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Charge movements in skeletal muscle

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In twitch muscle, an action potential propagating along the surface can lead to mechanical contraction of the entire cross section of the fibre. The processes involve a depolarization of the membranes of the transverse tubular system which, in turn, causes a release of calcium from its intracellular storage location, the sarcoplasmic reticulum. It seems that a change in potential across the first structure can trigger the release from the second, adjacent structure. If the time and voltage dependent ionic currents are blocked, small movements of charge can be detected when a fibre is depolarized from a normal resting potential to a potential at which contraction is activated. These charge movements, which do not behave as currents passing through ionic channels, may be part of a trigger mechanism.

Introduction

In skeletal muscle, contraction is normally activated by a depolarization of the membranes of the transverse tubular (T) system (Huxley & Taylor 1958). This activation occurs within a very narrow range of membrane voltage, -60 to -20 mV, in a muscle immersed in Ringers fluid at room temperature (Hodgkin & Horowicz 1960). The details of how this small change in me mbrane potential across the T-system causes a release of calcium from the adjacent sar coplasmic reticulum are not at all clear. One possibility would be to suppose that a charged molecule or part of a molecule, confined to the membrane, is free to move between two locations, one on either side of the membrane. The release of calcium would depend on the number of charged groups in the second or 'activating' position. The movement of charged groups following a change in membrane potential would contribute to the membrane current and if the density of charged groups were sufficiently high this component might be discernible.

In an attempt to detect such charge movements, Schneider & Chandler (1973) carried out voltage clamp experiments on frog sartorius fibres using the three microelectrode technique (Adrian, Chandler & Hodgkin 1970). Muscles were cooled to 2 °C in a solution containing 117.5 mM tetraethylammonium Cl (TEACl), 5 mM RbCl, 1.8 mM $CaCl_2$, 467 mM sucrose, 10^{-6} g/ml tetrodotoxin and 1 mM tris-maleate (pH = 7.1). TEA Cl and tetrodotoxin were used to block the voltage and time dependent changes in sodium and potassium currents which might obscure the observation of the charge movements. Sucrose was added to block mechanical movement. The strategy was to impose a voltage step from the holding potential, usually -80 mV, to a potential at which contraction would be activated, for example -20 mV. The current was measured and compared with that obtained for the same size voltage step in which the potential was confined to highly negative values, for example -140 to -80 mV. Differences in current between the first, or 'test', pulse and the second, or 'control', pulse consistently showed a transient outward component associated with depolarization and a transient inward component on repolarization. The time integrals of the two components were equal, indicating

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that the same amount of charge which moved on depolarization returned on repolarization. This equality held when the amount of charge moved was varied by changing either the voltage or the duration of the test pulse. The steady-state amount of charge which moved from the resting position followed a sigmoid curve when plotted as a function of membrane potential. These results showed that it is possible to detect the movement of charged groups of a molecule which is confined to the membrane phase but which is free to move between at least two separate locations.

Experimental results

The work discussed at the present meeting represents an attempt to learn more about the qualitative properties of the charge movement. The voltage-clamp technique and solution were the same as used previously.

Experiments were carried out on six fibres in which the effect of voltage on charge movement was studied. Although the differences (test − control) were usually averaged four times to improve the signal to noise ratio, the features of the charge movement could be seen clearly with a single difference. As shown previously the area of the 'on' transient was always equal to the area of the 'off'. The relation of charge moved to voltage could be expressed as

$$Q = \frac{Q_{\text{total}}}{1+\exp -(V-\bar{V})\,k}, \tag{1}$$

which is expected if the charge distributes itself between two possible positions according to the Boltzmann relation. Average values for $\bar{V}$ and k were -44 ± 3 mV (mean and s.e.) and 8 ± 1 mV respectively. If the total membrane potential is sensed between the two positions, the value for k implies that the charged group has a valence of magnitude 3.

The values of Q were divided by the total measured fibre capacitance to give an indication of charge per unit membrane area. The average of Q_{total} was 25 ± 2 nC/μF. If 1 cm² of membrane, whether it be surface or tubular, has a capacitance of 0.9 μF/cm² (Schneider 1970; Hodgkin & Nakajima 1972) the value of 25 nC/μF corresponds to roughly 1700 electronic charges/μm² or 600 charged groups/μm² with $k = 3$. If the charges are located in the T-system and are absent in the surface membrane the density would be about 700 groups/μm².

At this stage of the investigation it seemed more interesting to investigate the qualitative properties of the charge movement rather than to attempt a detailed study of the kinetics. One important question was whether the charges are located primarily in the surface membrane or in the T-system. If they are absent from the T-system they could not be expected to play a role in activating contraction.

Several attempts were made to sever the electrical continuity of the T-system from the surface by using the 'glycerol-shock' technique (Eisenberg & Gage 1969). Muscles were placed in Ringer + 400 mM glycerol at room temperature for 1 h, then transferred to Ringer + 5 mM Ca^{2+} + 5 mM Mg^{2+} (Eisenberg, Howell & Vaughan 1971); 20–30 min later the muscles were cooled to around 2 °C. At this point propagated action potentials without visible contractions were observed when fibres were electrically stimulated. Finally the muscles were transferred to an isosmotic solution with 117.5 mM TEA Cl, 5 RbCl, 1.8 mM $CaCl_2$, 10^{-6} g/ml tetrodotoxin, buffered to pH = 7.1 with tris-maleate. Voltage-clamp experiments were successfully carried

out in 14 fibres from five muscles. The glycerol treated fibres could be depolarized without producing visible contraction using pulses which would have produced contraction in untreated fibres. However, in every case movement occurred if the fibre was sufficiently depolarized with pulses lasting 100 ms. In only three fibres was it possible to make measurements at positive internal potentials. This failure to suppress contraction totally shows that the glycerol treatment was not completely successful in uncoupling contraction from electrical excitation. The results of cable analysis indicate that, at most, 0.4 of the tubular capacitance was eliminated, suggesting that less than half of the T-system had been isolated from the surface. Somewhat more surprising was the finding that a larger fraction, 0.7–0.8, of the charge movement was absent. This indicates that glycerol treatment can disrupt the charge movement process more than it disrupts the tubular continuity.

Although the experiment was not successful in localizing the charge, the results may explain a rather interesting finding of Dulhunty & Gage (1973). They reported that under certain conditions glycerol treatment was able to block the twitch but not alter the tubular capacitance. This would be expected from the present results if the charge movement is a necessary step in excitation–contraction coupling.

The next experiments were aimed at finding out whether the charge movement goes away during prolonged depolarization when the fibre becomes mechanically refractory (Hodgkin & Horowicz 1960). In four successful experiments, using hypertonic solutions, the holding potential was changed from -80 to -21 mV. In all cases the charge movement disappeared within 2 min. The disappearance time course was exponential with a time constant that varied in different fibres from 13 to 24 s at 1.5 °C. When the holding potential was restored to -80 mV the charge movement recovered to its initial level, also with an exponential time course (time constant of 21–53 s).

This result is of interest for two reasons. First, it shows that the ability of the charge to move disappears when the muscle is refractory to electrical stimulation, thereby raising the possibility that the disappearance is the cause of the refractory condition. The second point is that the reversible disappearance of the charge movement makes it unlikely that the electrical signal is an artefact of the method.

A disadvantage of the previous experiments is that contraction had to be blocked in order to make the charge movement measurements, to avoid mechanical movements which would dislodge the electrodes. Contraction was eliminated either by using hypertonic solutions or by glycerol-shock treatment. The finding that the charge movement is absent in a depolarized, mechanically refractory fibre and that it can be reprimed on hyperpolarization makes feasible a somewhat different approach, namely the comparison of the recovery of contraction and of charge movement in mechanically refractory fibres. In these experiments an isosmotic solution was used, 40 mM Rb_2SO_4, 55 mM $(TEA)_2SO_4$, 8 mM $CaSO_4$, tetrodotoxin (10^{-6} g/ml), buffered to pH = 7.0 with 1 mM tris-maleate, temperature 1–4 °C. Muscles placed in this solution depolarize to around -20 mV and become mechanically refractory.

The first experiments were designed to determine some of the necessary conditions to reprime a fibre to give a just visible contraction. After repriming fibres at -120 mV for a variable time, the potential was stepped to a value between -40 and $+100$ mV. For test potentials greater than $+40$ mV the repriming time required to produce a just visible contraction was constant. Longer times were required for more negative test potentials so that the relation between time and voltage resembles a rectangular hyperbola.

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This kind of experiment can be analysed on the assumption that an activator substance or mechanism A is reprimed according to

$$A = A_{\text{total}} [1 - \exp(-t/\tau)] \tag{2}$$

during the hyperpolarization, and that at the beginning of the test pulse V

$$A_{\text{activating}}/A = F(V). \tag{3}$$

A_{total} is the total amount of activator, τ is the time constant for repriming, $F(V)$ is the fraction in the activating position for a voltage V. If threshold requires a constant amount of $A_{\text{activating}}$, independent of V, then

$$A_{\text{activating}} = A_{\text{threshold}}, \tag{4}$$

and

$$\left[\frac{A_{\text{total}}}{\tau A_{\text{threshold}}}\right] F(V) = \frac{1}{\tau[1 - \exp(-t/\tau)]}. \tag{5}$$

For $t/\tau \ll 1$, the right side is approximately $1/t$, so the shape and position of the distribution function $F(V)$ can be determined by plotting $1/t$ against V. These plots are sigmoid, similar to those for Q in hypertonic solution, and can be fitted according to

$$\frac{1}{t} = \frac{(1/t)_{\max}}{1 + \exp -(V - \bar{V})/k}, \tag{6}$$

similar to equation (1). Values for $\bar{V}$ and k from five experiments were -22 ± 2 mV, 11 ± 1 mV respectively. If instead of assuming $t/\tau \ll 1$ a value of 10 s is used for τ, $\bar{V}$ becomes about 5 mV more negative and k is increased 1–3 mV.

The repriming results, then, are consistent with a model in which an activator is reprimed during hyperpolarization, and on subsequent depolarization is switched on according to membrane potential in a manner qualitatively similar to that found for the charge movement. Quantitatively, $\bar{V}$ is about 20–25 mV more positive for the activator A and k is slightly larger. Part of the difference, especially with respect to $\bar{V}$, may be due to differences in the solutions used in the two types of experiments, such as the threefold change in tonicity. In addition, there are rather large variations in the values of $\bar{V}$ and k in individual fibres which add to the difficulty in making the comparison.

Since the repriming of contraction can be explained rather nicely in terms of repriming an activator substance with properties similar to the charge, we undertook experiments to see whether, in fact, a detectable amount of charge movement was reprimed at the threshold for repriming the mechanical response. The procedure was first to determine the mechanical threshold, then to measure the appearance of charge movement as a function of repriming time. A voltage of -80 mV was usually used for repriming, with a test voltage of $+40$ mV. Within experimental error the charge movement increased linearly with short repriming times, consistent with the initial phase of an exponential recovery (equation (2)). Values of charge at the mechanical repriming threshold varied from 2 to 5 nC/μF with an average of 3.5 ± 0.4 nC/μF. Thus, a just-visible mechanical movement occurs when 8–20 % of the total charge is reprimed.

Conclusions

In many ways the properties of the charge movement are similar to those of sodium gating currents in squid axons, described in reports by Armstrong and by Rojas & Keynes at this meeting. The kinetics, however, are markedly different. In nerve the transient currents are

over within 1–2 ms whereas in muscle the time constants vary between 3 and 20 ms for voltages between −80 and 0 mV, entirely too slow to gate sodium channels.

It seems possible that the currents in muscle gate potassium channels rather than activate contraction. These possibilities would be difficult to distinguish since there are similarities between the turning-on of potassium conductance and the activation of contraction. For example, both processes occur at about the same potential (Costantin 1968) and both processes become refractory with maintained depolarization (Hodgkin & Horowicz 1960; Adrian, Chandler & Hodgkin 1970). It is therefore not surprising that the steady-state curve of n against V is similar to that of Q against V, and on this basis alone it would be reasonable to suppose that the charge movements are associated with potassium channels. One kinetic difference is clear, however. The time constants for $n(\tau_n)$ are considerably greater than those for $Q(\tau_Q)$. In several experiments in which Na^+ was used instead of TEA^+ it was possible to see delayed potassium current and charge movement in the same record. In all cases where the two components could be clearly resolved, τ_n was 1.6–4.3 times greater than τ_Q.

The experimental results obtained thus far seem entirely consistent with the notion that the charge movement plays a role in excitation–contraction coupling. On this basis activation of a threshold contraction with step depolarizations requires that 0.1–0.2 of the total resting amount of charge moves into an activating position. If this view is correct there is a rather interesting difference between the gating mechanisms which have been described in nerve and those in muscle. In nerve the gating currents move across the membrane in which the permeability change takes place. In muscle the currents move across the T-system membrane, but it is in the adjacent membrane of the sarcoplasmic reticulum where the permeability change to calcium is reckoned to occur.

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