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THE EFFECT OF CALCIUM ON CONTRACTION AND CONDUCTANCE THRESHOLDS IN FROG SKELETAL MUSCLE

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SUMMARY

1. The effect of extracellular calcium and magnesium on the contraction threshold and on the thresholds for an increase in sodium and potassium conductance with depolarization was studied in voltage-clamped frog muscle fibres.

2. A larger depolarization was required to reach each of the three thresholds when the concentration of divalent cation was increased.

3. The contraction and potassium conductance thresholds appeared to shift in parallel with alterations in calcium over the concentration range 0·2–10·0 mM and in magnesium over the concentration range 5·4–90·0 mM. The shift amounted to about 4 mV for a threefold change in concentration of divalent cation.

4. The sodium conductance threshold was much more sensitive to alterations in divalent cation concentration than was either the contraction or the potassium conductance threshold.

INTRODUCTION

In the squid giant axon, the threshold for the increase in sodium or potassium permeability with depolarization is dependent on the extracellular calcium concentration (Frankenhaeuser & Hodgkin, 1957); an increase in calcium is similar to hyperpolarization of the nerve membrane, that is, a larger depolarization is required to elicit the characteristic permeability changes when the extracellular calcium concentration is raised. In frog skeletal muscle, both the threshold for an increase in sodium or potassium permeability (Jenerick, 1959) and the contraction threshold (Lüttgau, 1963) are shifted by extracellular calcium so that a larger depolarization is required to activate both the permeability changes and the mechanical response when the calcium concentration is raised. When the results of the latter two studies are compared, it appears that the

quantitative effect of calcium on these three thresholds is quite different. Thus, a threefold increase in extracellular calcium results in an 18 mV shift in the contraction threshold (Lüttgau, 1963), while a tenfold increase in extracellular calcium shifts the sodium threshold by 20–25 mV and the potassium threshold by 15 mV (Jenerick, 1959). The present study was designed to reinvestigate the effect of extracellular calcium on these three thresholds and to extend the observations over a wider range of extracellular calcium concentrations.

METHODS

The sartorius muscle of *Rana temporaria* was employed throughout. The muscle was mounted at about 4/3 of its slack length, and a segment of muscle far from either end was chosen for study. All experiments were performed at room temperature (21–23° C).

A muscle fibre was impaled with a micro-electrode and the membrane potential recorded by backing off the observed potential with a calibrated voltage source. A second micro-electrode, which served to pass current, was then inserted within 100 μ from the first. The recorded membrane potential usually fell with insertion of the second micro-electrode; the mean fall in membrane potential was 4·6 mV, and the mean membrane potential with both electrodes in position was −83·8 mV.

Recording micro-electrodes were filled with 3 M-KCl and were selected for resistances in the 6–15 MΩ range and for low tip potentials. Electrodes were discarded if tip potentials exceeding −10 mV (electrode negative with respect to surrounding solution) developed in the course of the experiment. Stimulating electrodes were filled with 2 M potassium citrate, and their resistances were usually somewhat lower, about 4–12 MΩ.

The membrane potential was altered by a feed-back technique which is outlined in Fig. 1. The potential difference between the recording electrode and a large agar-filled electrode in the bathing solution was opposed by the calibrated voltage source and fed, via cathode followers, to a recording oscilloscope and to a feed-back amplifier. The output of the feed-back amplifier drove the second micro-electrode, so that, when the feed-back loop was closed, the potential difference between the recording electrode and the extracellular space was clamped at approximately the voltage of the calibrator. The vertical amplifier circuit of a Tektronix 502 oscilloscope served as the feed-back amplifier and provided a maximum open loop gain of 10,000. In general, the maximum gain could be employed without the development of oscillations in the feed-back loop, but on occasion it was necessary to decrease the open loop gain to 4000. Two measures were found useful in preventing oscillation of the feed-back loop. First, the stimulating electrode was carefully shielded by enclosing it in a brass tube to within 2–3 mm of its tip, and, second, the bathing solution was capacitatively coupled to earth by means of two platinum electrodes immersed at either end of the muscle chamber.

Fibres were clamped at a membrane potential of −90 mV, and stepwise depolarizations were produced by introducing an appropriate pulse into the feed-back loop. For a step input to the feed-back amplifier, a rise time of voltage at the recording electrode of less than 1 msec was readily obtained.

Total current through the fibre was recorded as an IR drop across a 4300 Ω resistor connecting the bath to earth. A capacitor shunted the resistor so that the time constant of this circuit was 0·5 msec.

It should be emphasized that the feed-back system clamped only that point of the fibre at the site of the recording electrode, so that an applied voltage step spread electrotonically

along the length of the fibre. The voltage–current relationship, therefore, reflected the total input impedance of the fibre.

Determination of the contraction threshold. In experiments in which the contraction threshold was to be determined, 10^{-6} g tetrodotoxin/ml. was added to the bathing solution to prevent action potentials. The site of electrode impalement was observed with a dissecting microscope at a total magnification of 100×. A single depolarizing pulse, 200 msec in duration, was applied, beginning with a step well below the contraction threshold, and the pulse magnitude was increased in 3 mV steps until a brisk local contraction was seen. The pulse

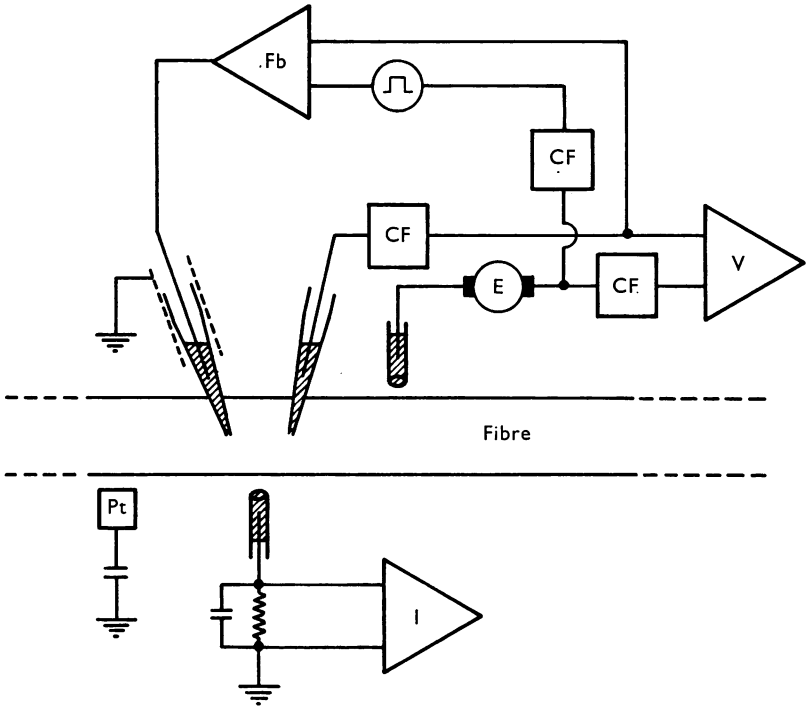

Fig. 1. Diagrammatic representation of feed-back circuit. The fibre was impaled by two closely spaced micro-electrodes, and two low resistance electrodes filled with agar containing 120 mM-NaCl were positioned in the bathing fluid. Fb = feed-back amplifier; CF = cathode follower; E = calibrated voltage source; V and I = dual-beam oscilloscope for the recording of membrane potential and total current through the muscle fibre; Pt = platinum-foil electrode in bathing solution. For details, see text.

was then diminished in 2 mV steps and the threshold was taken as 1 mV larger than the pulse which just failed to elicit visible motion. In general, all motion disappeared when the pulse had been decreased by 4–8 mV. As the threshold was approached, a series of 3 or 4 pulses at intervals of 2 sec or more was usually applied at each pulse step.

If the contraction threshold was approached more slowly, e.g. with 1 mV step increases in the magnitude of the depolarizing pulse, the threshold usually shifted 3–4 mV toward the resting potential after an initial contraction had been elicited. The cause of this shift is not clear, but, after it had occurred, the threshold appeared to be quite stable on repeated testing. On occasion, very large depolarizations produced an apparent threshold shift of as

much as 20 mV toward the holding potential of −90 mV, accompanied by a decrease in the current required to clamp the fibre at −90 mV. It seems likely that this apparent shift was an artifact due to the development of a large negative tip potential at the recording electrode secondary to the vigorous contraction.

Determination of the potassium conductance threshold. After the contraction threshold was determined, a series of 200 msec depolarizing pulses, increasing in 4–5 mV steps, was applied to the fibre, and current–voltage curves were plotted from the value of the current at the end of the pulse. Slight irregularities of the current record, presumably arising from muscle movement, consistently appeared with depolarizations beyond the contraction threshold. The irregularities were ignored in reading the current records, and it was assumed that the current changed smoothly toward its steady value. The records of about 5% of the fibres tested were discarded because of large movement artifacts. Depolarizations of greater than 15 mV beyond the contraction threshold produced gross irregularities, and this portion of the current–voltage curve could not be explored.

TABLE 1. Composition of solutions (mM)

	NaCl	KCl	$CaCl_2$	$MgCl_2$	$MgSO_4$	NaH_2PO_4	Na_2HPO_4	Tris*
A	115	2·5	1·8	—	—	0·85	2·15	—
B	117	2·5	0·2	—	—	0·85	2·15	—
C	110	2·5	5·0	—	—	0·28	0·72	—
D	106	2·5	10·0	—	—	0·14	0·36	—
E	114	2·5	1·8	—	—	—	—	4·0
F	109	2·5	—	5·4	—	—	—	4·0
G	92	2·5	—	18·0	—	—	—	4·0
H	48	2·5	—	34·0	20·0	—	—	4·0
J	—	2·5	—	55·0	35·0	—	—	4·0

*Tris(hydroxymethyl)aminomethane titrated to pH 7·2 with HCl.

Solutions. The solutions in which the muscle was bathed are given in Table 1. In each muscle studied, appropriate thresholds were determined in three groups of fibres; the first and third groups were obtained with the muscle in one solution and the second with the muscle in a different solution. No consistent difference in threshold was observed between the first and third groups, and they were considered together in all experiments. Solutions were changed by draining the muscle chamber, and this was repeated at least once during a 20 min waiting period before a group of fibres was studied in the new solution. One of the two different solutions to which each muscle was exposed was either solution *A* or solution *E*, both of which contained 1·8 mM calcium, so that the shift in threshold from the value at this calcium concentration could be determined directly in each muscle. Solution *A* served as the control solution when the test solution contained phosphate buffer, while solution *E* served the same purpose for the Tris-buffered test solutions. No difference in contraction threshold or in the sodium or potassium conductance thresholds was observed between muscles bathed in solution *A* or solution *E*.

Tetraethylammonium (TEA) or lithium solutions were prepared by replacement of NaCl in solution *A* with an equimolar amount of TEA chloride or LiCl (obtained as reagent grade salts from British Drug Houses, Ltd.). All other reagents were analytical grade. No attempt was made to eliminate trace amounts of calcium from the magnesium-containing solutions. The effect of zinc ion was studied by the addition of the desired amount of zinc acetate to solution *E*. Tris(hydroxymethyl)aminomethane was obtained from Sigma Chemical Co.

RESULTS

Effect of calcium

Contraction threshold. The contraction threshold in 1·8 mM calcium was $-48{\cdot}5 \pm 0{\cdot}5$ mV (S.E. of mean), and the effect of altering the calcium concentration is shown in Fig. 2. Over the range of calcium concentrations from 0·2 to 10·0 mM, the contraction threshold shifted about 15 mV; the

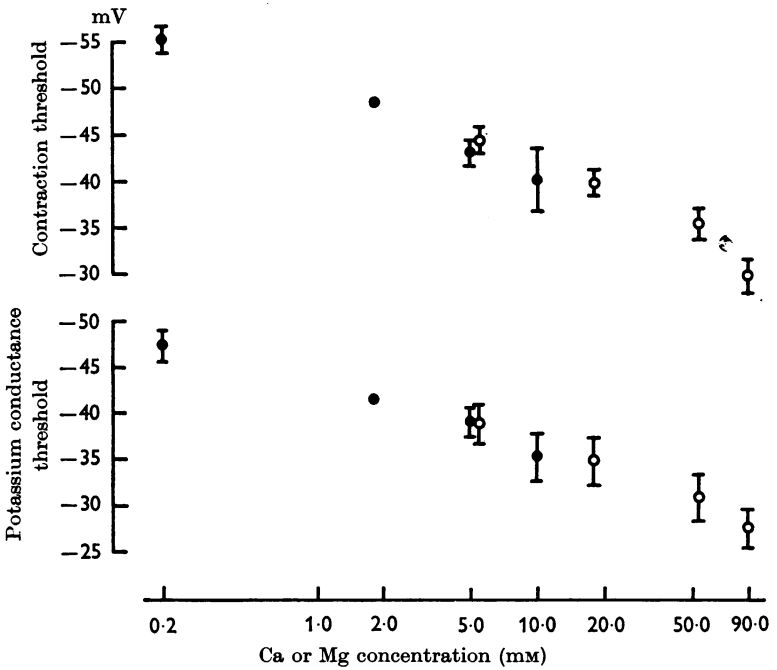

Fig. 2. Effect of divalent cations on the contraction threshold (above) and the threshold for the increase in potassium conductance with depolarization (below). In each muscle studied, thresholds were determined in 1·8 mM calcium and in one other concentration of calcium or magnesium (see Methods). The mean values for the thresholds in 1·8 mM calcium from all the muscles studied are shown. The shift, with alteration of the Ca or Mg concentration, in each threshold from its value in the same muscle in 1·8 mM calcium is plotted as an absolute membrane potential, and the brackets indicate 95 % confidence limits for the shift at the concentration of divalent cation given on the abscissa. Filled circles, calcium; open circles, magnesium.

relation between the contraction threshold and the log of the calcium concentration is approximately linear in Fig. 2 and amounts to a 4 mV shift in threshold for a threefold change in calcium concentration.

In 10·0 mM calcium solutions, many fibres developed a localized opacity at the site of electrode impalement after a few contractions had been elicited. These changes, which were less than 25 μ in extent, appeared to be

localized contractures. Since the membrane potential of these fibres was well maintained, the changes were probably not the result of extensive damage to the membrane by the impalement. It seemed more likely that the slight membrane damage normally produced by insertion of a microelectrode permitted an appreciable leak of calcium into the fibre when the extracellular calcium was high.

Potassium conductance threshold. A current–voltage curve obtained from a fibre in solution *A* is shown as the continuous line in Fig. 3. In this fibre, the contraction threshold was -48 mV, and a marked decrease in input resistance occurred at a membrane potential of -40 to -45 mV. This decrease in resistance, which has been examined in detail by Adrian, Chandler & Hodgkin (1966), was assumed in the present experiments to reflect a specific increase in the potassium conductance of the muscle membrane. The criterion for determining the threshold for this increase requires some discussion. Since the contraction threshold consistently occurred a few millivolts before the marked fall in input resistance, muscle movement made small changes in the slope of the current–voltage curve of doubtful significance in this region. Accordingly a criterion was chosen which imposed the requirement that a relatively large change in the slope of the current–voltage curve be present to be considered significant. In practice, the threshold was taken as the point at which the curve rose by 7×10^{-9} A above the tangent to the nearly linear curve for smaller depolarizations. The additional requirement was imposed that this increase in current occur within 5 mV or less, so that a conductance increase of at least 1·4 μmhos was required to define the threshold. This procedure for determining the threshold is shown in Fig. 3, and it can be seen that the actual membrane potential at which the input resistance of the fibre began to decrease was a few millivolts closer to the resting potential than the nominal threshold, T_k.

Although a larger percentage change in the conductance of the smaller muscle fibres was required for the detection of the potassium threshold, this seemed to have little effect on the final results. In 1·8 mM calcium, the mean input resistance, measured with 5 mV depolarizing pulses at the holding potential of -90 mV, was 460 kΩ. In fibres with resistances above this mean value, the difference between the contraction threshold and the threshold for a conductance increase was 7·5 mV, while, in fibres with resistances below the mean, this difference was 6·5 mV.

The steady-state relationship between the total current passing through the muscle membrane and the membrane current density at the site of the recording electrode is given by Cole's Theorem

$$I_m = kI_0(\mathrm{d}I_0/\mathrm{d}V_m),$$

where I_0 is the total current through the fibre, I_m and V_m are the membrane current and potential respectively at the electrode site, and k is a constant

(Cole, 1961). If this relationship is applied to the total current–voltage curve in Fig. 3, the membrane current–voltage curve (I_m) is obtained, and it can be seen that an appreciable increase in the membrane conductance seems to occur quite near the contraction threshold. This close correspondence between the contraction threshold and the onset of an increase in potassium conductance has been noted by R. H. Adrian, W. K. Chandler, and A. L. Hodgkin (Personal communication).

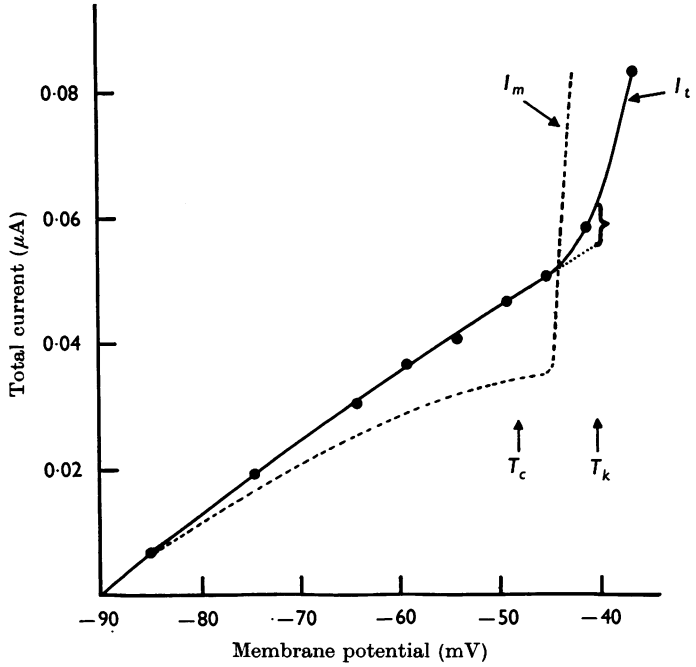

Fig. 3. Current–voltage relation of a fibre in 1·8 mM calcium (solution *A*) with tetrodotoxin (10^{-6} g/ml.). The fibre was clamped at a membrane potential of −90 mV, and 200 msec depolarizations to the potentials indicated on the abscissa were applied. The total clamping current, read at the end of a 200 msec pulse, was plotted, and the continuous curve I_t has been fitted by eye to these points. Current passing out of the fibre was taken as positive. T_c = contraction threshold; T_k = threshold for the increase in potassium conductance. T_k was determined as described in the text. I_m = membrane current density at the recording electrode. I_m has been derived from a Cole Theorem analysis of the total current curve I_t. The ordinate for I_m is in arbitrary units.

The effect of extracellular calcium on the potassium conductance threshold is shown in Fig. 2. The threshold in 1·8 mM calcium was $-41{\cdot}6 \pm 0{\cdot}4$ mV, and the shift when the extracellular calcium concentration was changed was quite similar to the shift in contraction threshold, that is, about 4 mV for a threefold change in extracellular calcium.

Potassium conductance threshold in hypertonic solution. The close correspondence between the contraction threshold and the threshold for an increase in potassium conductance raised the possibility that some motion artifact was responsible for the apparent conductance change. To investigate this, current–voltage curves were obtained in muscles exposed to solutions in which the tonicity had been increased to approximately twice isotonic by the addition of 340 mM sucrose. It was possible to depolarize fibres well beyond the conductance threshold in these solutions before the appearance of an obvious movement artifact on the current records. A current–voltage curve obtained from a fibre in hypertonic solution containing 1·8 mM calcium is shown in Fig. 4, together with a number of the original

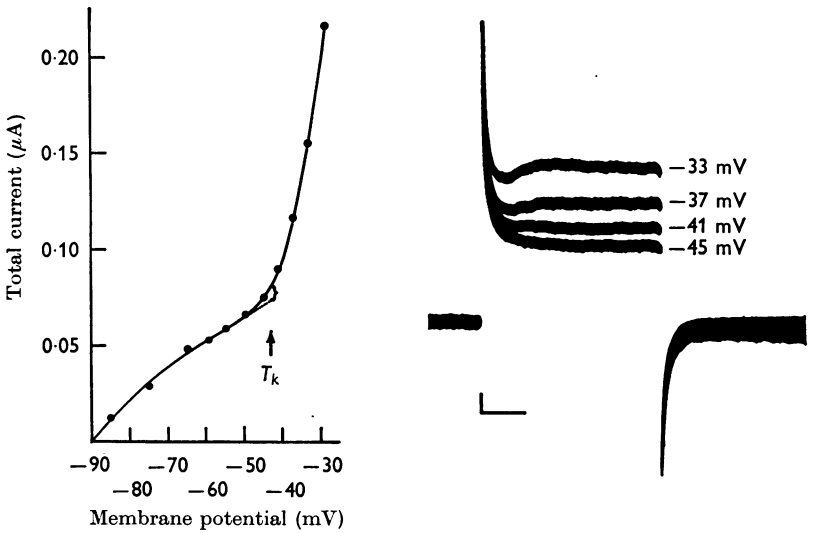

Fig. 4. Current–voltage relation of a fibre in hypertonic solution containing 1·8 mM calcium and tetrodotoxin (10^{-6} g/ml.). Left: total current–voltage curve obtained as described in Fig. 3. Right: four original current records from the same fibre which have been photographically superimposed. The potential to which the fibre has been depolarized is indicated on the right of each current trace. The over-shoot in the current (seen here as a thickening of the final portion of the current record) when the membrane potential was returned to −90 mV only occurred following the depolarization to −33 mV. Vertical line = 0·02 μA; horizontal line = 50 msec.

current records from the same fibre. The initial transient in the current records, which reflected the charging of the membrane capacity as the depolarizing step spread electrotonically along the length of the fibre, obscured the time course of the rise in potassium conductance. At the larger depolarizations, however, an increase in conductance was evident within 20 msec, and a progressive increase in current occurred for about 50 msec. The secondary fall in clamping current during the depolarizing

step to -33 mV probably reflects the time-dependent secondary inactivation of the potassium conductance characteristic of delayed rectification in frog skeletal muscle (Nakajima, Iwasaki & Obata, 1962). The possibility of a movement artifact as the cause of this secondary fall in current cannot be excluded, however, particularly since the current failed to return to its base line value following this pulse. With the exception of this relatively large depolarization step, no irregularity of the current records attributable to movement appears to be present.

To examine the conductance threshold in hypertonic solutions, each muscle was initially exposed to an isotonic solution with 1·8 mM calcium (Solution *A*), and the threshold determined in a group of fibres. The solution was replaced by a hypertonic solution containing 0·2, 1·8, or 10·0 mM calcium, and a second group of fibres was studied. The experiment was terminated after the muscle had been in the hypertonic solution for 2 hr. No change in threshold was observed over this time. The threshold was shifted by $-9{\cdot}2 \pm 2{\cdot}4$ mV in 0·2 mM calcium, $-4{\cdot}0 \pm 1{\cdot}4$ mV in 1·8 mM calcium, and $+3{\cdot}1 \pm 2{\cdot}6$ mV in 10·0 mM calcium (mean $\pm$ 95% confidence limits for the shift; a negative value represents a shift toward the holding potential of -90 mV). Thus at each calcium concentration the threshold in hypertonic solution was approximately 4 mV more negative than at the corresponding calcium concentration in isotonic solution, and the relation between the threshold and the log of the calcium concentration was again approximately linear with a slope of 4 mV for a threefold change in calcium concentration.

It is difficult to be certain that the slight displacement of the threshold toward the resting potential in hypertonic solution was not an experimental artifact. If movement of the muscle were to produce a transient hole in the membrane at the site of electrode impalement, the clamping current with depolarizing pulses beyond the contraction threshold would be decreased; this selective depression of the portion of the current–voltage curve beyond the contraction threshold would tend to shift the apparent conductance increase away from the resting potential, an effect which would be more marked in isotonic solutions where the contraction was much more vigorous. In a few fibres in isotonic solutions, the current–voltage curve actually developed a slight negative slope just beyond the contraction threshold, a result which would imply, by Cole's Theorem, an inward current in this region. This negative slope was usually associated with an over-shooting of the current base line upon return to the holding potential of -90 mV.

Sodium conductance threshold. When a muscle fibre which had not been treated with tetrodotoxin was depolarized beyond about -55 mV, a transient early decrease in current during the depolarizing pulse was seen (Fig. 5). This decrease was assumed to reflect the appearance of an inward sodium current resulting from an increase in the sodium conductance of the muscle membrane. To determine the threshold for this conductance increase, the current records of progressively increasing depolarization

steps were traced and superimposed, and the depolarization step was found in which the current at some time during the step was less than the level attained during the previous step. The average of these two depolarizations was taken as the threshold. In the fibre shown in Fig. 5, for example, the current within the first 50 msec at a potential of -51 mV was less than that at a potential of -55 mV, and the threshold was taken to be -53 mV. Further small depolarizations produced a progressive increase in the inward current until the feed-back circuit was inadequate to clamp the fibre and an action potential was generated. Pulses of 50–200 msec duration were employed in the sodium threshold experiments.

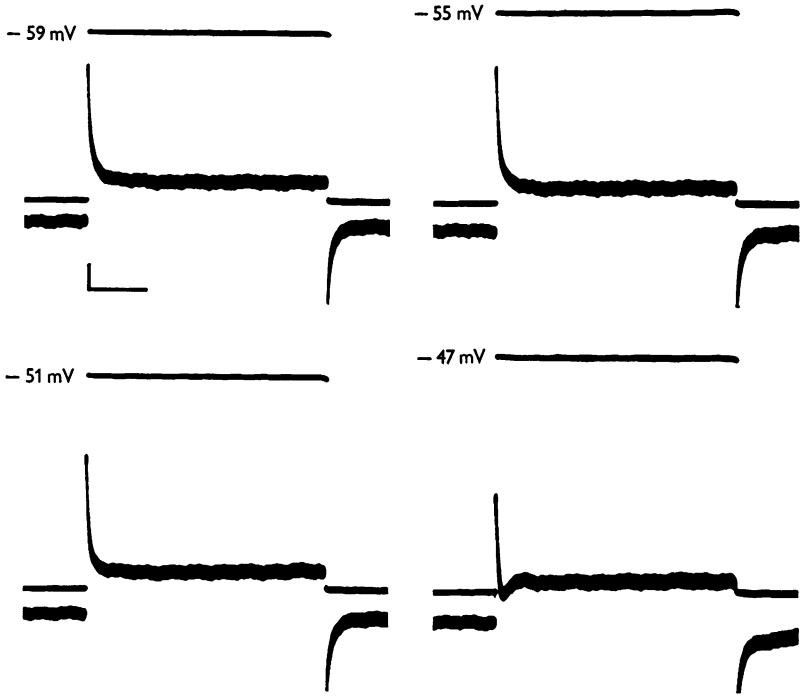

Fig. 5. Oscillographic records of total fibre current and membrane potential with progressive depolarizations. Muscle in solution *E* with no added tetrodotoxin. Upper trace, membrane potential. The fibre was clamped at a membrane potential of -90 mV, and the membrane potential to which the fibre was depolarized has been indicated to the left of each potential trace. Lower trace, total current. Current passing out of the fibre indicated by an upward deflexion. Vertical line = 0·02 μA; horizontal line = 50 msec.

In 1·8 mM calcium, the threshold for the increase in sodium conductance was $-53{\cdot}6 \pm 0{\cdot}6$ mV. The sodium threshold was much more sensitive to a change in extracellular calcium than was either the contraction threshold or the potassium threshold; the sodium threshold was shifted by more than

30 mV (Fig. 6) while the shifts in the contraction and potassium thresholds were 15 and 12 mV respectively (Fig. 2) over the range of calcium concentrations from 0·2 to 10·0 mM. In 10·0 mM calcium the contraction threshold was nearer the resting potential than was the sodium conductance threshold, and local contractions could be produced without the appearance of an action potential.

Effect of magnesium

Since fibres in 10·0 mM calcium developed small opacities at the site of electrode impalement, the effect of higher calcium concentrations was not examined. Magnesium, however, proved to be an adequate substitute for calcium in the present experiments. The optical changes seen in 10·0 mM calcium were not found even with magnesium concentrations of 90·0 mM, and the mean membrane potential in 5·4 mM magnesium (solution *F*) was −88·2 mV. Like calcium, magnesium shifted the sodium threshold (Fig. 6)

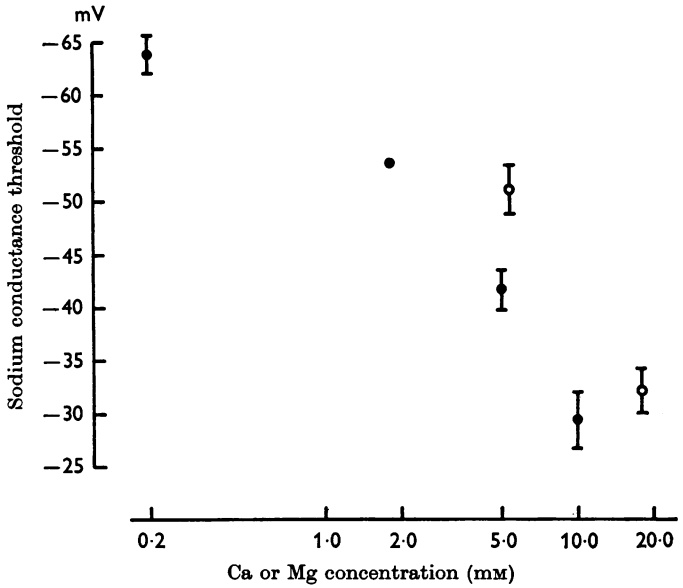

Fig. 6. Effect of divalent cations on the threshold for the increase in sodium conductance with depolarization. The results have been plotted as in Fig. 2. Filled circles, calcium; open circles, magnesium.

much more than the contraction or potassium threshold (Fig. 2). The values for the sodium threshold in 5·4 and 18·0 mM magnesium indicated that magnesium was about one half as effective as calcium, while the two ions seemed more nearly alike in their effect on the contraction and potassium thresholds. However, the shift in the latter two thresholds with alteration of divalent cation concentration was small relative to the un-

certainty of the experimental measurements, and it is not clear that this apparent difference in the relative effectiveness of magnesium and calcium on the contraction or potassium threshold and on the sodium threshold reflected selectivity in the action of the two cations on these thresholds.

As shown in Fig. 2, a progressive increase in the magnesium concentration of the bathing solution resulted in a further shift of both the contraction threshold and the threshold for an increase in potassium conductance. Up to a magnesium concentration of 54·0 mM, the shift in both thresholds was similar to that seen with altered extracellular calcium, that is, a shift of about 4 mV for a threefold change in extracellular magnesium.

Over the entire range of thresholds studied in both calcium and magnesium solutions, the contraction threshold shifted slightly more than did the potassium threshold. Thus in 0·2 mM calcium the two thresholds were separated by 7·8 mV and in 90·0 mM magnesium, they were separated by only 2·2 mV. It is not clear whether this represented a real difference in the effect of divalent cations on the two thresholds, as was apparently the case with the contraction threshold and the sodium threshold, or whether it was simply a reflexion of the difficulty in determining the potassium threshold in the present experiments. When fibres in solutions with low concentrations of divalent cations were depolarized to less than the potassium threshold (see Figs. 3 and 4), the current–voltage curve showed a slight increase in resistance which presumably reflected a fall in potassium permeability with depolarization (Katz & Lou, 1947). In the high magnesium solutions, on the other hand, a progressive decrease in resistance was usually seen with depolarization, so that the additional fall in fibre resistance at the potassium threshold might have been more readily detectable in the high magnesium solutions.

Other cations

Zinc 50 μM added to solution *E* had no significant effect on either the contraction threshold or the potassium threshold, while 0·5 mM zinc produced a small shift in both thresholds. Tetraethylammonium chloride, in concentrations of 57·5 or 115 mM, shifted both the contraction and potassium thresholds toward the resting potential, while lithium chloride was without significant effect on either threshold. These results are summarized in Table 2.

DISCUSSION

The present experiments confirm the observation (Jenerick, 1959) that the sodium conductance threshold is more sensitive to a change in extracellular calcium concentration than is the potassium conductance threshold. While the shift in potassium threshold in the present experiments

was somewhat less than that observed by Jenerick, the two studies are not strictly comparable, since Jenerick's results were obtained at calcium concentrations of 1·8 and 18·0 mM. Localized contractions were seen in the present experiments with an extracellular calcium concentration of 10·0 mM, and the concentration range was therefore limited to 0·2–10·0 mM.

The effect of extracellular calcium on the contraction threshold which was observed in the present experiments was quite different from the results of Lüttgau (1963). An increase in extracellular calcium from 1·8 to 5·0 mM in the present study shifted the contraction threshold from −48·5 to −43·2 mV, whereas Lüttgau reported a shift from −35 to −18 mV between the same two calcium concentrations. Unlike the present study,

TABLE 2. Effect of various cations on contraction and potassium thresholds. Values represent the mean ±95% confidence limits for the shift in threshold from the value in 1·8 mM calcium. A shift toward the holding potential of −90 mV is taken as negative

	0·05 mM zinc	0·5 mM zinc	57·5 mM TEA	115 mM TEA	115 mM lithium
Contraction threshold (mV)	+0·7±2·2	+3·5±2·0	−6·4±1·7	−8·6±2·1	+0·5±1·7
Potassium threshold (mV)	+0·5±2·8	+3·5±2·6	−4·2±2·0	−5·7±2·4	+0·7±1·8

Lüttgau examined the contraction threshold in single fibres depolarized by high potassium solutions, and the membrane potential was inferred by parallel micro-electrode recordings made on whole muscle; but Hodgkin & Horowicz (1960), employing a similar technique, found a contraction threshold of −50 mV in 1·8 mM calcium, a value in good agreement with that obtained in the present experiments. It would seem unlikely, therefore, that this difference in the experimental procedure could account for the discrepancy between Lüttgau's results and the present ones, and the reason for the discrepancy is not apparent.

The close correspondence between the contraction threshold and the threshold for an increase in potassium conductance which was found over a wide range of calcium and magnesium concentrations motivated the attempt to dissociate these two phenomena by exposure of the muscle to other ions. Both zinc (Mashima & Washio, 1964) and TEA (Hagiwara & Watanabe, 1955) are known to prolong the falling phase of the action potential, and it has been suggested that this effect might arise from interference by these ions with the increase in potassium conductance during the action potential. In the present study, 50 μM zinc, a concentration which produces a marked prolongation of the action potential, had no effect on either the potassium or the contraction threshold, and a concentration of 0·5 mM produced a slight shift in both thresholds. TEA, somewhat surprisingly, shifted both thresholds towards more negative membrane

potentials, but no definite evidence of dissociation of the two thresholds was found with either ion. If, as seems likely, the effect of these ions on the action potential is due to interference with the potassium permeability, it would seem to result from a decrease in the magnitude of the change in potassium permeability with large depolarizations rather than from a shift at all membrane potentials in the relation between potassium permeability and membrane potential.

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