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THE ELECTROMOTIVE ACTION OF ACETYLCHOLINE AT THE MOTOR END-PLATE

By PAUL FATT

From the Biophysics Research Unit, University College, London

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Since the work of Dale and his co-workers (Dale, Feldberg & Vogt, 1936; Brown, Dale & Feldberg, 1936; Brown, 1937), evidence has accumulated which shows that acetylcholine (Ach) plays an important part in the transmission of impulses from motor nerve to muscle. According to the now accepted view Ach is liberated on the arrival of an impulse at the nerve terminations and then acts on the muscle fibre so as to give rise to a propagated impulse and contraction. The immediate action of Ach on the muscle fibre is a reduction of the normal resting potential of the fibre membrane (Cowan, 1936) similar to that produced by a stimulating electric current. Kuffler (1943) showed that Ach in low concentrations exerts this depolarizing action only at the end-plate regions of the fibre.

If, in accordance with the classical view, the membrane potential arises from a selective permeability toward different ions, the role of Ach at the motor end-plate could be explained by either of two possible reactions (see Katz, 1942, p. 182). First, the membrane might be rendered permeable to previously non-penetrating ions, which on diffusing across would reduce the resting potential; or, secondly, the Ach ion (Ach^+) itself might move inward across the membrane at a rate sufficiently high to produce a depolarization. The first possibility is of special interest since the investigations of Hodgkin & Katz (1949) and Nastuk & Hodgkin (1950), following the early work of Overton (1902) on the electrical excitability of nerve and muscle, suggest that an increased permeability toward, and entry of, sodium ions is responsible for the rise of the action potential. If the permeability of the end-plate membrane were increased in a similar way by Ach, the sodium ions on the outside of the muscle fibre would move inward across the membrane and thereby reduce the resting potential.

To test this hypothesis the effect of sodium withdrawal on the depolarizing action of Ach was studied, with the result that Ach was found to produce its local electrical effect even in the complete absence of sodium ions from the

solution bathing the muscle. It becomes necessary then to discuss whether the second mechanism is feasible; that is, whether Ach ions could themselves in very low concentrations produce an electrical discharge of the end-plate membrane.

As an alternative or auxiliary mechanism, the same result could be achieved if Ach were involved in a reaction at the end-plate whereby other ions capable of penetrating the membrane are made available. In any case, if the concentrations and permeabilities of pre-existing diffusible ions are known (notably of potassium and chloride, see Boyle & Conway, 1941), the rate of passage of the 'depolarizing ion' required for a certain steady potential change can be calculated by the method of Goldman (1943). Applying this procedure to the hypothesis that the movement of Ach^+ alone provides the electromotive force for depolarization, the required 'permeability' of the membrane toward Ach can be calculated.

METHOD

Preparation. Various skeletal muscles of the frog, *Rana temporaria*, were used. The m. rectus abdominis and m. sartorius served as the experimental material in preliminary experiments in which mechanical responses alone were recorded. For recording electric potential changes the m. sartorius and m. extensor longus digiti quarti were used. Extreme care had to be taken in the dissection for those experiments in which electrical recording was employed. Visible damage of only a few fibres caused an appreciable negativity to appear at the injured region. All such injured muscles had to be discarded.

There appeared to be no difference in the results obtained with winter and summer frogs. The experiments were carried out at room temperature, varying between 14 and 21° C.

Apparatus. The experimental arrangement employed with the sartorius for the simultaneous recording of electrical and mechanical changes has already been described (Fatt, 1949). In all other experiments the muscle was mounted vertically in a glass chamber so constructed that bathing solutions could be changed through a connexion at the bottom of the vessel. The mechanical response was recorded by attaching the thread tied to the tendon at one end of the muscle to an isotonic lever.

The chamber as used for electrical recording with the extensor digiti IV is shown in Fig. 1. The syringe served to apply a stream of test solution, usually containing Ach, which flowed down the length of the muscle. The leading-off electrodes in contact with the muscle were made of cotton threads stiffened with agar and connected to chlorided spirals of silver. The electrical apparatus consisted of a high impedance input stage, a balanced d.c. amplifier, and a clockwork-driven pen recorder. The stability of the recording system, including the leading-off electrodes, was equivalent to a potential change at the input of not more than 0·2 mV. during the 60 sec. required to take a record.

Experimental procedure. Potentials were recorded by a method similar to that used by Cowan (1936) and Kuffler (1943), with the difference that Ach was applied to the whole muscle rather than locally and that the two recording electrodes were placed, respectively, on the regions of greatest and least end-plate density. (Although there is no recognizable morphological 'end-plate' in the Amphibia, the term is used here in a physiological sense to designate the receptive region of the muscle fibre.) In order to determine the distribution of end-plates a preliminary record was obtained of the potential differences along the surface of each muscle before and after bathing in Ach-containing Ringer solution.

In the case of the sartorius one of the electrodes was kept at the pelvic end while the other was moved from point to point along the surface of the muscle, potential readings being taken every 1–2 mm. An example of the results obtained by this method is illustrated in Fig. 2. The variation

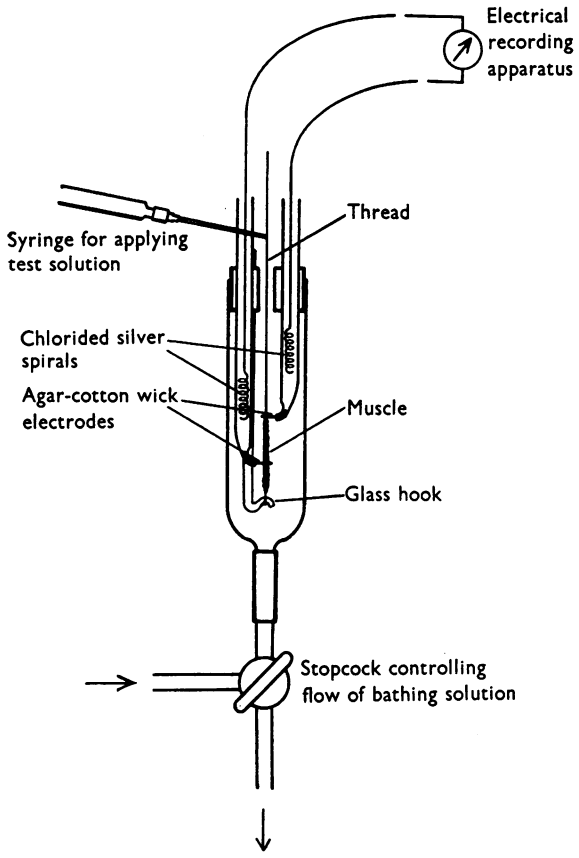

Fig. 1. Apparatus used for measuring potential differences on the m. extensor longus dig. IV.

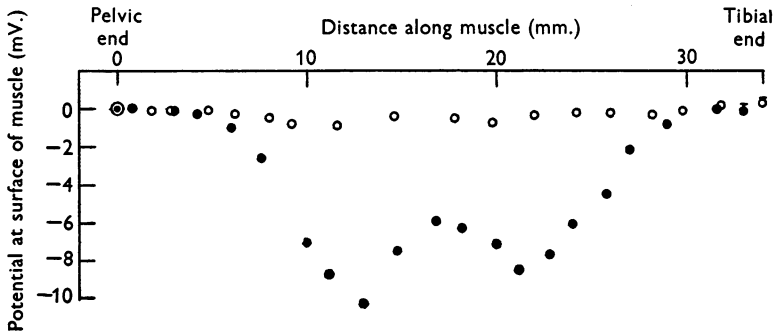

Fig. 2. Potentials measured along a sartorius muscle (○) after immersion in Ringer solution plus eserine sulphate 10^{-5} and (●) a few minutes after adding Ach chloride 10^{-6}. The potentials recorded are those between the moving electrode and the one fixed at the pelvic end. Just before and after each series of readings the bath was flooded and the potential difference between the electrodes found. The change in this potential difference is indicated by a cross-bar above the tibial potential readings.

of depolarization along the muscle agrees with the distribution of neuromuscular junctions as found by other means of electrical recording (Eccles, Katz & Kuffler, 1941) and by histological methods (Pézard & May, 1937).

In the case of the extensor digiti IV, using the chamber of Fig. 1, one electrode was kept at the upper (proximal) end of the muscle, while the other remained near the bottom of the chamber in contact with the solution. With part of the muscle immersed in saline the potential difference recorded was that between the upper end and a point on the muscle at the level of the bathing solution. The solution which initially covered the whole muscle was allowed to flow out of the chamber at an approximately constant rate. With the falling level of the bath serving as a uniformly moving contact on the muscle, the pen recorder gave a continuous tracing of the potential distribution along the muscle surface.

Once having located the position of maximum depolarization, and hence maximum end-plate density, one electrode was placed on that point while the other remained at a region relatively free of end-plates. In both the sartorius and extensor digiti IV the latter position corresponded to the proximal end of the muscle. The time course of the potential change between the two electrodes was then followed as Ach, dissolved in a solution of otherwise the same composition as had previously been bathing the muscle, was applied to the muscle surface. With the sartorius two or three drops of Ach-containing solution were used. The solution rapidly spread along the whole muscle and was diluted by that already present on its surface and in the interstitial spaces. In the case of the extensor digiti IV, to insure that the Ach concentration being used actually reached the end-plates, the drug was applied in a prolonged series of drops which, flowing down the muscle, mixed with and gradually replaced the solution adhering to its surface. Droplets, formed at the points where the electrodes made contact with the muscle, served as reservoirs which maintained a constant composition of the solution bathing the fibres.

At all times except when potentials were being recorded the muscle was kept immersed in solution.

Solutions. Unless otherwise noted in the sodium-free solution, NaCl (0·675%) was replaced by 3·70% glucose. The other constituents of frog-Ringer solution remained unchanged: 2·0 mM. K^+, 1·8 mM. Ca^{++}, 1·0 mM. phosphate, and Cl^- to make up the anion balance. The pH was about 6·8. In some experiments on the sartorius eserine sulphate in a concentration of 10^{-5} was used; in most of those on the extensor digiti IV prostigmine bromide in a concentration of 10^{-6} was used.

An internal micro-electrode as described by Graham & Gerard (1946) and Hodgkin & Nastuk (1949) was used to obtain information on the effect of the removal of sodium ions on the resting potential. Frog sartorii served as the experimental material.

RESULTS

Sodium ions and mechanical response

In preliminary experiments on the rectus abdominis the mechanical response was observed which could be evoked by Ach chloride in concentrations of 10^{-6} to 10^{-5}. Ach was applied first after bathing in normal Ringer solution and again after bathing in sodium-free solution. After soaking the muscle in the sodium-free medium for 40 min. it was found that Ach no longer elicited a contracture. This result was confirmed on the sartorius, and it agrees with the findings of Wachholder & Matthias (1933), Cicardo (1938) and others in various frog-muscle preparations.

That the removal of sodium did not abolish the contractility of the muscle was shown by the fact that application of potassium chloride (20–50 mM.) still produced a contracture. With the rectus abdominis the KCl contractures did not differ markedly whether sodium was present in the solution or not.

With the sartorius the pronounced initial twitching, which is normally produced by excess potassium, was abolished when sodium had been removed leaving only the contracture response. Direct electrical stimulation was, on the other hand, very ineffective in producing a mechanical response in the absence of sodium. Using six pairs of stimulating electrodes along the length of a sartorius muscle, condenser discharges (RC product = 1 msec.) could produce only very weak local contractions. Similar results were obtained by Overton (1904) with muscles in sodium-free solutions provided some calcium salts were present.

Sodium ions and acetylcholine depolarization

Although these experiments showed that the presence of sodium was necessary for the appearance of the mechanical response to Ach, such observations alone are inconclusive in that they cannot reveal the direct action of Ach. Kuffler (1943) showed that in the concentrations used here Ach depolarizes only the regions of the muscle fibre around the neuromuscular junction. The removal of sodium by stopping propagation would prevent the spread of depolarization farther along the fibre, and it is therefore conceivable that an insufficient length of each fibre might have been depolarized to give an observable mechanical response. The action of Ach would be similar to an electrical stimulus acting at one or a few points along each fibre. On the other hand, if the entire fibre is depolarized, as by potassium chloride, there is a visible contraction even in the absence of a propagating mechanism.

It was therefore necessary to study the action of Ach more directly, and to record electric potential changes at the end-plate focus of the muscle. When Ach in a concentration of $1–2 \times 10^{-6}$ was applied to a muscle previously soaked in Ringer solution the focal region became depolarized (see Fig. 3*a*). Simultaneously, propagated action potentials were set up which, though not faithfully reproduced by the recording apparatus, complicated the record by being led off at both electrodes. After propagated impulses had ceased the true level of depolarization could be determined. The depolarization was maintained over a period of minutes, its amplitude depending on the Ach concentration. In the absence of anti-cholinesterases it is unlikely that all the end-plates would be reached, or, at any rate, uniformly acted on. The depolarization at an end-plate focus of the sartorius produced by Ach in the stated concentrations amounted under these circumstances to 5–10 mV.

A difficulty was encountered when the muscles were soaked in sodium-free solutions. Although the surface of uninjured muscles was normally, i.e. after immersion in Ringer solution, at almost uniform potential (differences not exceeding 1 or 2 mV., see Fig. 2), when NaCl had been replaced by glucose or sucrose, potential differences of up to 10 mV. appeared, which varied in an irregular manner along the muscle surface and were very unsteady. Similar effects have previously been described by Fenn (1931), who suggested that they

might be responsible for changes in the mechanical state of the muscle. With the sartorius these potential differences usually vanished after prolonged soaking in the sodium-free solution (1–3 hr.). Other investigators (Lorente de Nó, 1947; Nastuk & Hodgkin, 1950) have been able to overcome such difficulties in measurements of the resting potential of nerve and muscle by using choline chloride, rather than a non-electrolyte, to replace NaCl. But choline has itself a depolarizing action on end-plates, and was therefore not suitable, nor has any other 'inert' cation been found which could be used.

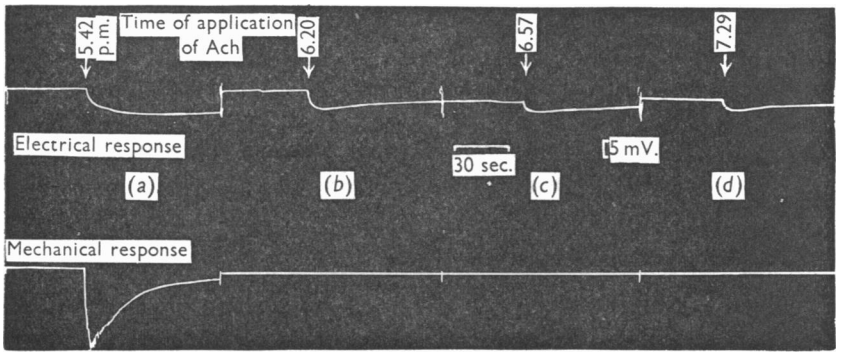

Fig. 3. Electrical and mechanical responses of a sartorius muscle to Ach 2×10^{-6}: (*a*) after bathing in Ringer solution; (*b*) after bathing in a mixture of 5% Ringer solution and 95% sodium-free solution; and (*c*) and (*d*) after bathing in sodium-free solution. The muscle was immersed in the solutions for about 35 min. before Ach was applied. Each test with Ach was immediately preceded by application of some of the bathing solution. Individual spikes do not appear in the electrical record because of the slow response of the recording instrument.

After sartorius muscles had been immersed in sodium-free solution long enough for the initial potential differences to subside, Ach still evoked a depolarization of the end-plate focus. The maximum depolarization was, however, only about 50% of that occurring in the presence of sodium, in spite of the fact that the sodium-free solution had a much lower conductivity and therefore caused less external shunting of the true membrane depolarization. The depolarization was considerably increased when only 5–10% of the NaCl contained in Ringer solution was added to the sodium-free medium. This is seen in Fig. 3, where Ach was applied in a concentration of 2×10^{-6} to a sartorius muscle after soaking (*a*) in Ringer solution, (*b*) in a solution containing 5·8 mM. sodium (i.e. 5% of the sodium content of Ringer solution), and (*c*) and (*d*) in sodium-free solution.

After each Ach application the solution in the bath was changed and the muscle allowed to equilibrate in the new solution for about 35 min. before another test. Immediately preceding each test with Ach a few drops of the bathing solution were applied to the muscle. Although in the experiment illustrated the initial potential gradients occurring in electrolyte-deficient solutions subsided rapidly, there is a small deflexion of the electrical record at the beginning of test (*b*)

caused by a shunting of the potential difference between the points under the two electrodes. The two tests (*c*) and (*d*) in sodium-free solution show that no further change attributable to a continued loss of sodium took place following the initial periods of soaking.

When, instead of adding sodium, the calcium ion content of the sodium-free solution was increased to three times that in Ringer solution, or both the calcium and potassium content raised (calcium three times, potassium two times), a decrease in the Ach-depolarization was observed. This is of interest because it confirms that the potentiation of the Ach effect was specifically due to sodium ions. The reduction of the recorded depolarization by added calcium or calcium plus potassium was probably largely the effect of increased shunting, although a specific action of the ions cannot be excluded. Adding then small amounts of sodium to the solution of high calcium and potassium content caused an increase of the depolarization.

Removal of sodium not only reduced the maximum recorded effect of Ach, but also necessitated two to three times higher Ach concentrations in order to attain this maximum.

It is clear from the foregoing that the presence of sodium is not essential for the occurrence of a depolarization at the end-plate, but that it somehow serves to potentiate the action of Ach. This might be explained if sodium ions are normally responsible for the setting up of a 'local response' in the neighbourhood of the end-plate, that is, for an active reinforcement of the depolarization produced by Ach. Hodgkin & Katz (1949) have suggested that sodium ions mediate in the excitatory process by entering the fibre as it becomes depolarized, thereby intensifying the initial potential change and causing the depolarization to spread to a greater distance than would be possible simply by means of the passive cable properties of the fibre. Because of the scattered distribution of end-plates in a muscle a portion of the observed potential change must be due to spread of the depolarization from end-plates situated a short distance away from the recording lead. Sodium ions would therefore cause an increased potential change in two ways: first, by augmenting the depolarization immediately at the end-plate; and, secondly, by allowing more end-plates to contribute, due to an increased spread of depolarization.

Relation between depolarization and acetylcholine concentration

Having found that sodium ions reinforce, but are not essential for the electrical action of Ach, further experiments were made in the absence of sodium. The electrical changes so recorded give probably a simpler and more direct indication of the reaction between Ach and the end-plate membrane.

To obtain consistent quantitative measurements some improvements in the stability of the preparation were necessary. The m. extensor digiti IV provided certain advantages. Potential differences which occurred when preparations were immersed in sodium-free solutions subsided more quickly in this small

muscle, while the individual fibres survived as well as in the sartorius. The results became also more consistent when prostigmine (as the bromide salt) in a concentration of 10^{-6} was added to the solutions. In this way, with an anti-cholinesterase present, Ach was more likley to exert a uniform effect throughout the muscle, and to reach the deeper as well as the superficial fibres. As a result the observed potential changes became appreciably larger.

With these improvements it became possible to measure the relation between Ach concentration and depolarization. Muscles were bathed in sodium-free solutions, and Ach was applied in various concentrations at intervals of 15–20 min. In Fig. 4 tracings of several records obtained on a single muscle are

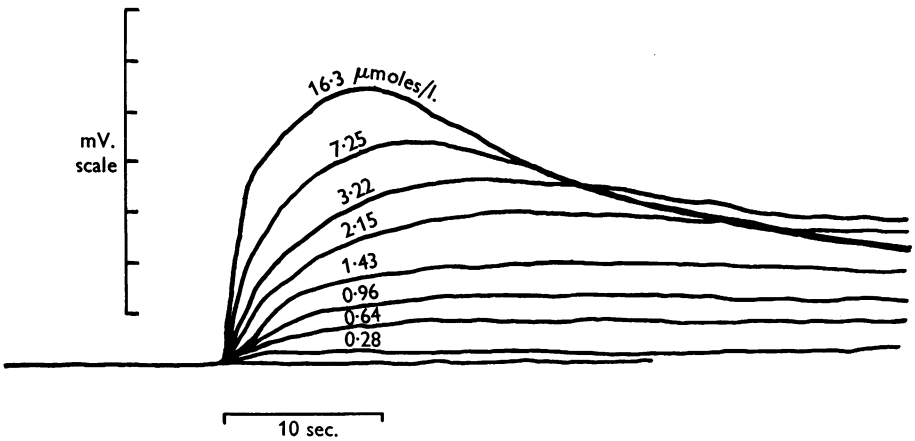

Fig. 4. Tracings of electrical records showing the depolarization at the end-plate focus of m. extensor digiti IV when applying various concentrations of Ach. The muscle had previously been bathed in sodium-free solution containing prostigmine bromide 10^{-6} and was kept in that solution between tests. The Ach concentration is indicated on each trace (1 μmole/l. corresponds to a concentration for Ach chloride of $1{\cdot}82 \times 10^{-7}$).

superimposed. The Ach concentration is shown on each trace in μmoles/l. The concentrations range from one which produced a barely detectable depolarization to one which gave a maximum effect. Of six muscles examined in this way all gave essentially similar results. The relative changes in depolarization were the same within the same concentration range. The absolute value of depolarization, however, varied from muscle to muscle, depending, no doubt, on the local density of end-plates.

An interesting phenomenon was that with high concentrations of Ach the initial depolarization was not maintained. This had also been observed, although much less marked, in the presence of sodium. Further addition of Ach, following the decline of depolarization, did not restore it to its original value. In fact, long periods of washing in Ach-free solution were necessary for the depression of the end-plate sensitivity to pass away, and even then

after concentrations exceeding 30 μmoles/l. complete recovery was usually not obtained. Measurements made with such large doses, although not wholly reproducible, were necessary as they indicated the size of the maximum depolarization which could be obtained.

In Fig. 5 the depolarization reached after each application of Ach is plotted against Ach concentration. The results have been taken from the experiment illustrated in Fig. 4. For convenience Ach concentration is plotted on a logarithmic scale. The exact shape of the curve is probably of no special significance; it is drawn to a maximum at concentrations which were found to

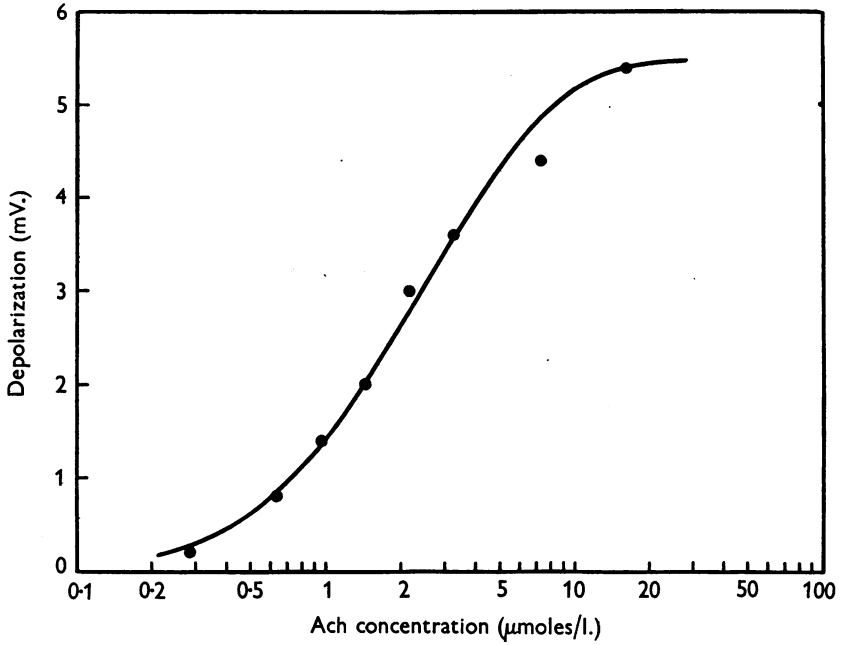

Fig. 5. Height of depolarization produced in the muscle of Fig. 4 plotted against Ach concentration. Ach concentration is plotted on a logarithmic scale.

produce a maximum depolarization in several similar experiments. What is important is the range of concentrations in which Ach begins to be effective on the end-plates. One-half its maximum effect occurs at a concentration of about 2 μmoles/l.

Some comment is required on the recovery of membrane potential when Ach was removed from the bath. The time course is illustrated in Fig. 6, where a muscle was washed with Ach-free solution 50 sec. after the application of Ach in a concentration of $1{\cdot}2 \times 10^{-6}$. Depolarization was only partly reversed immediately after the removal of Ach. There was a residual effect which required prolonged washing and then gradually disappeared, and this was found at all Ach concentrations used.

Bathing the muscle in D-tubocurarine chloride in a concentration of 10^{-5} greatly reduced the depolarizing effect of Ach. It is interesting that eserine sulphate in a concentration of 10^{-4} produced the same effect. This observation agrees with those of Eccles & MacFarlane (1949), who found that eserine, even in moderate concentrations (i.e. concentrations necessary for complete cholinesterase inhibition), produces a depression of end-plate potentials. With the concentrations used in the present experiments neither curarine nor eserine had a depolarizing action themselves.

As substitutes for NaCl, glucose, sucrose, choline chloride, LiCl, and mixtures of glucose and $CaCl_2$ were tried. Sucrose gave in all respects the same results as glucose. Choline chloride, when used in a concentration of 0·12 M. produced an immediate end-plate depolarization which slowly disappeared. After 2 hr. in this medium the excitability of the end-plates was irreversibly abolished.

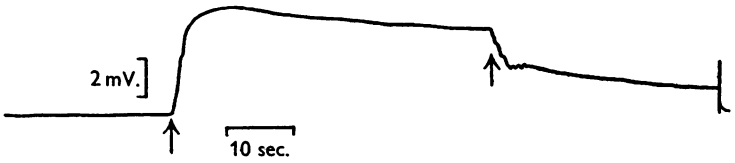

Fig. 6. Record of depolarization at the end-plate focus of a muscle bathed in sodium-free solution containing prostigmine bromide 10^{-6}. At the first arrow Ach $1{\cdot}2 \times 10^{-6}$ was applied. At the second arrow the muscle was washed with solution free of Ach. The 'jerky' deflexions during the washing arise from transient shunting when individual drops flowed along the muscle.

Lithium chloride had the same effect as NaCl, as Overton (1902) had already found in his studies on the excitability of frog muscle. Increasing the $CaCl_2$ in the sodium-free solution to 22 mM., while reducing the initial instability of potential, caused an irreversible decrease in the excitability of the muscle, again confirming Overton (1904).

DISCUSSION

The experiments described in this paper rule out the suggestion that Ach might act primarily by making the end-plate more permeable to sodium ions. Sodium is, nevertheless, important for the normal process of neuromuscular transmission in that it amplifies the initial local depolarization and enables it to lead to a propagated potential change and associated contraction. As was shown for direct electrical stimulation, only very weak local contractions can be set up in the absence of sodium by depolarizing at a few points, while when the muscle fibre membrane is depolarized along its entire length, as by potassium chloride, the contraction is much the same whether or not sodium ions are present.

Since the movement of sodium ions is not the immediate cause of the end-plate depolarization the next possibility to consider is whether Ach^+ ions could themselves discharge the membrane to the observed extent. The only specialized property of the end-plate region of the muscle fibre necessary for this mechanism

would be a specifically high permeability to Ach. For the membrane potential to be reduced by any given amount a certain rate of flow of electric charge across unit area of membrane must be provided by the depolarizing agent. When Ach^+ ions move inward the electric potential difference across the membrane which normally maintains the high internal potassium concentration (see Ussing, 1949) is reduced, and an outward flow of potassium ions must occur. For every observed steady level of membrane potential the inward flow of Ach must equal the outward flow of potassium. Chloride ions need not be considered, as in the sodium-free solution their concentration was reduced to only 4 mM. compared to 120 mM. in Ringer solution.

If Ach is to be an effective depolarizing agent in concentrations of only a few μmoles/l., while diffusible potassium ions are present within the fibre in a concentration of about 100 mmoles/l., the membrane will have to be exceedingly permeable to Ach, in any case, very much more so than to potassium. Some approximate calculations can be made to inquire whether such a mechanism is at all feasible for the concentrations involved. The only mathematical treatment at present available for the purpose is that of Goldman (1943). According to this theory the net outward flux, ϕ, for a univalent cation, defined as its rate of transfer across unit area of membrane, is given by

$$\phi = -P\frac{EF}{RT}\frac{c_o - c_i e^{-EF/RT}}{1 - e^{-EF/RT}}, \qquad (1)$$

where P is a permeability coefficient for the ion due to osmotic forces alone; E is the membrane potential; c_o and c_i are the outside and inside concentrations of the ion; and R, T and F are the gas constant, the absolute temperature and the faraday, respectively. A similar equation with c_o and c_i reversed applies for anions. (For the assumptions involved in its derivation see Goldman, 1943; and also Hodgkin & Katz, 1949).

The relationships expressed in equation (1) may be used to calculate the increased outward flux of potassium as the membrane is depolarized in the absence of sodium. If the end-plate region is assumed to have a potassium permeability equal to that of other regions of the fibre, the permeability coefficient P can be obtained from studies in which radioactive tracers have been used. From the results of the 'soaking in' experiments of Harris & Burn (1949), together with a membrane potential in Ringer solution of 87 mV. (Hodgkin & Nastuk, 1949), the permeability coefficient P for potassium ions is calculated to be $2{\cdot}7 \times 10^{-7}$ cm./sec. By means of capillary microelectrodes the membrane potential was observed after the removal of sodium and was found to be rather less than in Ringer solution. The potentials were highly variable, ranging between 55 and 80 mV. On returning to Ringer solution after 2 hr. the membrane potential recovered its original value of 80–90 mV. The fact that in the sodium-free solution the membrane potential was less than

for a potassium concentration cell, i.e. $RT/F \log_e c_i/c_o$, indicates that the fibres are losing potassium. This leak can be allowed for in the mathematical treatment by assigning to c_o a value higher than the true external potassium concentration. In any case, the initial size of the resting potential has, over a wide range, only a very small effect on the relations calculated here. In Fig. 7 a curve is shown, calculated from equation (1), which relates membrane depolarization to extra potassium flux resulting from the depolarization.

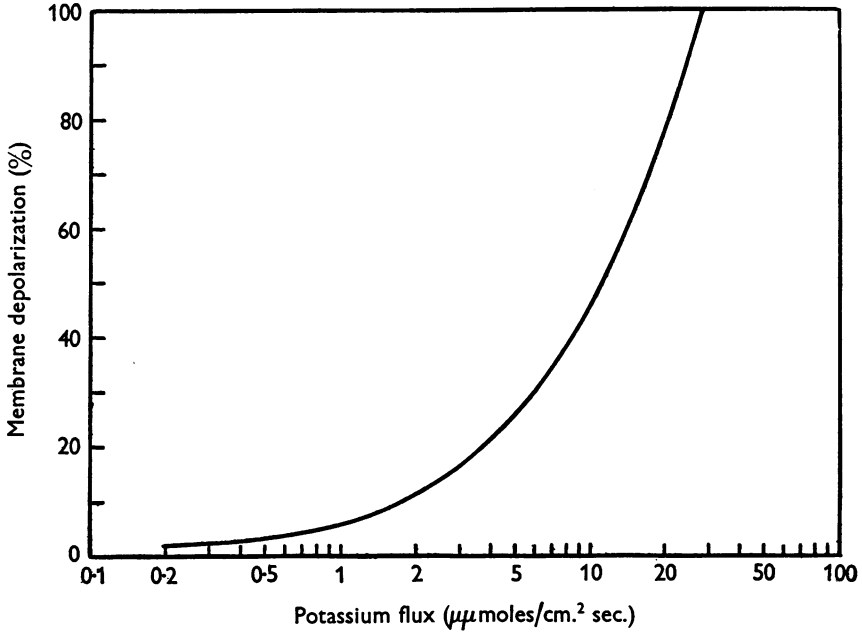

Fig. 7. Membrane depolarization, in percentage, plotted against outward potassium flux calculated from equation (1). Potassium flux is plotted on a logarithmic scale.

In order to apply this curve to the experimental findings some assumption must be made which will permit the actual magnitude of membrane depolarization to be derived from the observed potential changes. The simplest assumption is that the maximum effect observed with high Ach concentrations corresponds to a complete depolarization of the end-plate membrane.

Since the inward flux of Ach at each steady level of depolarization must be equal to the extra outward flux of potassium, the relation of Ach flux to Ach concentration can be obtained by comparing Figs. 5 and 7. The relative ordinate scales must first be adjusted so that maximum depolarization in Fig. 5 corresponds to 100% depolarization in Fig. 7. It is then seen that over the region where the curves are determined with greatest accuracy, i.e. at about one-half depolarization, the ratio of inward Ach flux to external Ach concentration is approximately 5×10^{-3} cm./sec. This quantity represents a form of permeability

coefficient for Ach. It should be noted, however, that it is based only on the concentration existing in the solution around the muscle and not on the true difference in concentration across the membrane. With so high a ratio of flux to concentration it must be inquired whether diffusion of Ach in the solution external to the end-plate membrane would be sufficiently rapid to allow the proposed mechanism to operate. In order to form a quantitative estimate a rough guess must be made regarding the size and shape of the end-plate. This estimate (see Appendix) suggests that the required ratio is, indeed, close to that set by diffusion. In other words, the factor limiting the flux of Ach is not the barrier imposed by membrane permeability, but rather the rate at which Ach in the immediate neighbourhood of the end-plate is replaced by diffusion from the large volume of external solution. If, then, the view is accepted that Ach depolarizes by direct penetration at the end-plates, a mechanism must be provided whereby any Ach^+ ion that approaches the end-plate receptors is immediately transferred with its positive charge into the interior of the fibre. This result would be accomplished by the presence in the membrane of a carrier, in temporary combination with which Ach and certain other depolarizing substances would cross. A similar mechanism was invoked by Hodgkin & Katz (1949) to account for the apparently high sodium permeability during the action potential. The action of curarine, and to a lesser extent of eserine, would be to compete with Ach for the carrier substance without being transported by it across the membrane.

The passage of Ach across the membrane may conceivably be only the first step in the depolarization process. Some secondary reaction quite apart from the entry of sodium might then occur which amplifies the potential change produced by Ach. An internal anion which diffuses outward whenever the membrane becomes partly depolarized by Ach, could constitute such a mechanism.

For more direct evidence on the processes occurring at the end-plate a better experimental preparation will have to be used. Small groups of muscle fibres were dissected out of the m. adductor magnus and used in a modification of the procedure described for exploring the extensor digiti IV. Depolarizations around end-plates were recorded, but the fibres were in poor condition and did not survive soaking in the different solutions. The microelectrode technique was also tried, but certain difficulties in the procedure have so far precluded its use in more critical experiments.

SUMMARY

1. The origin of the depolarization which acetylcholine produces at the motor end-plates of muscle fibres is discussed. One of the suggested mechanisms which requires an increase of the sodium permeability of the end-plate membrane is subjected to experimental test.

2. When frog muscles are kept in sodium-free solutions, Ach in concentrations of 10^{-7} and 10^{-5}, although not eliciting any visible mechanical response, still produces a local depolarization at the end-plate.

3. The presence of sodium increases the depolarization produced by Ach and leads to the setting up of propagated impulses. Sodium in concentrations below that necessary for propagated impulses already exerts a marked effect on the magnitude of the local depolarization.

4. The quantitative relation between Ach concentration and end-plate depolarization is investigated in the absence of sodium. Its theoretical significance is discussed from the point of view that the depolarization may be due to a direct penetration of the end-plate membrane by Ach^+ ions.

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APPENDIX

Mathematically the simplest assumption which can be made to calculate the rate of diffusion of Ach up to the end-plate would be to represent the end-plate as a spherical sink in an infinite volume of solution. It can then be shown that, starting with a uniform concentration c^0 of a substance with diffusion coefficient D throughout the infinite volume, and maintaining at all times zero concentration at the surface of the sphere of radius ρ^0, the concentration c at any time t and at any distance ρ from the centre of the sphere ($\rho > \rho^0$) will be given by

$$c = c^0 - \frac{\rho^0 c^0}{\rho}\left(1 - \operatorname{erf}\frac{\rho - \rho^0}{2\sqrt{Dt}}\right). \tag{2}$$

From equation (2) the ratio of flux across the surface to concentration at an infinite distance is found to be

$$\frac{\phi}{c^0} = \frac{D}{\rho^0} + \sqrt{\frac{D}{\pi t}}. \tag{3}$$

(Cf. Carslaw & Jaeger (1947) for the derivation of an equation analogous to equation (2) applied to heat conduction in solids.)

For Ach the condition that the concentration at the end-plate surface remains zero will be fulfilled if Ach is immobilized or removed in some way as rapidly as it enters the fibre so that it cannot diffuse back across the membrane. From the equivalent conductance found for Ach^+ in dilute solutions (29 $\Omega.^{-1}$ cm.^{2}) the diffusion coefficient is calculated to be $7{\cdot}6 \times 10^{-6}$ cm.^{2}/sec. The selection of a value for ρ^0 can only be made very approximately. If ρ^0 is taken as of the same order as the radius of the fibre, say 5×10^{-3} cm., a steady state of Ach flux will be reached with ϕ/c^0 equal to $1{\cdot}5 \times 10^{-3}$ cm./sec.

If this figure is compared with the theoretically required Ach 'permeability' of the end-plate membrane, i.e. 5×10^{-3} cm./sec. (p. 419), it is seen, as far as the accuracy of this procedure will allow, that the two are of the same order of magnitude. Thus, while the limit set by diffusion in the external medium may not make the theory altogether untenable, it does become the factor deciding the rate at which Ach will pass the end-plate membrane under the conditions of the experiments described in this paper.

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