J. Physiol. (1968), **198**, *pp.* 81–90
With 6 text-figures
Printed in Great Britain

A STUDY OF THE END-PLATE POTENTIAL IN SODIUM DEFICIENT SOLUTION

BY M. KORDAŠ*

From the Department of Biophysics, University College London, Gower Street, London, W.C. 1

(*Received* 20 *March* 1968)

SUMMARY

1. The effect of anticholinesterases in lengthening the end-plate potential is much more pronounced in a low-sodium solution than in an ordinary Ringer-curare solution.

2. To investigate this difference, intracellular recordings of end-plate potentials and of end-plate currents in voltage-clamped muscle fibres were used.

3. It was found that the very large lengthening of the end-plate potential in a low-sodium solution was due to a combination of three factors: (i) lengthening of the underlying end-plate current, (ii) an increased membrane resistance and (iii) marked inward-going (anomalous) rectification of the muscle membrane.

INTRODUCTION

It is well known that, after anticholinesterases are added to the bathing solution, the end-plate potential (e.p.p.) in a curarized muscle becomes larger and more prolonged. This effect has been attributed to the accumulation of the transmitter acetylcholine (Brown, Dale & Feldberg, 1936; Feng, 1940; Eccles, Katz & Kuffler, 1942; Eccles & MacFarlane, 1949; Fatt & Katz, 1951; Takeuchi & Takeuchi, 1959). However, a much more striking lengthening of the e.p.p. occurs if prostigmine is added to a nerve–muscle preparation which has been kept in a sodium deficient solution: the rise time of the e.p.p. is increased to 10 msec or more (about 3 msec in the curarized and prostigmine-treated preparation) and the whole e.p.p. lasts for about 100 msec or more (about 40 msec in the curarized and prostigmine-treated preparation; Fatt & Katz, 1951). Takeuchi & Takeuchi (1959) reported that under this condition the active phase of the e.p.p. is longer than in the curarized preparation. However, in their experiments

* Permanent address: Institute of Pathophysiology, University of Ljubljana, Ljubljana 5, Yugoslavia.

the effect of anticholinesterase was not as striking as that reported by Fatt & Katz (1951).

The aim of the present work was to examine the prostigmine-induced lengthening of the low-sodium e.p.p. in more detail. The time course of the e.p.p. is determined by, among other factors, the time course of its active phase (end-plate current, e.p.c., Takeuchi & Takeuchi, 1959, 1960*a*) and the membrane resistance of the muscle fibre. In this study the e.p.p.s and the underlying e.p.c.s were recorded at different membrane potential levels and the membrane resistance of the muscle fibres was measured in different ionic media.

METHODS

The procedure for recording the e.p.p. and measuring the input resistance of the muscle fibre was similar to that described by Fatt & Katz (1951). In addition, voltage-clamp conditions were used for e.p.c. measurements as described by Takeuchi & Takeuchi (1959, 1960*a*; see also Kordaš, 1968).

During the clamping procedure, the e.p.p. was always reduced to less than 10% of the 'unclamped' size (Figs. 1, 2 and 3). The input resistance was also measured under clamp conditions, by applying known voltage steps to the cathode follower input of the recording system, and measuring the resulting clamping current.

A technical difficulty arose in low-sodium solutions because the resting potential of muscle fibres, impaled with two low-resistance micro-electrodes, was not usually very well maintained. But when micro-electrodes of about 20–30 MΩ were used, the resting potential during the course of an experiment fell by only a few millivolts. Under these conditions, with recording and current-passing micro-electrodes inserted into the same muscle fibre, the time constant of the feed-back loop was 150–200 μsec, which was adequate for the present purpose. The distortion of the intracellularly recorded e.p.p.s by the e.p.p.s in nearby end-plates was minimized by recording the e.p.p. in end-plates which were remote from other end-plates.

'Normal Ringer' (NaCl 116 mM, KCl 2 mM, $CaCl_2$ 3·6 mM, Tris 4 mM, HCl 3, 3 mM; pH 7·4; ionic strength, I = 0·132 M) and 'low-sodium Ringer' (NaCl 23 mM, KCl 2 mM, $CaCl_2$ 3·6 mM, sucrose 168 mM, Tris 4 mM, HCl 3·3 mM; pH 7·4; I = 0·039 M) were used. Prostigmine, tetra-ethylpyrophosphate (TEPP) and tetrodotoxin (TTX) were added in different experiments to make final concentrations of 10^{-6} M, 10^{-4} M and 10^{-6} g/ml., respectively.

All experiments were carried out at room temperature (18–20° C) on sciatic nerve–sartorius muscle preparations of the frog (*Rana pipiens*).

RESULTS

The effect of anticholinesterases on the e.p.c. in sodium deficient solution. Figure 1 illustrates the change brought about by prostigmine in curarized muscle and Fig. 2 the change in sodium deficient solution. It is clear that the anticholinesterase induced lengthening of the e.p.p. is mainly due to a prolongation of underlying e.p.c. The effect is much more striking in low-sodium than in curarized muscle, which confirms the results of Fatt & Katz (1951) and Takeuchi & Takeuchi (1959). In the anticholinesterase-

treated curarized preparations the rise and half-decline time of the e.p.c are about 1·5 and 2·8 msec, respectively (Takeuchi & Takeuchi, 1959; Kordaš, 1968), whereas in the similarly treated low-sodium preparation the corresponding values are 2·2 msec (mean of 18 experiments, range 1–5 msec) and 13·3 msec (mean of 18 experiments, range 7·5–23 msec), respectively. In lengthening the e.p.c., prostigmine and TEPP had similar effects, though different concentrations were required (prostigmine, 10^{-6} M, TEPP, 10^{-4} M for 120 min).

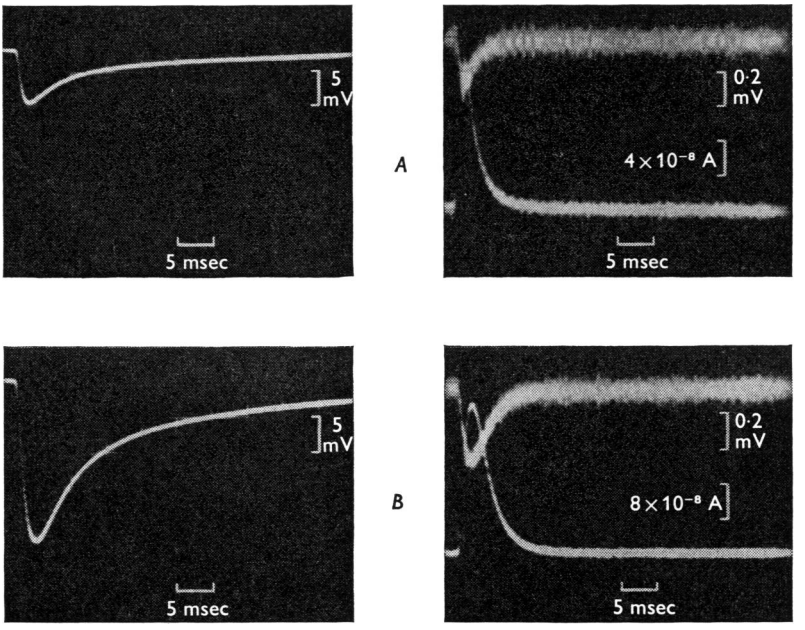

Fig. 1. End-plate current and end-plate potential in a curarized muscle before (*A*) and after (*B*) treatment of the muscle with prostigmine. In the left-hand blocks the traces are intracellular recordings of the end-plate potential (inside positive deflexion downwards). In right-hand blocks the upper traces are intracellular recordings of the reduced membrane potential change during the flow of the 'clamping current', which is recorded on the lower trace (inward current upwards). All records from the same end-plate.

The reason for the striking effect of anticholinesterases in low-sodium solutions is not clear. One possibility is that a larger number of quantal packets might be released, possibly leading to saturation of junctional receptors in the presence of an anticholinesterase. A saturation effect might easily lead to a protracted time course and slower decay of the e.p.c. To test this suggestion, two nerve impulses were set up in rapid successions and e.p.p.s and underlying e.p.c.s were recorded. When two e.p.p.s are evoked at brief intervals, the second e.p.p. is larger than the

first (Schaefer & Haas, 1939; Feng, 1940; Eccles, Katz & Kuffler, 1941; Eccles & MacFarlane, 1949), owing to facilitation of transmitter release (Hutter, 1952; Eccles, 1952; del Castillo & Katz, 1954*a*). As shown in Fig. 3*A*, such a facilitation occurs also in low-sodium Ringer, provided prostigmine is absent. In the presence of prostigmine, however, the second e.p.p. and e.p.c. are smaller and slower than the first, the total amplitudes

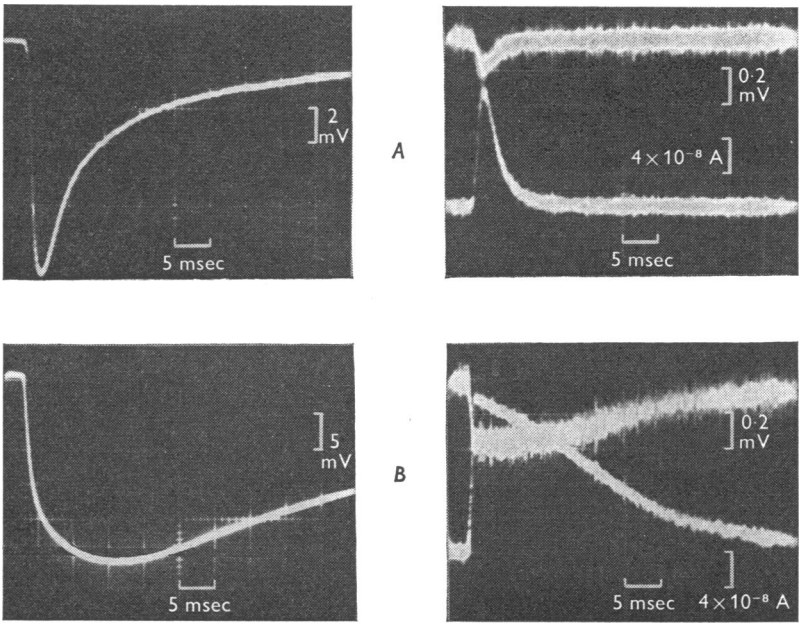

Fig. 2. End-plate current (right, lower traces) and end-plate potential (left) in a muscle soaked in sodium deficient solution before (*A*) and after (*B*) treatment of the muscle with prostigmine.

being only slightly larger than those evoked by a single nerve impulse (Fig. 3*B*). When the time interval was increased, the second e.p.p. and e.p.c. gradually became similar in shape to the first one. These observations tend to support the suggestion that under the present experimental conditions junctional receptors may approach saturation with transmitter, thus giving rise to a flat-topped and prolonged e.p.c.

The muscle fibre membrane resistance in sodium deficient solution. The time course of the end-plate potential is determined not only by the underlying e.p.c. but also by the resistance and time constant of the muscle fibre. Hence, if in sodium deficient Ringer the membrane conductance is reduced, a slowing of the time course of the e.p.p. would result.

As all experiments were made with 'chloride Ringer', sodium deficiency was accompanied by a reduction of chloride concentration. Because of the

relatively high chloride permeability of the muscle membrane (Boyle & Conway, 1941; Hodgkin & Horowicz, 1959, 1960; Adrian & Freygang, 1962), membrane resistance increases when the Cl^- concentration in the bathing solution is diminished (Hutter & Padsha, 1959; Hutter & Noble, 1960).

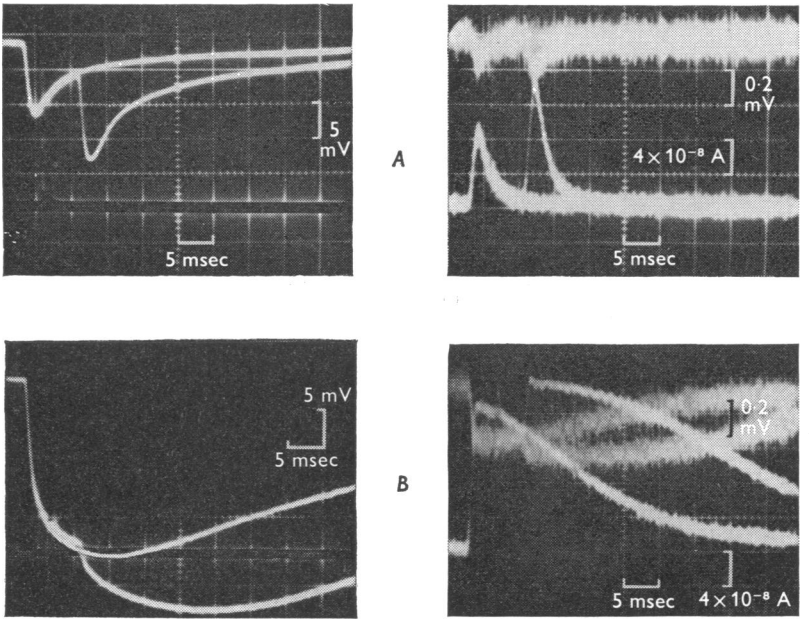

Fig. 3. End-plate currents (right, lower traces) and end-plate potentials (left) evoked by double nerve stimuli in a muscle kept in low-sodium solution before (*A*) and after (*B*) treatment with prostigmine.

The input resistance (the effective resistance between inside and outside of a muscle fibre (Fatt & Katz, 1951)) was measured in normal Ringer solution and again in the same fibres after 1–3 hr soaking in sodium deficient solution. In normal Ringer solution the input resistance in fourteen fibres was 170–600 KΩ. In sodium deficient Ringer the input resistance increased in twelve out of fourteen fibres, on the average by 27 % (range 10–40 %). Furthermore, the slope of the current–voltage relation was greater for outward than for inward currents (Fig. 4*A*). This might be due to a regenerative 'local response', or to a passive rectification of the fibre membrane. The former was eliminated by adding TTX, 10^{-6} g/ml., (Furukawa, Sasaoka & Hosoya, 1959; Narahashi, Deguchi, Urukawa & Okhubo, 1960) to the solution. In a series of experiments in which TTX was used in normal-sodium Ringer, the input resistance was 350–790 KΩ. In low-sodium Ringer with TTX the resistance increased on the average

by 18% (range 5–50%), and the voltage–current relation for outward current still had a steeper slope (Fig. 4*B*), indicating the presence of inward-going (anomalous) rectification (Katz, 1949; Adrian & Freygang, 1962). When the muscle membrane was further depolarized (by more than 30–40 mV) the slope often diminished.

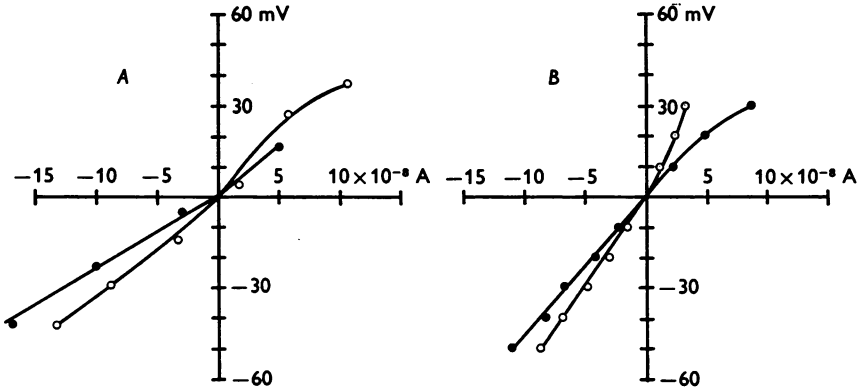

Fig. 4. *A*: Voltage–current relation of a muscle fibre kept in normal (●) and low-sodium (○) Ringer. *B*: Voltage–current relation of a muscle fibre in normal (●) and low-sodium (○) Ringer, both with tetrodotoxin. Hyperpolarization downwards.

It is clear that an increased membrane resistance contributed to the antiesterase-induced lengthening of the e.p.p. The extent of this contribution was not measured in the present experiments. Further, the inward-going rectification of the muscle membrane added to this lengthening of the e.p.p. This factor became apparent when the e.p.p. was evoked in the same fibre at different levels of membrane potential (Figs. 5 and 6). This was done in eighteen muscle fibres, and simultaneously the voltage–current relation was recorded. During hyperpolarization the rise time of the e.p.p. was reduced by up to 5 msec and the half-decline time by 4–20 msec.

Other observations. It is known that the 'equilibrium potential' for the e.p.p. moves to larger inside-negative potentials in sodium deficient solutions (del Castillo & Katz, 1955; Takeuchi & Takeuchi, 1960*b*; Takeuchi, 1963). The 'low-sodium' e.p.p., in the presence of prostigmine, is often of large amplitude. Therefore if the e.p.p. is large enough to approach the equilibrium potential closely, one would expect its time course to show a 'flat top' (Fatt & Katz, 1951; Katz & Miledi, 1967). To examine whether this factor contributed significantly to the flat top and protracted time course of the low-sodium e.p.p., the equilibrium potential was determined in eight muscle fibres by plotting e.p.p. and e.p.c. amplitude against membrane potential. Extrapolation of the observed relations

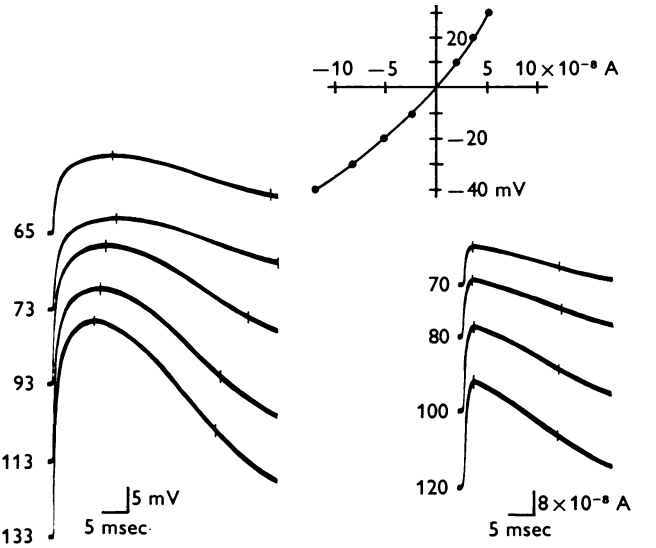

Fig. 5. Tracings of records of end-plate currents (right), tracings of records of end-plate potentials (left) and the voltage current relation (top), recorded from a muscle fibre in sodium deficient solution with prostigmine. The numbers indicate the membrane potential level (negative inside). The rise and half-decline time are marked in each record.

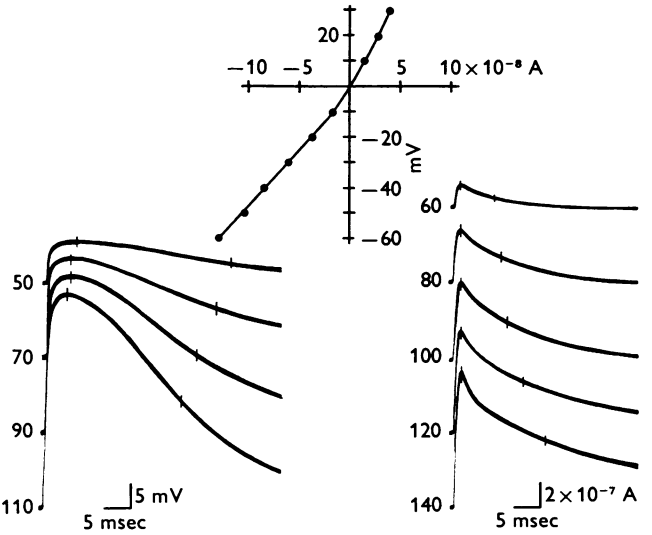

Fig. 6. Tracings of records of end-plate currents (right), tracings of records of end-plate potentials (left) and the voltage-current relation (top), recorded from another muscle fibre in sodium deficient solution with prostigmine. The numbers indicate the membrane potential level. This experiment illustrates lengthening of the half-decline time of e.p.c. with hyperpolarization.

for e.p.p.s and e.p.c.s crossed the abscissa at $-28{\cdot}5 \pm 6$ mV and $-29{\cdot}7 \pm 6$ mV (mean $\pm$ S.D.), respectively. It may be concluded that in muscle fibres with a resisting potential of 80–90 mV even the largest observed e.p.p. (about 30 mV) did not approach the equilibrium level sufficiently closely for this factor to become very important in affecting the time course. It may be noted that the present value of the 'equilibrium potential' is somewhat lower than that reported by Takeuchi & Takeuchi (1960) and Takeuchi (1963).

An interesting feature was sometimes observed, when the muscle fibre was hyperpolarized. There was on occasions a lengthening in the half-decline time of the e.p.c. At a few end-plates this value became almost twice as large as at the resting potential (Fig. 6), whereas in other fibres no such change was seen (Fig. 5). The reason for this lengthening is not known, but a similar effect has previously been observed in the e.p.c. of a curarized preparation (Takeuchi & Takeuchi, 1959; see also Katz & Miledi, 1965*a*).

DISCUSSION

While there are several factors which contribute to the prolongation of the end-plate potential by anticholinesterases in sodium deficient solutions, the main effect is due to a very marked lengthening of the inward end-plate current, that is of the phase of transmitter action. The question why anticholinesterases prolong this active phase much more in low-sodium than in curarized preparations remains to be investigated further. The reason for this difference may lie in the action of curare, which is known not only to reduce end-plate potential but also to shorten the period of post-synaptic transmitter action (see Eccles *et al.* 1942; Beranek & Vyskočil, 1968). One would expect, then, that in the absence of curare-like agents the effect of anticholinesterases would generally resemble that observed in the sodium deficient medium. This seems to be the case in muscles kept in normal Ringer solution (Eccles *et al.* 1942) or treated with excess Mg^{2+} (del Castillo & Katz, 1954*b*) and also in experiments in which Ca^{2+} was applied iontophoretically to a single junction (Katz & Miledi, 1965*b*).

The fact that an added second e.p.p. had a smaller amplitude and slower time course (Fig. 3*B*) might be explained by saturation of junctional receptors with excess transmitter, possibly because of increased quantal release in sodium deficient medium (Kelly, 1965; Birks & Cohen, 1965; Rahamimoff & Colomo, 1967). Under these conditions, diffusion of transmitter towards more distant receptors (Miledi, 1959, 1960) may occur and contribute to the observed prolongation of end-plate current and end-plate potential.

The author would like to thank Professor B. Katz and Professor R. Miledi for suggesting this study, for their continuous interest and help throughout the course of this work, and for assistance in the preparation of the manuscript. The excellent services of Miss Audrey Paintin and Mr L. J. Ward are very much appreciated.

This work was done during the tenure of a World Health Organisation Fellowship, and was also supported by the Boris Kidrič Foundation, Ljubljana, and the Federal Research Council, Belgrade, Yugoslavia.

REFERENCES

ADRIAN, R. H. & FREYGANG, W. H. (1962). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

BERANEK, R. & VYSKOČIL, F. (1968). The effect of atropine on the frog sartorius neuromuscular junction. *J. Physiol.* **195**, 493–503.

BIRKS, R. I. & COHEN, M. W. (1965). The role of sodium ions in the metabolism of acetylcholine. In: *Muscle*, ed. PAUL, W. M., DAVID, E. E., KAY, C. M. & MONCKTON, A., pp. 403–420. Oxford: Pergamon.

BOYLE, P. J. & CONWAY, E. J. (1941). Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1–63.

BROWN, G. L., DALE, H. H. & FELDBERG, W. (1936). Reactions of normal mammalian muscle to acetylcholine and eserine. *J. Physiol.* **87**, 394–424.

DEL CASTILLO, J. & KATZ, B. (1954*a*). Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol.* **124**, 574–585.

DEL CASTILLO, J. & KATZ, B. (1954*b*). Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573.

DEL CASTILLO, J. & KATZ, B. (1955). Local activity at a depolarized nerve-muscle junction. *J. Physiol.* **128**, 396–411.

ECCLES, J. C. (1952). *The Neurophysiological Basis of Mind*, pp. 89 seq. Oxford: Clarendon Press.

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.

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

FENG, T. P. (1940). Studies on the neuromuscular junction XVIII. The local potentials around n-m junctions induced by single and multiple volleys. *Chin. J. Physiol.* **15**, 367–404.

FURUKAWA, T., SASAOKA, T. & HOSOYA, Y. (1959). Effects of tetrodotoxin on the neuromuscular junction. *Jap. J. Physiol.* **9**, 143–152.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & HOROWICZ, P. (1960). The effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. *J. Physiol.* **153**, 370–385.

HUTTER, O. F. (1952). Post-tetanic restoration of neuromuscular transmission blocked by D-tubocurarine. *J. Physiol.* **118**, 216–227.

HUTTER, O. F. & NOBLE, D. (1960). The chloride conductance of frog skeletal muscle. *J. Physiol.* **151**, 89–102.

HUTTER, O. F. & PADSHA, S. M. (1959). Effect of nitrate and other anions on the membrane resistance of frog skeletal muscle. *J. Physiol.* **146**, 117–132.

KATZ, B. (1949). Les constantes électriques de la membrane du muscle. *Archs Sci. physiol.* **3**, 285–300.

KATZ, B. & MILEDI, R. (1965*a*). Propagation of electric activity in motor nerve terminals. *Proc. R. Soc.* B **161**, 453–482.

KATZ, B. & MILEDI, R. (1965*b*). The effect of calcium on acetylcholine release from motor nerve terminals. *Proc. R. Soc.* B **161**, 496–503.

KATZ, B. & MILEDI, R. (1967). Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc.* B **167**, 8–22.

KELLY, J. S. (1965). Antagonism between Na^+ and Ca^{++} at the neuromuscular junction. *Nature, Lond.* **205**, 296–297.

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.* (In the Press.)

MILEDI, R. (1959). Acetylcholine sensitivity of partially denervated frog muscle fibres. *J. Physiol.* **147**, 45–46*P*.

MILEDI, R. (1960). Junctional and extra-junctional acetylcholine receptors in skeletal muscle. *J. Physiol.* **151**, 24–30.

NARAHASHI, T., DEGUCHI, T., URUKAWA, N. & OKHUBO, Y. (1960). Stabilization and rectification of muscle fibre membrane by tetrodotoxin. *Am. J. Physiol.* **198**, 934–942.

RAHAMIMOFF, R. & COLOMO, F. (1967). Inhibitory action of sodium ions on transmitter release at the motor end-plate. *Nature, Lond.* **215**, 1174–1176.

SCHAEFER, H. & HAAS, P. (1939). Über einen lokalen Erregungsstrom an der motorischen Endplatte. *Pflügers Arch. ges. Physiol.* **242**, 364–381.

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

TAKEUCHI, A. & TAKEUCHI, N. (1960*a*). An analysis of end-plate potential. In *Electrical activity of single cells*, ed. KATSUKI, Y., pp. 207–216. Tokyo: Igaku Shoin.

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

TAKEUCHI, N. (1963). Some properties of conductance changes at the end-plate membrane during the action of acetylcholine. *J. Physiol.* **167**, 128–140.