplate potential of frog muscle. *J. Neurophysiol.* **12**, 59–80.

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*). Capacitance of the surface and transverse tubular membrane of frog sartorius muscle fibers. *J. gen. Physiol.* **53**, 265–278.

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

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 skeletel muscle: morphological alterations which interrupt excitation-contraction coupling. *Fedn Proc.* **26**, 553.

HUBBARD, J. I., JONES, S. F. & LANDAU, E. M. (1971). The effect of temperature upon transmitter release, facilitation and post-tetanic potentiation. *J. Physiol.* **216**, 591–609.

HUBBARD, J. I., LLINAS, R. & QUASTEL, D. M. J. (1969). *Electrophysiological Analysis of Synaptic Transmission*, pp. 312–315. London: Edward Arnold.

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). 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. (1970). The effect of procaine on neuromuscular transmission. *J. Physiol.* **209**, 689–699.

KORDAŠ, M. (1972). An attempt at an analysis of the factors determining the time course of the end-plate current. I. The effects of prostigmine and of the ratio of Mg^{2+} to Ca^{2+}. *J. Physiol.* **224**, 317–332.

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

MAENO, T., EDWARDS, C. & HASHIMURA, S. (1971). Difference in effects on the end-plate potentials between procaine and lidocaine as revealed by voltage-clamp experiments. *J. Neurophysiol.* **34**, 32–46.

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, N. (1958). The effect of temperature on the neuromuscular junction of the frog. *Jap. J. Physiol.* **8**, 391–404.

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

WERMAN, R. (1969). An electrophysiological approach to drug-receptor mechanisms. *Comp. Biochem. Physiol.* **30**, 997–1017.