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ON THE ROLE OF JUNCTIONAL CHOLINESTERASE IN DETERMINING THE TIME COURSE OF THE END-PLATE CURRENT

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SUMMARY

1. The effect of membrane potential on the half-time ($t_{\frac{1}{2}}$) of the falling phase of the end-plate current was studied both in the absence and the presence of anticholinesterase.

2. In absence of anticholinesterase the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear at negative, and not linear at positive levels of membrane potential. This relation was not affected by the quantity of the released transmitter.

3. In presence of an irreversible anticholinesterase the relation between log $t_{\frac{1}{2}}$ and membrane potential was shifted towards higher values of log $t_{\frac{1}{2}}$. This shift could be counteracted by decreasing the quantity of the released transmitter.

4. It is concluded that in presence of anticholinesterase the decay of the end-plate current is slowed down because in this experimental condition the elimination of transmitter from the synaptic cleft is slowed down.

5. These observations were made in muscles bathed in Tris-HCl buffered Ringer solution. If a phosphate buffered Ringer solution was used, the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear throughout the range of membrane potentials studied. The reason why different buffers give different results is not clear and should be further studied.

INTRODUCTION

The molecular events underlying the mediator-receptor interactions have been studied electrophysiologically by using two different approaches. One was to examine the time course and amplitude of the end-plate current (e.p.c.) in various experimental conditions (e.g. Takeuchi & Takeuchi, 1959; Kordaš, 1969; Magleby & Stevens, 1972*a*, *b*; Gage & McBurney, 1975). The other approach was to study the membrane 'noise', evoked by focal application of various drugs to the end-plate region (e.g. Katz & Miledi, 1970, 1971, 1972, 1973; Anderson & Stevens, 1973). The results thus obtained suggested that the simplified two-state model can be applied to the receptor-transmitter interactions (cf. Katz & Miledi, 1972, 1973; Anderson & Stevens, 1973; for review see Colquhoun, 1975; Rang, 1975). At present it is believed that in the factors determining the time course of the e.p.c. there is a single rate-limiting step causing the e.p.c. or the miniature e.p.c. to decay in a simple exponential fashion. This process has been shown to be highly dependent on temperature and on membrane potential (Kordaš, 1969, 1972*b*; Magleby & Stevens, 1972*a*, *b*; Anderson & Stevens, 1973), and also on the pH of the bathing medium (Scuka, 1975). These authors suggested that it is not diffusion, but receptor-transmitter interaction which is the rate-limiting step.

This concept seems to explain the time course of the e.p.c. in various experimental conditions. However, great difficulties are encountered when the same idea is used in an attempt to explain the prolongation of the e.p.c. due to cholinesterase inhibition. It was found by Katz & Miledi (1972, 1973) that the anticholinesterase prostigmine did not prolong the ionic 'gating time'. Consequently, the prolongation of the e.p.c. in this experimental condition was attributed to a delayed clearance of transmitter. Because it is thought that clearance of transmitter from the synaptic cleft is not voltage-sensitive, then the effect of membrane potential on the half-time of the e.p.c. might be expected to be much reduced in the presence of anticholinesterase (cf. Rang, 1975). This, however, was not found (Magleby & Stevens, 1972*a*; Gage & McBurney, 1975).

The present study was undertaken in an attempt to provide further data on the mechanism of prolongation of the e.p.c. due to cholinesterase inhibition. To achieve this the irreversible anticholinesterase methanesulphonylfluoride (MSF) was used. It was reported that by using this drug cholinesterases in frog skeletal muscle can be almost completely inhibited, while junctional receptors seemed not to be affected (Kordaš, Brzin & Majcen, 1975). In the majority of present experiments the e.p.c. was recorded at various levels of membrane potential in absence and in presence of MSF, and the voltage dependence of the half-time ($t_{\frac{1}{2}}$) of the

falling phase of the e.p.c. studied. A brief account on results thus obtained was published elsewhere (Kordaš, 1976).

METHODS

All experiments were carried out on m. extensor long. dig. IV of the frog (*Rana esculenta*). To abolish contraction of muscles, they were 'glycerol-pretreated' (Howell & Jenden, 1967) by using 400–800 mM glycerol in Ringer solution as described (Kordaš *et al.* 1975). Details about temperature are given in Figs. 1–7.

In the great majority of experiments Tris-HCl buffered (pH 7·4) Ringer solution was used (Kordaš *et al.* 1975). In one experiment this buffer was replaced by phosphate buffer (pH 7·2; Magleby & Stevens, 1972*a*; Anderson & Stevens, 1973; Gage & McBurney, 1975). The quantity of the released transmitter was increased either by evoking two e.p.c.s in rapid succession, or decreased by adding $MgCl_2$ to the bathing solution to make up a final concentration of 1–2 mM. In this way it was possible to enhance, or to reduce, the amplitude of the e.p.c. by about 100 or 50 %, respectively.

Junctional cholinesterase was inhibited by using MSF (Eastman Organic Chemicals) at a concentration of 10^{-3} M for 1 hr before experiment, which was then started without washing out the drug. In a few experiments the muscle was curarized by using (+)-tubocurarine (Nutritional Biochemical Corporation) at a concentration of 5×10^{-7} M.

For recording of the e.p.c. the voltage-clamp method described earlier (Kordaš, 1968*a*) was slightly modified. Membrane potential changes were recorded by Tektronix 502A and R 5031 oscilloscopes, connected in parallel. The former was used as a feed-back amplifier (open loop gain 10^4 to 2×10^4), the latter as a convenient recording oscilloscope. To change the membrane potential under voltage-clamp conditions, the 'command' voltage was fed either to the input of the cathode follower stage of the recording system, or to one side of the input of the feedback amplifier (oscilloscope).

RESULTS

The effect of membrane potential on the e.p.c. both in absence and in presence of MSF is shown in Fig. 1, in which an unexpected finding could be observed. When, in absence of MSF, the results were presented in the way suggested by Magleby & Stevens (1972*a*), the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear only at negative, and not linear at positive levels of membrane potential. This phenomenon was seen in all muscles bathed in Tris-HCl buffered Ringer solution.

After irreversible inhibition of muscle cholinesterase with MSF the relation between log $t_{\frac{1}{2}}$ and membrane potential was shifted towards higher levels of log $t_{\frac{1}{2}}$ and at some end-plates the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear throughout the range of membrane potentials studied. The slope of the linear part of this relation was not noticeably affected by anticholinesterase treatment.

The effect of membrane potential on the relation between log $t_{\frac{1}{2}}$ and membrane potential at different temperatures is shown in Fig. 2. Treatment of

the muscle with MSF resulted again in a shift of the relation between log $t_{\frac{1}{2}}$ and membrane potential towards higher values of log $t_{\frac{1}{2}}$, but the linear slope of this relation was affected only by temperature changes.

The effect of quantity of the released transmitter on the relation between log $t_{\frac{1}{2}}$ and membrane potential in muscles treated with MSF. It is well known that in the muscle treated with an anticholinesterase the time

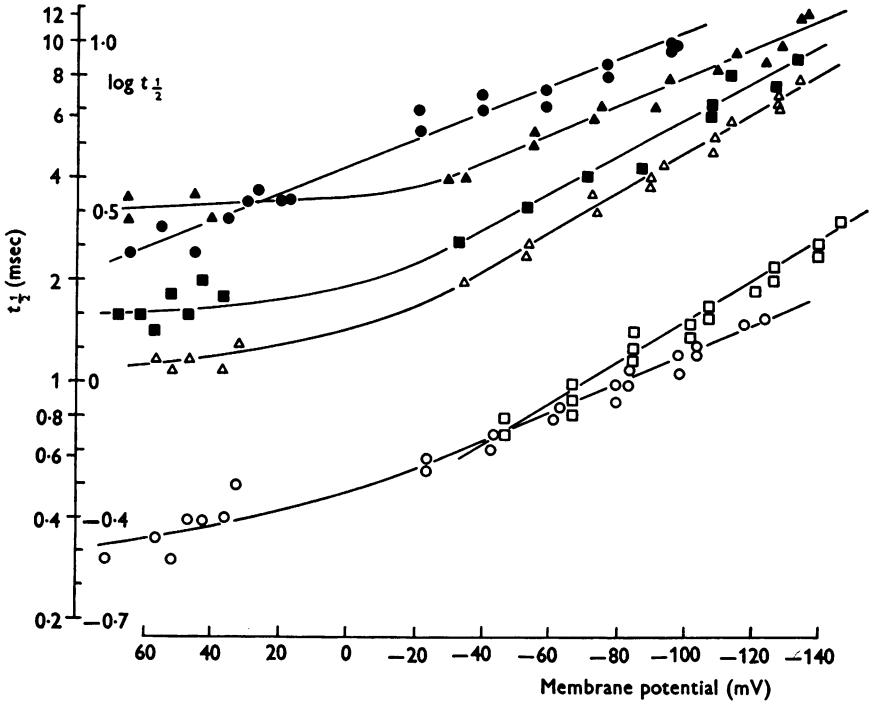


Fig. 1. The effect of membrane potential on the half-time of the falling phase of the e.p.c. in three end-plates before (open symbols) and after (filled symbols) treatment of the muscle with MSF. Note that before treatment with this drug the relation between log $t_{1/2}$ and membrane potential is linear only at negative levels of membrane potential. After treatment with MSF this relation is shifted towards higher values of log $t_{1/2}$. The slope of the linear part is not noticeably changed. Squares 21·1°, triangles 11·7° and circles 22·2° C.

course of the e.p.c. depends also on the quantity of the released transmitter. If it is decreased, the half-time of the e.p.c. is reduced, and vice versa (Kordaš, 1972*a*; Magleby & Terrar, 1975; Hartzell, Kuffler & Yoshikami, 1975). It was of interest to establish whether a diminution in the amount of released transmitter would cause a change of the relation between log $t_{\frac{1}{2}}$ and membrane potential in MSF-treated muscle.

The results are shown in Fig. 3*A*. The shift of the relation between

log $t_{\frac{1}{2}}$ and membrane potential towards higher values of log $t_{\frac{1}{2}}$, effected by MSF, could be counteracted by reducing the amount of released transmitter. The slope of the linear part, however seemed to be very little affected. In some MSF-treated muscles, at membrane potentials more negative than -100 mV, the amplitude of the e.p.c. exceeded 1 μA. In this condition the e.p.c. appeared to decay in two steps (Fig. 3*B*).

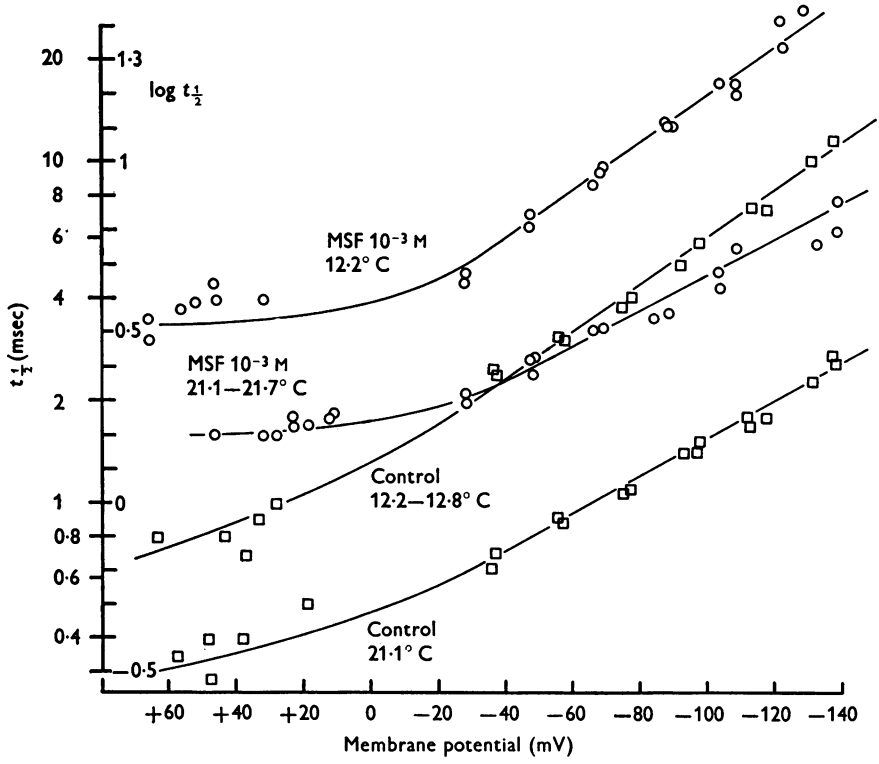


Fig. 2. The effect of membrane potential on the half-time of the e.p.c. at different temperatures before (control, squares) and after treatment with MSF (circles). Note that the slope of the linear part of the relation between log $t_{1/2}$ and membrane potential is affected by temperature changes only. All data from one end-plate.

The effect of quantity of the released transmitter on the relation between log $t_{\frac{1}{2}}$ and membrane potential in muscle not treated with MSF is shown in Fig. 4. By adding $MgCl_2$ to the bath, the amplitude of the e.p.c. was depressed by about 50%. However, there was little, if any change in the half-time of the e.p.c. Again the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear at negative, and not linear at positive levels of membrane potential.

The quantity of the released transmitter was also changed by evoking

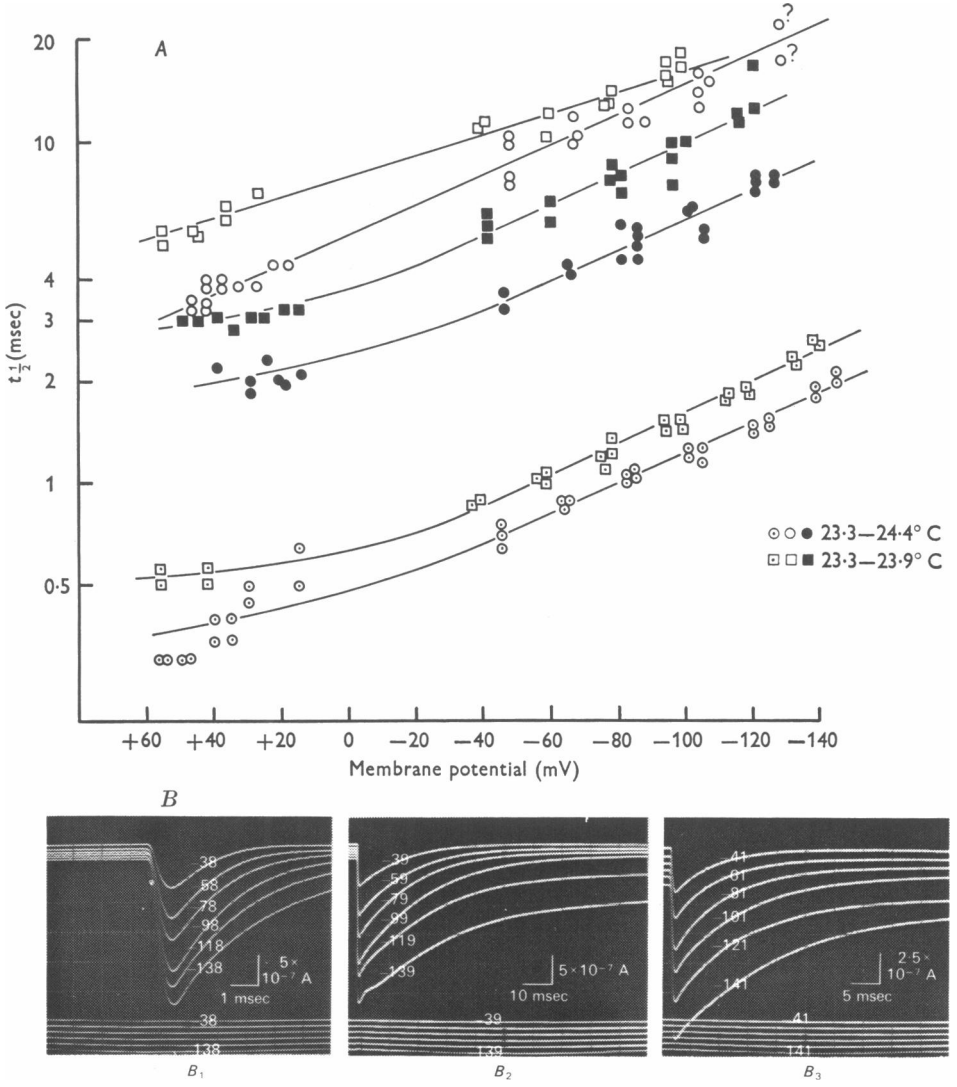


Fig. 3. *A*: the effect of membrane potential on the half-time of the e.p.c. in two end-plates before (⊙, ⊡) and after (○, □) treatment with MSF. Note that when in this condition the quantity of the released transmitter is decreased (●, ■), the relation between log $t_{1/2}$ and membrane potential is shifted towards smaller values of log $t_{1/2}$. *B*, the effect of membrane potential on the e.p.c. before (B_1) and after treatment with MSF (B_2), and when the quantity of the released transmitter is decreased (B_3). Note that, at membrane potentials more negative than −100 mV, after treatment with MSF the e.p.c. decayed in two steps. See also in *A* the points marked with '?'. The membrane potential changes are recorded on the lower beam, while the e.p.c. is recorded on the upper beam. Membrane potential indicated by numbers.

two e.p.c.s in rapid succession; it is well known that the second e.p.c. is larger because of larger transmitter release (del Castillo & Katz, 1954; Kordaš, 1972*a*; Magleby & Terrar, 1975). As shown in Fig. 5, there is little, if any change in the half-time of the e.p.c.

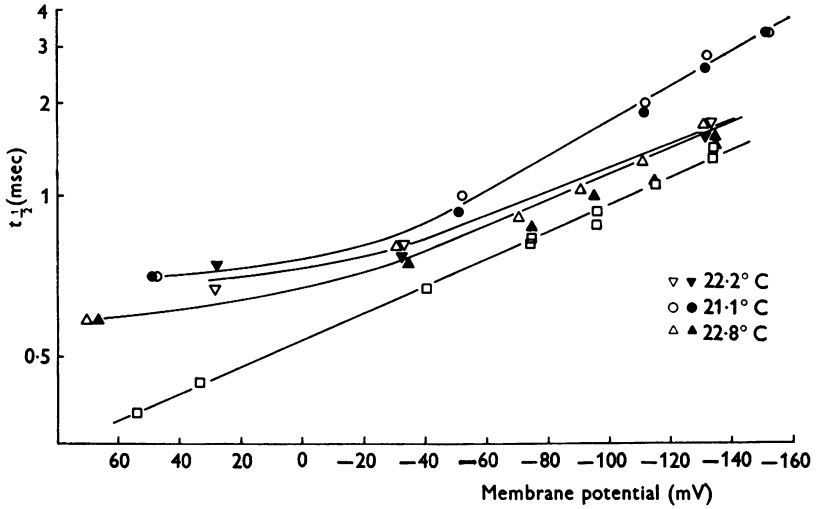


Fig. 4. The effect of membrane potential on the half-time of the e.p.c. recorded in absence of MSF in Tris-HCl buffered Ringer solution when the quantity of the released transmitter was relatively high (●, ▲, ▼) or relatively low (○, △, ▽). In one experiment a phosphate-buffered Ringer solution was used and data shown by □.

Other observations. The non-linear relation between log $t_{\frac{1}{2}}$ and membrane potential was observed in all muscles bathed in Tris-HCl buffered (pH 7·4) Ringer solution. If, however, a phosphate buffered (pH 7·2) Ringer solution was used, this relation was linear throughout the range of membrane potentials studied (Fig. 4).

In the present experiments it was also found that the e.p.c. amplitude was a linear function of the membrane potential. The reversal potential of the e.p.c. was $-1{\cdot}4 \pm 3{\cdot}5$ mV (mean ± S.D., fourteen experiments, Fig. 6).

In some experiments the effect of curare on the half-time of the e.p.c. was studied. As shown in Fig. 7, this drug depressed the half-time of the e.p.c. at all levels of membrane potentials in some end-plates. In the others, this shortening was hardly measurable.

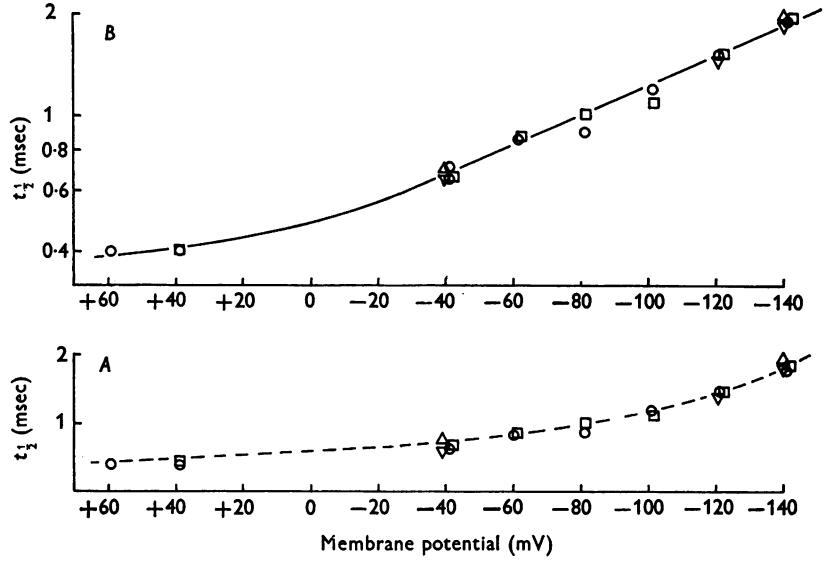


Fig. 5. The effect of membrane potential on the half-time of the e.p.c. in absence of MSF in an end-plate while the quantity of the released transmitter was changed by evoking two e.p.c.s in rapid succession, or by adding $MgCl_2$ to the bath. ○, first e.p.c., $CaCl_2$ 1·8 mM; □, second e.p.c., $CaCl_2$ 1·8 mM; ▽, first e.p.c., $CaCl_2$ 1·8 mM, $MgCl_2$ 2 mM; △, second e.p.c., $CaCl_2$ 1·8 mM, $MgCl_2$ 2 mM. Temperature 23·3–23·9° C. Results are shown both on linear (*A*) and logarithmic (*B*) amplitude scale.

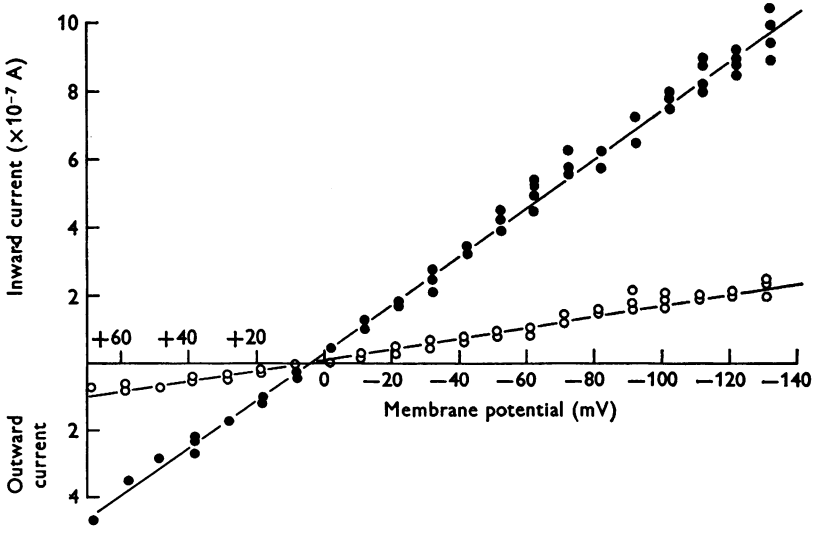


Fig. 6. The effect of membrane potential on the amplitude of the e.p.c. at one end-plate if the quantity of the released transmitter was relatively high (●) or relatively low (○). Temperature 22·2° C.

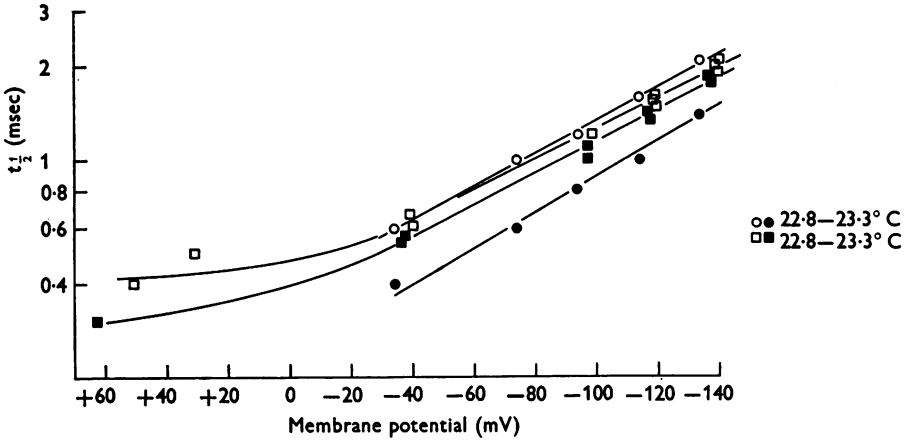


Fig. 7. The effect of membrane potential on the half-time of the e.p.c. in two end-plates before (○, □) and after (●, ■) treatment of the muscle with curare 5×10^{-7} M.

DISCUSSION

Summarizing the results it was found (i) that the relation between log $t_{\frac{1}{2}}$ and membrane potential was linear at negative and not linear at positive levels of membrane potential, where it seemed to approach a lower limit of $t_{\frac{1}{2}}$; (ii) after junctional cholinesterase inhibition with MSF this relation was shifted towards higher values of log $t_{\frac{1}{2}}$ without greatly changing the slope of its linear part; (iii) the shift of this relation towards higher values of log $t_{\frac{1}{2}}$ could be counteracted by decreasing the quantity of the released mediator; (iv) in a muscle with active junctional cholinesterase the relation between log $t_{\frac{1}{2}}$ and membrane potential was very little, if at all, affected by the quantity of the released mediator.

It seems that some of the present findings can be explained along general lines, derived from the kinetic scheme shown in the Appendix. Present information suggests that the step $A_nR \leftrightharpoons A_nR'$ is voltage sensitive (Magleby & Stevens, 1972*a*, *b*; Anderson & Stevens, 1973; for other possibilities see Gabrovec, Kordaš & Popović, 1975; Colquhoun, 1975), while clearance of transmitter from the synaptic cleft is probably voltage insensitive (Magleby & Stevens, 1972*a*; Kordaš, 1972*b*). The non-linear relation between log $t_{\frac{1}{2}}$ and membrane potential indicates that the voltage dependence of the half-time of the e.p.c. is less at positive, than at negative levels of membrane potential. This could mean that during severe depolarization the voltage sensitive process (the conformational step of receptor-transmitter interaction) may become so fast that the voltage insensitive process (the over-all diffusion of transmitter from the synaptic cleft) becomes dominant in determining the decay of the e.p.c.

The non-linear relation between log $t_{\frac{1}{2}}$ and membrane potential was

observed in all muscles bathed in Tris-HCl buffered Ringer solution (pH 7·4), but not in muscles bathed in phosphate buffered (pH 7·2) Ringer solution (Fig. 4; Magleby & Stevens, 1972*a*; Anderson & Stevens, 1973; Gage & McBurney, 1975). This discrepancy is probably not due to a difference in pH. Scuka (1975) studied the effect of membrane potential on the half-time of the e.p.c. at pH levels ranging from 5·4 to 9·4, using Tris-HCl buffered Ringer solution. If these results are analysed in the way suggested by Magleby & Stevens (1972*a*) it is found that the relation between log $t_{\frac{1}{2}}$ and membrane potential is linear at negative and not linear at positive levels of membrane potential. Apparently some other mechanism underlies this discrepancy, and should be further studied.

The present findings agree with observations of Magleby & Stevens (1972*a*) and Gage & McBurney (1975) that after treatment of the muscle with anticholinesterase the relation between log $t_{\frac{1}{2}}$ and membrane potential is shifted towards higher values of log $t_{\frac{1}{2}}$ without greatly changing the slope of the linear part. It seems unlikely (cf. Katz & Miledi, 1973, 1975) that a change in the receptor–transmitter interaction is the underlying mechanism. The present experiments suggest that not only junctional cholinesterase inhibition, but also the quantity of the released transmitter is important in determining the relation between log $t_{\frac{1}{2}}$ and membrane potential. This again agrees very well with earlier observations that in a muscle treated with anticholinesterase the decay of the e.p.c. becomes dependent on the quantity of the released transmitter (Kordaš, 1968*b*, 1972*a*; Magleby & Terrar, 1975; Katz & Miledi, 1975; Hartzell *et al.* 1975).

The effect of anticholinesterase on the decay of the e.p.c. can be explained according to the kinetic scheme shown in the Appendix. If the transmitter is cleared from the synaptic cleft by diffusion and hydrolysis, the decay of the computed e.p.c. is relatively fast and determined predominantly by the backward rate constant of the conformational transition of the receptor-mediator complex. If, however, the hydrolysis of transmitter is partially or completely blocked, its clearance from the synaptic cleft is slowed down. In this condition the decay of the computed e.p.c. is relatively slow and determined by several rate constants. The analysis of the computed e.p.c. also gives an approximately exponential decay. Its half-time is longer if clearance of transmitter from the synaptic cleft is slower.

This explanation is similar to those of Katz & Miledi (1973, 1975), Barnard (1974), Kuffler & Yoshikami (1975) and Hartzell *et al.* (1975), that diffusion of mediator can be important in determining the decay of the e.p.c. However, other explanations such as Magleby & Terrar (1975) and Sterz, Dreyer & Paper (1976) are not excluded.

The analysis of the kinetic scheme in the Appendix also helps to explain why the decay of the e.p.c. is independent of the quantity of the released transmitter, provided junctional cholinesterase is active. Similar results were obtained also by Magleby & Stevens (1972*a*) and Hartzell *et al.* (1975). But the present kinetic scheme does not explain why the decay of the e.p.c. becomes dependent on the quantity of the released transmitter when junctional cholinesterase is inhibited. Apparently in this condition the e.p.c. depends also on factors not included in this scheme. One of these factors is, as pointed out by Katz & Miledi (1973), the geometry of the synaptic cleft and spatial distribution and activation of junctional receptors. This was studied in great detail by Hartzell *et al.* (1975). They concluded that if junctional cholinesterase is inhibited the clearance of transmitter is slowed down. Therefore transmitter, which is quantally released at different points of presynaptic membrane, spreads laterally along the receptive membrane. Areas of activated receptors overlap and further prolong the decay of the e.p.c. It seems that this process is the mechanism underlying the shift of the relation between log $t_{\frac{1}{2}}$ and membrane potential. Thus, if the quantity of the released transmitter is decreased, overlapping is decreased and the relation between log $t_{\frac{1}{2}}$ and membrane potential is shifted towards lower values of log $t_{\frac{1}{2}}$.

The two-step decay of the e.p.c. observed in presence of MSF during large hyperpolarization is similar to that described earlier (Kordaš *et al.* 1975; Hartzell *et al.* (1975). The underlying mechanism might be, as suggested by Hartzell *et al.* (1975) related to the time required for the adjacent quanta to diffuse to overlapping areas.

It was repeatedly shown that in frog sartorius muscle the relation between membrane potential and e.p.c. amplitude is convex, i.e. the increase in e.p.c. amplitude is less than expected from a linear voltage–current relation (Kordaš, 1969; Magleby & Stevens, 1972*a*, *b*; Scuka, 1975). It was therefore somewhat unexpected that in the present experiments the voltage–current relationship was linear. It seems, however, that this discrepancy can be explained by the results of Dionne & Stevens (1975), Scuka (1975) and Mallart, Dreyer & Peper (1976). The voltage–current relationship may be convex, linear or concave, depending on channel life-time and on time course of transmitter concentration at receptors. A decrease in channel life-time, or a slower time course of transmitter concentration would thus tend to change a convex voltage–current relation into a linear relation (cf. Mallart *et al.* 1976, fig. 5*A* and *B*). If this is so, it could be speculated that the time course of transmitter concentration is faster in end-plates of m. sartorius than in those of m. ext. long. dig. IV.

It is well known that curare has a shortening effect on the end-plate potential and e.p.c. (Eccles, Katz & Kuffler, 1941; Oomura & Tomita,

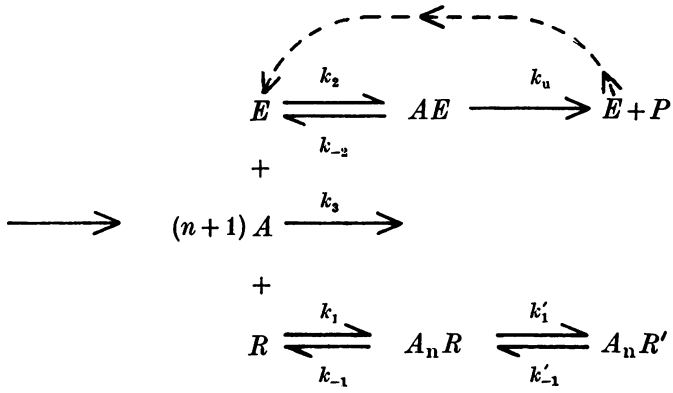
1960; Beranek & Vyskočil, 1967). This effect is very pronounced in the presence, and slight in the absence of anticholinesterase (Eccles, Katz & Kuffler, 1942; Kordaš, 1968*a*). In the present experiments performed in absence of anticholinesterase the shortening effect was variable. In some end-plates it was relatively pronounced, while in others it was hardly measurable. The underlying mechanism is not clear. However, bearing in mind that in presence of anticholinesterase the shortening effect is very pronounced, it is possible that curare, by decreasing the number of activable receptors, decreases the overlapping of areas of activated receptors, thus shortening the half-time of the e.p.c.

APPENDIX

By P. Jakopin and M. Kordaš

The results described are very complex and not easy to understand. It was therefore of interest to analyse diffusion and hydrolysis of transmitter and transmitter–receptor interactions in a more quantitative way.

The main features of these processes can be summarized in the kinetic scheme which is a modification of those already described (Kordaš, 1972*a*;



Katz & Miledi, 1972, 1973; Anderson & Stevens, 1973; Gabrovec *et al.* 1975; Gage & McBurney, 1975; Scuka, 1975). The released mediator (A) undergoes three processes: In a reversible combination of n molecules of A with one receptor (R) the receptor–mediator complex (A_nR) and its active conformation (A_nR') is formed; by binding to junctional cholinesterase (E), A is hydrolysed into products of hydrolysis (P); part of the released A is eliminated from the synaptic cleft by diffusion.

Assuming that the time course of A_nR' reflects the time course of the e.p.c., the following points are of interest:

(i) What changes in the time course of the e.p.c. occur if the quantity of the released transmitter is changed while junctional cholinesterase is either active or inhibited, assuming that one transmitter molecule interacts with one receptor;

(ii) What changes in the time course of the e.p.c. occur in conditions as described in (i), but assuming that more than one transmitter molecule interacts with one receptor (Dreyer & Peper, 1975; Peper, Dreyer & Müller, 1976).

The present model is similar to that described earlier (Kordaš, 1972*a*). However, in the present one the quantal release was better simulated. The release of one quantum was assumed to be instantaneous; the quantity of the released transmitter was thus changed by changing the number of quanta released in a constant period of time. The quantity of the released transmitter (m) was thus directly proportional to the frequency of release.

In these calculations the initial concentration of receptors was 1·0 U (arbitrary unit); the quantum size was 0·1 U; the number of quanta released was 20, 40, 80; the release time of all quanta was 0·5 msec. Simulating an active junctional cholinesterase, $E = 1{\cdot}0$ U; simulating an irreversibility, partially or completely, inhibited junctional cholinesterase $E = 0{\cdot}1$ U or $E = 0{\cdot}0$ U.

The equations describing the above kinetic scheme are as follows:

$$\frac{dA}{dt} = -k_1 . A_1^n . R - k_2 . A . E - k_3 . A + k_{-1} . A_n R + k_{-2} . AE, \quad (1)$$

$$\frac{dR}{dt} = -k_1 . A_1^n . R + k_{-1} . A_n R, \quad (2)$$

$$\frac{dA_n R}{dt} = -k'_1 . A_n R - k_{-1} . A_n R + k_1 . A^n . R + k'_{-1} . A_n R', \quad (3)$$

$$\frac{dA_n R'}{dt} = k'_{-1} . A_n R' + k'_1 . A_n R, \quad (4)$$

$$\frac{dE}{dt} = -k_2 . A . E + (k_{-2} + k_u) . AE, \quad (5)$$

$$\frac{dAE}{dt} = -(k_{-2} + k_u) . AE + k_2 . A . E. \quad (6)$$

In these equations, $n = 1$, assuming the reaction $A + R \leftrightharpoons AR$, and $n = 3$, assuming the reaction $3A + R \leftrightharpoons A_3 R$. To solve these equations the digital computer CYBER 72 was used.

The data on the amplitude and time course of the computed e.p.c. for various values of rate constants and $n = 1$ are given in Table 1. As expected, at $E = 1{\cdot}0$ U (simulating an active junctional cholinesterase) the

TABLE 1. Amplitude (arbitrary units, U), rise time (msec) and half-time (msec) of the computed e.p.c. ($n = 1$) as a function of E (U) and m. (*): the computed e.p.c. seems to decay in two steps

No. of computation	k_1 (sec^{-1}. U^{-1})	k_{-1} (sec^{-1})	k'_1 (sec^{-1})	k'_{-1} (sec^{-1})	k_2 (sec^{-1}. U^{-1})	k_{-2} (sec^{-1})	k_3 (sec^{-1})	k_U (sec^{-1})	E (U)	Amplitude (U)/rise time (msec)/$t_{1/2}$ (msec) At $m = 20$	At $m = 40$	At $m = 80$
1	10^6	10^5	10^4	10^3	10^6	10^3	10^4	10^3	1·0	0·52/0·66/1·0	0·86/0·62/1·0	0·89/0·59/1·0
									0·1	0·84/0·63/2·60	0·88/0·58/2·55	0·90/0·58/2·65
									0·0	0·84/0·60/5·00*	0·89/0·58/5·20*	0·90/0·58/—
2	10^6	10^5	10^4	10^3	10^6	10^3	10^4	10^4	1·0	0·17/0·51/0·85	0·43/0·53/0·80	0·87/0·54/0·85
									0·1	0·80/0·60/1·35	0·88/0·57/1·35	0·90/0·57/1·40
3	10^6	10^5	10^4	10^3	10^6	10^3	10^4	10^5	1·0	0·13/0·53/0·90	0·24/0·52/0·85	0·41/0·51/0·80
									0·1	0·59/0·55/1·30	0·80/0·53/1·35	0·89/0·53/1·30
4	10^6	10^5	10^4	10^3	10^6	10^4	10^4	10^5	1·0	0·13/0·53/0·85	0·25/0·51/0·85	0·43/0·52/0·80
									0·1	0·60/0·55/1·35	0·80/0·52/1·35	0·88/0·52/1·30
									0·0	0·84/0·60/4·30*	0·88/0·58/4·30*	0·90/0·58/4·60*
5	10^6	10^4	10^3	10^3	10^6	10^4	10^4	10^5	1·0	0·10/0·67/0·85	0·15/0·65/0·85	0·22/0·62/0·85
									0·1	0·27/0·86/1·65	0·32/0·78/1·65	0·35/0·76/1·65
									0·0	0·39/1·34/7·25*	0·41/1·26/8·05*	0·42/1·22/7·95*
6	10^6	10^4	10^3	10^3	10^6	10^4	10^3	10^5	1·0	0·10/0·67/0·90	0·16/0·65/0·85	0·23/0·62/—
									0·1	0·29/0·87/1·80	0·33/0·80/1·75	0·39/0·88/1·65

decay of the computed e.p.c. is exponential, its half-time being determined predominantly by k'_{-1}. Its value was set to 10^3 sec^{-1} as suggested by the work of Magleby & Stevens (1972*a*, *b*), Anderson & Stevens (1973) and

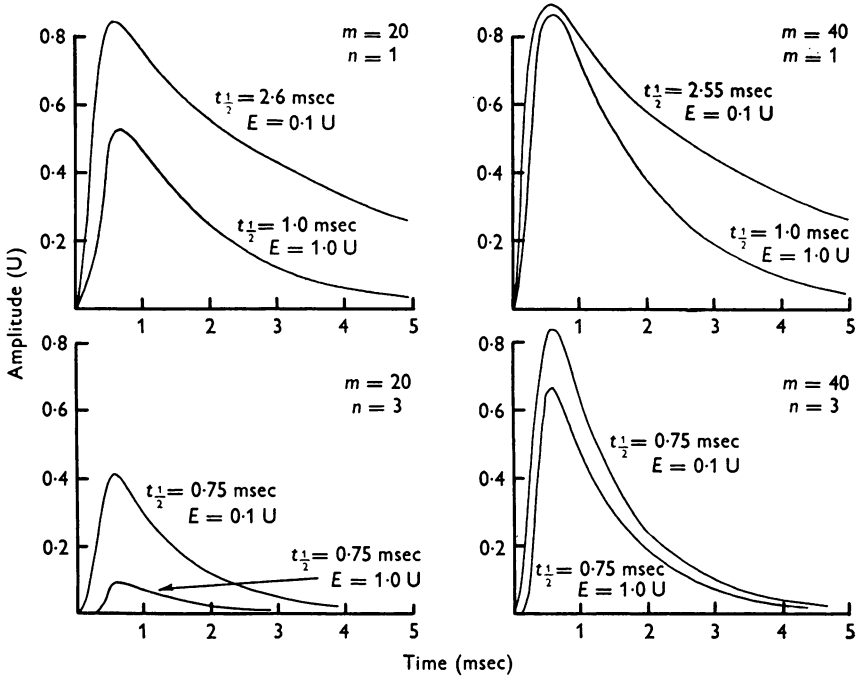


Fig. 8. The time course of the computed e.p.c. (cf. Table 1, computation no. 1), assuming $n = 1$ and $n = 3$. Note that the decay of the computed e.p.c. is exponential, its half time being dependent on relative cholinesterase concentration, E, provided $n = 1$. If $n = 3$, the half-time of the computed e.p.c. is very little changed if E is decreased.

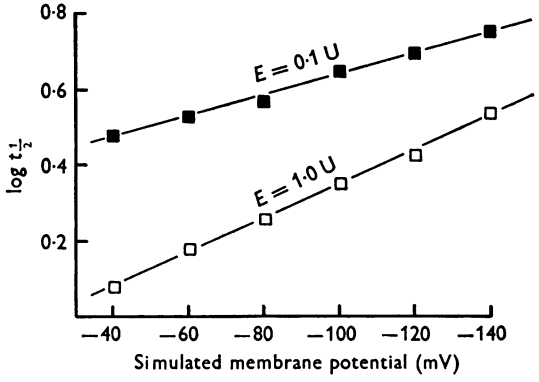


Fig. 9. Simulation of the effect of membrane potential on the half-time of the e.p.c. if $E = 1{\cdot}0$ U and $E = 0{\cdot}1$ U. Note that in the latter condition the effect of voltage on $t_{1/2}$ is only slightly attenuated.

Katz & Miledi (1973). For this reason the time course of the computed e.p.c. was similar to that recorded in isolated muscle. If $E = 0{\cdot}1$ U or $E = 0{\cdot}0$ U (simulating the partially or completely inhibited cholinesterase), the decay of the computed e.p.c. was still exponential but had a longer half-time. It was not determined only by k'_{-1}, but also by other rate constants. Simulating a change in the quantity of the released transmitter the half-time of the computed e.p.c. remained practically constant. This occurred when $E = 1{\cdot}0$ U, and when $E = 0{\cdot}1$ U or $E = 0{\cdot}0$ U. The former simulation is in agreement with present observation in isolated muscle. The latter simulation, however, is not in agreement with present observations in isolated muscle. Apparently, when junctional cholinesterases are inhibited the geometry of the synaptic cleft, not included in the present simulations, becomes an important factor in determining the decay of the e.p.c. (Katz & Miledi, 1973; Hartzell *et al.* 1975).

The rise time of the computed e.p.c remained practically constant if E was decreased. Similarly, in isolated and glycerol-treated muscle the rise time of the e.p c. is only slightly lengthened by MSF treatment (Kordaš *et al.* 1975).

The time course of the computed e.p.c., assuming $n = 1$ and $n = 3$ is shown in Fig. 8. If in the former condition E is decreased, both the amplitude and half-time of the computed e.p.c. increase. However, if in the latter condition E is decreased, the amplitude of the computed e.p.c. is increased but its half-time hardly affected.

Finally, it seemed of interest to simulate the effect of membrane potential on the half-time of the e.p.c., both when $E = 1{\cdot}0$ U and $E = 0{\cdot}1$ U. For a given level of membrane potential (in brackets), the following value of k'_{-1} was chosen: 814 sec^{-1} (−40 mV); 660 sec^{-1} (−60 mV); 531 sec^{-1} (−80 mV); 425 sec^{-1} (−100 mV); 374 sec^{-1} (−120 mV); 275 sec^{-1} (−140 mV). As shown in Fig. 9, if $E = 0{\cdot}1$ U, the relation between $\log t_{\frac{1}{2}}$ and simulated membrane potential level was shifted towards higher values of $\log t_{\frac{1}{2}}$. The slope of this relation was only slightly decreased.

These results are similar to those obtained by Caldwell (reported by Gage, 1976) and could sometimes be observed also in isolated muscle (Fig. 1). The simulation experiments thus support the assumption that in the presence of anticholinesterase the decay of the e.p.c. is lengthened because in this condition the clearance of transmitter from the synaptic cleft is slowed down.

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