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Ca^{2+} CURRENT AND CHARGE MOVEMENT IN ADULT SINGLE HUMAN SKELETAL MUSCLE FIBRES

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SUMMARY

1. The Vaseline-gap technique was used to record calcium currents (I_{Ca}) and charge movement in single cut fibres from normal human muscle. Experiments were carried out in 2 or 10 mM-extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) and at 17 or 27 °C.

2. The passive electrical properties of the fibres with this technique were: membrane resistance for unit length $r_m = 59{\cdot}4$ kΩ cm; longitudinal resistance per unit length $r_i = 4{\cdot}9$ MΩ/cm; longitudinal resistance per unit length under the Vaseline seals $r_e = 438$ MΩ/cm; specific membrane resistance $R_m = 1{\cdot}176$ kΩ cm^2; input capacitance = 5·53 nF; specific membrane capacitance = 8·9 μF/cm^2.

3. The maximum amplitude of I_{Ca} at 17 °C was: in 2 mM $[Ca^{2+}]_o$, −0·42 μA/μF and in 10 mM $[Ca^{2+}]_o$, −1·44 μA/μF. At 27 °C and in 10 mM $[Ca^{2+}]_o$, it increased to −3·04 μA/μF. The calculated temperature coefficient (Q_{10}) for the increase in amplitude from 17 to 27 °C was 2·1.

4. Ca^{2+} permeability (P_{Ca}) was calculated using the Goldman–Katz relation; in 2 mM $[Ca^{2+}]_o$ at 17 °C, $P_{Ca} = 1{\cdot}26 \times 10^{-6}$ cm/s; in 10 mM $[Ca^{2+}]_o$ at 17 °C, $P_{Ca} = 2{\cdot}23 \times 10^{-6}$ cm/s; in 10 mM $[Ca^{2+}]_o$ at 27 °C, $P_{Ca} = 4{\cdot}03 \times 10^{-6}$ cm/s.

5. The activation curve calculated from the P_{Ca} was shifted by 10 mV to positive potentials when raising $[Ca^{2+}]_o$ from 2 to 10 mM. Increasing the temperature did not change the curve. The mid-point potentials ($V_{a\frac{1}{2}}$) and steepness (k) of the activation curves were: at 17 °C, in 2 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = -1{\cdot}53$ mV and $k = 6{\cdot}7$ mV; in 10 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = 9{\cdot}96$ mV and $k = 6{\cdot}8$ mV; at 27 °C and 10 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = 11{\cdot}3$ mV and $k = 7{\cdot}7$ mV. The activation time constant in 10 mM $[Ca^{2+}]_o$ reached a plateau at potentials positive to 10 mV, with a value of 93·8 ms at 17 °C and 17·4 ms at 27 °C. The calculated Q_{10} was 4·5.

6. The deactivation of the current was studied from tail currents at different membrane potentials in 10 mM $[Ca^{2+}]_o$. For potentials from −120 to −50 mV, the deactivation time constant ranged between 5 and 7 ms at 17 °C and between 7 and 14 ms at 27 °C. For potentials positive to −50 mV the deactivation became much slower at 27 °C.

7. I_{Ca} was reversibly reduced by the addition of 0·2 or 0·5 μM-nifedipine and completely blocked by 2 μM-nifedipine.

8. Charge movement was recorded in 2 or 10 mM $[Ca^{2+}]_o$ at 17 °C. Increasing

$[Ca^{2+}]_o$ caused an increase of the maximum amount of charge moved (Q_{max}) and a positive shift of the curve. The parameters ($V_{q\frac{1}{2}}$ = mid-point activation and k = steepness) for the voltage–charge relationship were: 2 mM $[Ca^{2+}]_o$, Q_{max} = 5·2 nC/μF, $V_{q\frac{1}{2}} = -46{\cdot}0$ mV and k = 12·9 mV; 10 mM $[Ca^{2+}]_o$, Q_{max} = 9·8 nC/μF, $V_{q\frac{1}{2}} = -28{\cdot}8$ mV and k = 15·2 mV.

9. The fact that the activation curves of the I_{Ca} and the charge movement differ widely in the mid-point of activation can be explained by the presence of several closed states before the channel opens. In addition, it is also possible that only part of the charge movement corresponds to the gating of the Ca^{2+} channel.

INTRODUCTION

Electrophysiological study of human muscle is of primary importance in normal and diseased states. Previous studies of human skeletal muscle have been carried out in excised muscles using intracellular microelectrodes (see for example, Kwiecinski, Lehmann-Horn & Rüdel, 1984) or the loose-patch clamp technique (Zite-Ferenczy, Matthias, Taylor & Rüdel, 1986), and in cultured cells using the whole cell or inside-out patch clamp configurations (Burton, Dörstelmann & Hutter, 1988). An advantage of tissue culture is that the cells may be maintained for hours or days in an appropriate environment. However, myoblasts are immature muscle cells, derived from the satellite cells and may exhibit different functional characteristics.

An important issue in adult muscle is the characterization of the membrane Ca^{2+} access pathways and their relationship with disease. In this paper, we present Ca^{2+} currents (I_{Ca}) and charge movement recorded from human muscle cells. The importance of these experiments resides in the search for the role of Ca^{2+} channels and charge movement in the excitation–contraction coupling mechanism in normal human muscle. Furthermore, the characterization of charge movement and Ca^{2+} channels in normal human muscle is a necessary initial step to study their possible role under pathological conditions.

METHODS

Fibre preparation. Experiments were performed in single fibres isolated from human muscle (six from vastus lateralis and one from biceps). Biopsies were obtained under local anaesthesia from patients with non-specific complaints who were later found, after detailed testing, not to have muscle or nerve diseases. Experiments were performed with the understanding and consent of each individual and the approval of the local Baylor Ethics Committee. The results were obtained from fifteen fibres from six subjects. Immediately after obtaining the specimen, it was stored in a beaker containing normal Krebs solution (see solution composition below). A bundle of muscle fibres was dissected and transferred to a chamber with the dissecting solution. A 0·5–1 cm segment of muscle fibre was isolated in this solution, which was then transferred to the experimental chamber containing the mounting solution. The chamber consisted of three compartments separated by Vaseline strands. Two seals around the positioned fibre were made and a cover-slip was put over the fibre. After, the mounting solution was replaced by the extracellular solution in the middle pool, and by the intracellular solution in the end pools. Fibres were mounted at slack length in the experimental chamber and movement was prevented by the presence of 10 mM-EGTA in the intracellular solution. The width of the seals ranged from 150–300 μm.

Electrical recordings. The double-seal Vaseline-gap voltage clamp technique described by Francini & Stefani (1989) was used. Briefly, agar bridges equilibrated with the end-pool or middle-

pool solution provided electrical connections between each compartment and separate wells that were filled with 3 M-KCl and fitted with an Ag–AgCl pellet. We could switch from a voltage pulse mode to a voltage clamp mode. In the voltage clamp mode, the command pulses referred to earth were applied at the central pool. The current was injected into one of the end pools (I) via a variable-gain feedback amplifier. The negative input of the amplifier was connected to the other end pool (E). Membrane potential was measured between the middle pool and compartment E, and the current as the voltage drop across a 100 kΩ resistance. In the voltage pulse mode, constant current pulses were applied to compartment I and the feedback amplifier was earthed. Passive properties were recorded according to Irving, Maylie, Sizto & Chandler (1987), with the voltage clamp and the voltage pulse mode at 0 and −90 mV. The membrane potential was held at −90 mV.

Data collection. Data were collected on-line by a laboratory microcomputer with a 12-bit AD–DA converter (Axolab-1, Burlingame, CA, USA). Signals were filtered at 0·3 of the sampling frequency (−3 dB point) with a 4-pole Butterworth filter. Linear components were digitally subtracted by automated scaling of control pulses which were −1/4 of the test pulses. Data were stored in discs for deferred analysis.

Solutions. The following solutions were used (mM). Normal Krebs: NaCl 145, KCl 5, $CaCl_2$ 2·5, $MgSO_4$ 1, Na-HEPES 10, glucose 10. Dissecting: K_2SO_4 95, $MgCl_2$ 10, $CaCl_2$ 0·4, Na-HEPES 10. Mounting: potassium glutamate 150, $MgCl_2$ 2, K_2-EGTA 1, K-HEPES 10. Extracellular: (TEA) tetraethylammonium-CH_3SO_3 150, $CaCl_2$ 2 or 10, $MgCl_2$ 2, TEA-HEPES 5, tetrodotoxin (TTX) 0·0005. Intracellular: sodium glutamate 120, Na_2-EGTA 10, Mg-ATP 3, disodium phosphocreatine 5, Na-HEPES 10, glucose 10. pH was adjusted to 7·4 in all the solutions. Osmolarity was corrected to 300 mosM with sucrose when necessary. Nifedipine was diluted in ethanol and stored in light-resistant containers. Experiments with this drug were carried out under dim red light. Nifedipine and chemicals were added from stock solutions to the extracellular solution in the middle pool at the desired concentration. Temperature was set at 17 or 27 °C and it was monitored with a thermistor probe positioned close to the fibre in the middle pool. In these conditions fibres lasted in stable conditions for about 2–3 h. Raising the temperature to 37 °C resulted in an immediate increase of the leak current and a rapid deterioration of the fibre. Results are given as means ± S.E.M. with the number of observations given in parentheses.

RESULTS

Passive electrical properties

Passive electrical properties of the fibres were obtained 10–20 min after changing the extracellular and intracellular solutions. All the ionic conductances but calcium were blocked by ion substitution or specific channel blockers. The extracellular solution contained 2 mM-Ca^{2+} and the temperature was 17 °C. We initially measured the membrane potential in the voltage pulse mode with the I pool disconnected. Typical values ranged between −14 and −27 mV. After this, a 20 mV voltage pulse was applied to the I end, which was recorded as a fraction of this potential between the central and the E end pool. The fraction recorded varied between 16 and 19 mV. Thereafter, we switched to the voltage clamp mode and polarized the membrane to −90 mV, which was the holding potential throughout the experiment. The capacitance of the fibres was calculated from the integral of current transients obtained in response to a 20 mV voltage step from this potential. The input capacitance was 5·7 ± 0·8 nF (15) and the specific membrane capacitance was 8·9 ± 0·85 $\mu F/cm^2$ ($n = 15$). The area of fibre membrane in the middle pool was obtained from the length and the width of the segment of fibre and assuming cylindrical shape. Its value was $6{\cdot}4 \times 10^{-4} \pm 0{\cdot}33$ cm^2 (15).

The same voltage pulses and the passive responses of the membrane were used to calculate the fibre membrane resistance for unit length (r_m), the internal resistance per unit length (r_i), and the longitudinal external resistance per unit length under the

Vaseline seals (r_e) following the procedure of Irving *et al.* (1987). The corresponding values were: $r_m = 59{\cdot}4 \pm 6{\cdot}5$ kΩ cm (15); $r_i = 4{\cdot}9 \pm 0{\cdot}6$ MΩ/cm (15); $r_e = 438 \pm 45$ MΩ/cm (15). Once the values of r_m, r_e and r_i were derived, we calculated the specific membrane resistance (R_m) and the ratio $r_e/(r_e + r_i)$ which reflects the quality

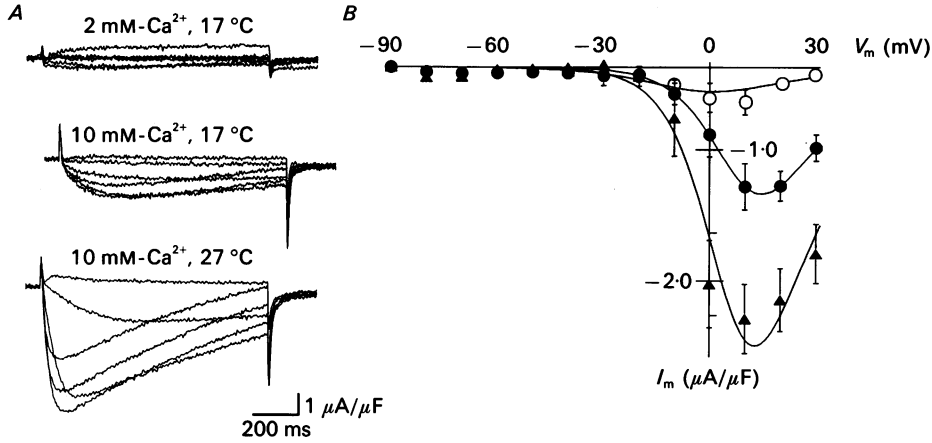

Fig. 1. Inward Ca^{2+} currents in normal human muscle. *A*, inward currents were elicited by depolarizing steps of 1 s from −90 mV. The traces correspond to membrane potentials form −20 to 30 mV in 10 mV steps. Uppermost traces were obtained in 2 mM $[Ca^{2+}]_o$ at 17 °C; middle traces in 10 mM $[Ca^{2+}]_o$ at 17 °C; and lower traces in 10 mM $[Ca^{2+}]_o$ at 27 °C. The traces correspond to the same fibre. *B*, peak inward current–membrane potential relationship for the same conditions as in *A*. Each point represents the average ± S.E.M. of seven fibres in 2 mM $[Ca^{2+}]_o$ (○), nine fibres in 10 mM $[Ca^{2+}]_o$ (●), both at 17 °C, and six fibres in 10 mM $[Ca^{2+}]_o$ at 27 °C (▲). The smooth curve was drawn by fitting the experimental data to eqn (1).

of the Vaseline seal resistance in relation to the longitudinal myoplasmic and transverse membrane resistances. R_m had a value of $1{\cdot}176 \pm 0{\cdot}125$ kΩ cm² (15) which is much smaller than the data reported for human intercostal muscle fibres obtained with different extracellular ionic composition (5·970 kΩ cm², Kwiencinski *et al.* 1984). The value of R_m is also smaller when compared to frog skeletal muscle (6·363 kΩ cm², Irving *et al.* 1987) and other mammalian skeletal muscle (6·355 kΩ cm², Delbono, Garcia, Appel & Stefani, 1991) obtained under similar conditions. The calculated ratio $r_e/(r_e + r_j)$ was $0{\cdot}987 \pm 0{\cdot}001$ (15). This ratio was very close to 1 indicating the adequacy of this method for membrane current measurements in human skeletal muscle.

Inward Ca^{2+} current (I_{Ca})

Figure 1*A* shows current traces obtained with pulses of 1 s duration from the holding potential (−90 mV) to different membrane voltages at 17 °C. The outward current at the onset of the pulse corresponds to the charge movement, which increased with further depolarizations and saturated at positive potentials. Charge movement was followed by a small inward current in 2 mM $[Ca^{2+}]_o$ (upper panel).

This current increased in amplitude during the pulses and at the tail after raising the $[Ca^{2+}]_o$ to 10 mM (middle panel), indicating that it was carried by Ca^{2+}. I_{Ca} amplitude remained stable during the experiment as it was reported for I_{Ca} recorded under similar conditions in rat skeletal muscle fibres (Delbono *et al.* 1991). I_{Ca} had a peak average amplitude of $-0{\cdot}42 \pm 0{\cdot}15$ $\mu A/\mu F$ (7) at 10 mV in 2 mM $[Ca^{2+}]_o$ and increased to $-1{\cdot}44 \pm 0{\cdot}28$ $\mu A/\mu F$ (7) at 10 mV in 10 mM $[Ca^{2+}]_o$. I_{Ca} decayed very slowly during the pulses at this temperature. After changing the temperature to 27 °C and with 10 mM $[Ca^{2+}]_o$ (lower panel), the I_{Ca} maximum amplitude increased to $-3{\cdot}04 \pm 0{\cdot}42$ $\mu A/\mu F$ (9) at 10 mV. In addition, the activation and decay of the current became faster. The temperature dependence of I_{Ca} amplitude was evaluated from the following expression: $X_2/X_1 = [Q_{10}](T_2 - T_1)/10$ K, where X_2 is the peak I_{Ca} amplitude at 27 °C (T_2) and X_1 is peak I_{Ca} at 17 °C (T_1) (Cota, Nicola-Siri & Stefani, 1983). The calculated Q_{10} for the change in amplitude with the temperature was 2·1.

The current–voltage relationships for I_{Ca} recorded in 2 or 10 mM $[Ca^{2+}]_o$ and 17 or 27 °C are shown in Fig. 1*B*. The graph shows the increase in I_{Ca} when raising external Ca^{2+} to 10 mM. A further increase of I_{Ca} was observed by elevating the temperature to 27 °C. There were no changes in the voltage dependence.

In order to calculate the Ca^{2+} permeability P_{Ca} we fitted the experimental data to the following equation:

$$(1 + (\exp((V_{a\frac{1}{2}} - V_m)/k))^{-1}\, 2zF^2V_m P_{Ca}[Ca^{2+}]_o)/(RT)$$
$$(\exp((zF(V_m - V_{Ca})/(RT)) - 1)/(\exp(zFV_m/(RT)) - 1), \quad (1)$$

where z is the valency of the molecule, F, R and T have the usual thermodynamic meanings, k is the slope of the curve, $V_{a\frac{1}{2}}$ the half-activation potential (see below), V_m is the pulse potential, P_{Ca} the calcium permeability, $[Ca^{2+}]_o$ the external calcium concentration and V_{Ca} is the reversal potential for I_{Ca} which was assumed to be 150 mV (Sánchez & Stefani, 1983). The left expression of this equation corresponds to a Boltzmann relation of the activation form, while the right one is the Goldman–Katz equation. The smooth curves in the graph (Fig. 1*B*) were drawn according to this fitting. The calculated P_{Ca} from this method was as follows: in 2 mM $[Ca^{2+}]_o$ at 17 °C, $P_{Ca} = 1{\cdot}26 \times 10^{-6}$ cm/s; in 10 mM $[Ca^{2+}]_o$ at 17 °C, $P_{Ca} = 2{\cdot}23 \times 10^{-6}$ cm/s; in 10 mM $[Ca^{2+}]_o$ at 27 °C, $P_{Ca} = 4{\cdot}03 \times 10^{-6}$ cm/s. This current has a similar voltage dependence to the one described in rat and rabbit muscle (Donaldson & Beam, 1983; Lamb & Walsh, 1987). However, the activation phase is slower in human muscle (see below). In addition, P_{Ca} is smaller in these muscles when recorded under equivalent conditions. For instance, the values of P_{Ca} for rat muscle obtained with 2 mM $[Ca^{2+}]_o$ at 17 °C varied between $1{\cdot}95 \times 10^{-5}$ cm/s (Mejía-Alvarez, Fill & Stefani, 1991) and $2{\cdot}76 \times 10^{-5}$ cm/s (Delbono *et al.* 1991) and for frog muscle with 10 mM $[Ca^{2+}]_o$ obtained at 22–26 °C were $1{\cdot}4 \times 10^{-4}$ cm/s (Sánchez & Stefani, 1983).

Time and voltage dependence of Ca^{2+} channel activation

The voltage-dependent Ca^{2+} channel activation was calculated using the values of P_{Ca}. Data were normalized to the maximum P_{Ca} which was attained at 30 mV. Figure 2 shows the activation curve of the I_{Ca} with 2 (○) or 10 mM $[Ca^{2+}]_o$ at 17 °C (●) and

with 10 mM $[Ca^{2+}]_o$ at 27 °C (▲). This relationship is an indication of the fraction of channels available for activation at each membrane potential. Increasing $[Ca^{2+}]_o$ causes a positive shift of the curve of about 10 mV, as expected for an effect on surface charges (Cota & Stefani, 1984). On the other hand, an increase of temperature

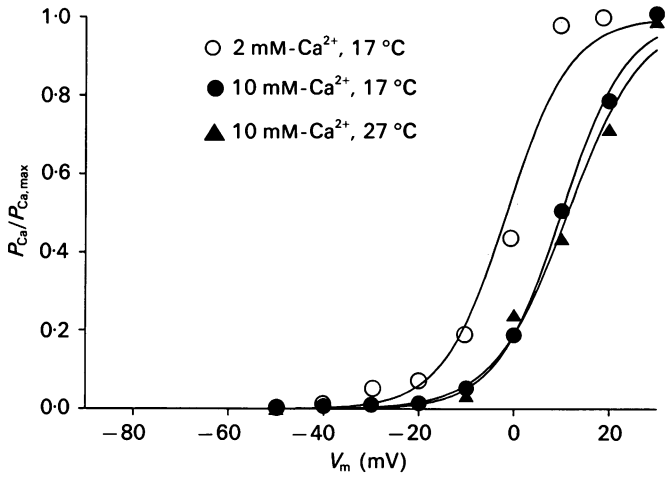

Fig. 2. Activation curve of the Ca^{2+} current. Ca^{2+} permeability was obtained for each membrane potential and normalized to the maximum permeability in each condition. Data were fitted to eqn (2) and the continuous curve was drawn according to the best fit. Raising $[Ca^{2+}]_o$ caused a positive shift of about 10 mV while increasing the temperature did not cause major modifications.

did not appreciably modify the activation curve. The continuous curve was drawn by fitting the mean of the experimental data to a Boltzmann relationship of the form:

$$P_{Ca}/P_{Ca,max} = 1/(1+\exp(V_{a\frac{1}{2}}-V/k)), \quad (2)$$

where $V_{a\frac{1}{2}}$ is the potential at which 50% of the channels may be activated, and k is the steepness of the curve. The values for these two parameters obtained by the fitting were: at 17 °C, in 2 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = -1{\cdot}53$ mV and $k = 6{\cdot}7$ mV; in 10 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = 9{\cdot}96$ mV and $k = 6{\cdot}8$ mV; at 27 °C and 10 mM $[Ca^{2+}]_o$, $V_{a\frac{1}{2}} = 11{\cdot}3$ mV and $k = 7{\cdot}7$ mV.

To estimate the dependency of the activation rate of the I_{Ca} with the membrane potential, we fitted the rising phase of the records to an exponential function with different exponents, from 1 to 3. As an example, Fig. 3*A* shows records of I_{Ca} during a depolarization step to 10 mV, either at 17 (upper traces) or at 27 °C (lower traces) for the same fibre. This procedure was not performed in the records obtained with 2 mM $[Ca^{2+}]_o$ because the current was very small. The fitted trace is superimposed on the current records, and can be better appreciated from the expanded records in the bottom part of each pair of traces. The current records were accurately fitted with a single exponential at all membrane potentials and at both temperatures. Thus, the value of the fitting can be taken directly as the time constant of activation, τ_m. A similar behaviour has been found in rat muscle, where one exponential is enough to

describe the activation of the I_{Ca} (Mejía-Alvarez *et al.* 1991; Delbono *et al.* 1991). On the other hand, an exponent of 3 better describes the I_{Ca} time course in frog muscle (Sánchez & Stefani, 1983). Since Ca^{2+} channels have been located in the membranes of the transverse tubular system of frog muscle and developing myoblasts in culture

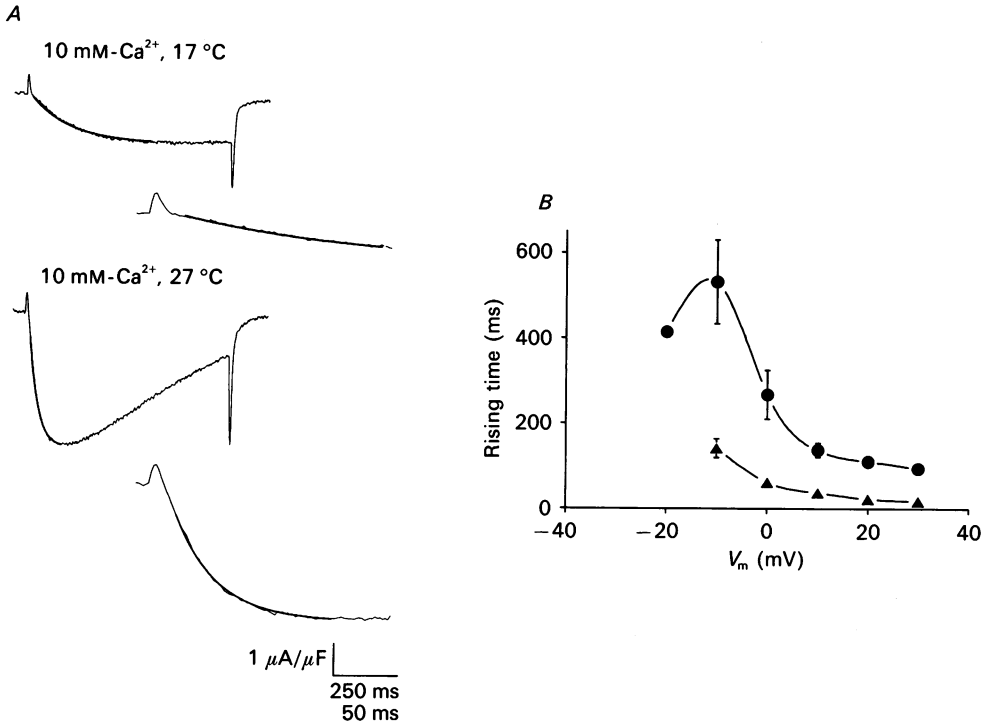

Fig. 3. Time constant of activation of the Ca^{2+} current. *A*, current traces obtained for a pulse to 10 mV from the holding potential in 10 mM $[Ca^{2+}]_o$, either at 17 °C (upper traces) or at 27 °C (lower traces). Same fibre in both cases. The rising phase of the current was fitted to a single exponential which is overimposed on the traces. Bottom traces in each pair show the current expanded five times in order to better appreciate the fit. *B*, τ_m as a function of membrane potential for different fibres at both temperatures.

(Siri, Sánchez & Stefani, 1980; Romey, Garcí, Dimitriadou, Pinçon-Raymond, Rieger & Lazdunski, 1989), it is reasonable to think that in human muscle the Ca^{2+} channels are likewise located in the tubular membrane. This would represent a problem if the luminal resistance of the tubules is very large as it may cause non-controlled variations in the tubular membrane potential, thus altering the kinetics of the Ca^{2+} channels. We can disregard this difficulty based on both the single exponential fitting to the activation phase of the current and by the smooth rise of the current–voltage relationship in the phase of the negative slope (see Fig. 1*B*).

The voltage dependence of τ_m calculated in this way is plotted in Fig. 3*B*. The curve obtained from the data at 17 °C is bell shaped and centred at −10 mV with a value of 532·3 ± 98·3 ms (4). As expected, the values at 27 °C were smaller. At 30 mV, the average value of τ_m was 93·8 ± 7·22 ms (9) at 17 °C and 17·4 ± 3·04 ms (10) at 27 °C. The calculated Q_{10} for this parameter was 4·5.

Decay of the tail current

Figure 4*A* shows an experiment performed to study the deactivation time constant as a function of voltage. Command pulses of 150 ms duration were delivered from the holding potential (−90 mV) to 10 or 20 mV. After this pulse, the membrane

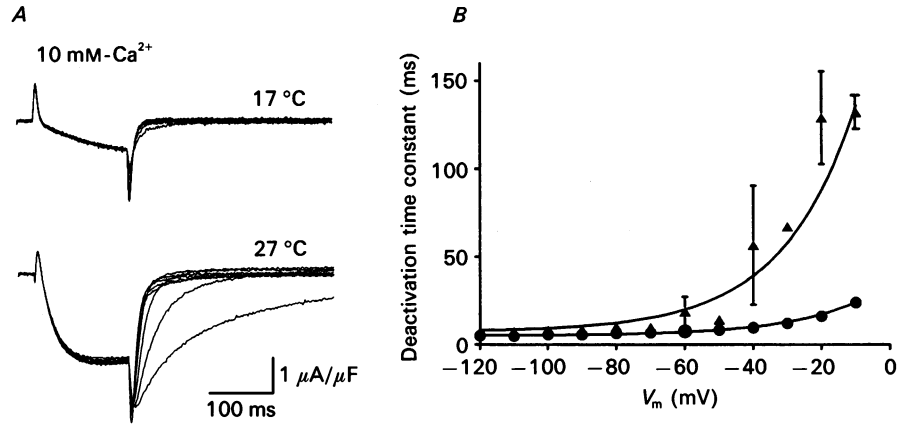

Fig. 4. Deactivation of the Ca^{2+} current. *A*, tail currents were recorded at different membrane potentials after return of 150 ms pulses to 10 or 20 mV in 10 mM $[Ca^{2+}]_o$. Upper records were obtained at 17 °C and the lower ones at 27 °C from the same fibre. The returning level after the pulses was varied between −120 and −10 mV. The records correspond to voltages from −90 to −20 in 10 mV steps. To calculate the deactivation time constant, an exponential curve was fitted to the tails for each membrane potential. *B*, voltage dependence of the deactivation time constant at both temperatures. The difference between the temperatures becomes more evident for potentials positive to −40 mV.

potential was returned to different voltages from −120 to −10 mV. Upper records correspond to the I_{Ca} recorded with 10 mM $[Ca^{2+}]_o$ at 17 °C and the lower ones at 27 °C. By fitting exponential functions to the tail currents we were able to obtain the deactivation time constants as a function of membrane potential. Tails were well fitted to a single exponential. It was not possible to differentiate between the decay time constant of the I_{Ca} and the charge movement since both decay with a similar time constant (Delbono *et al.* 1991). At 17 °C, for membrane potentials from −120 to −50 mV, the exponential had a value between 5 and 7 ms; for potentials more positive than −50 mV, the time constant became larger reaching a maximum value at −10 mV of 23·8±9 ms (4). At 27 °C for potentials from −120 to −50 mV the value of the time constant varied between 7 and 14 ms, which is very similar to the data obtained at 17 °C. Differences in the decay were apparent for potentials positive to −50 mV, when the decay at 27 °C became slower (Fig. 4*B*). This indicates that the return to the closed state from the open state was slowed by raising the temperature and that the channel remains in the open state for longer times at 27 °C. This finding is opposed to that found in rat muscle, where the deactivation of the current becomes faster by increasing the temperature (O. Delbono & E. Stefani, unpublished observations).

The inward current is sensitive to nifedipine

Since in other muscle cells a I_{Ca} with similar voltage dependence is sensitive to dihydropyridines (DHP; see Bean, 1989) we tested the sensitivity of our current to nifedipine. Nifedipine was applied to the extracellular solution in two experiments at concentrations of 0·2 and 0·5 μM and at 27 °C. The current was reduced by 57 and 52% in the presence of 0·2 μM-nifedipine, and 76 and 84% with 0·5 μM-nifedipine. In addition, in two other fibres we used a higher concentration (2 μM) and the current was completely blocked. The effect of nifedipine was partially reversed after the wash-out of the drug, although it was more persistent with the higher concentration. These results are in agreement with the experiments done in myoballs by Cognard, Lazdunski & Romey (1986).

Charge movement from human skeletal muscle

Charge movement was recorded in 2 or 10 mM $[Ca^{2+}]_o$ at 17 °C. The stimulating procedure for recording charge movement was the same as for the I_{Ca} except in the

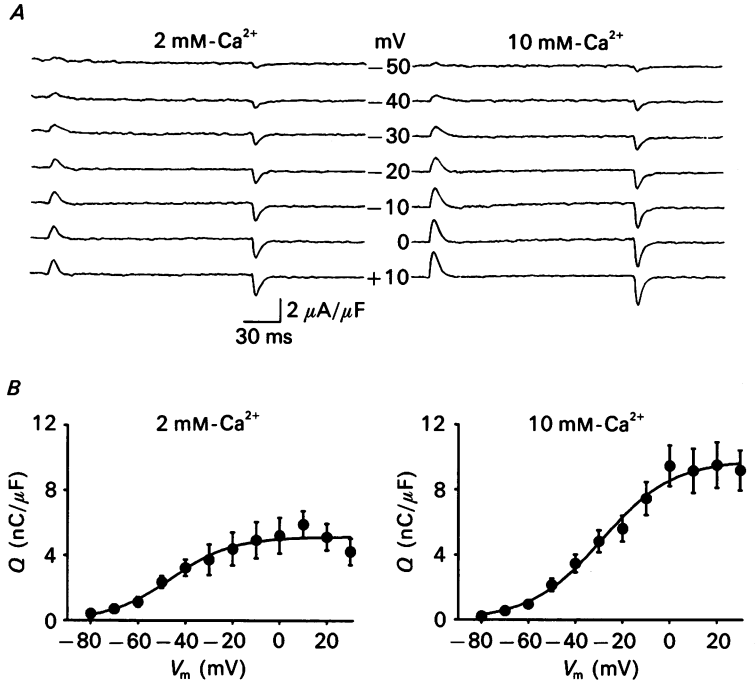

Fig. 5. Charge movement from normal human skeletal muscle. *A*, charge movement recorded from the same fibre at 17 °C in 2 mM $[Ca^{2+}]_o$ (left) or 10 mM $[Ca^{2+}]_o$ (right). Depolarizing pulses were delivered to the membrane potentials indicated between the traces from the holding potential. Records were corrected with a sloping baseline fitted to the second half of the trace since this fibre had a small inward current at this temperature. The amount of charge moved at each potential was calculated as the integral of the area below the transient at the start of the depolarization. *B*, voltage–charge movement relationship. Experimental data were fitted to eqn (3). An increase in the $[Ca^{2+}]_o$ caused an enhancement of the maximum amount of charge moved and a positive shift of the curve of about 18 mV.

pulse duration which was shorter for charge movement and thereby did not elicit a large I_{Ca} at positive potentials. Records in Fig. 5*A* show the charge movement obtained at 2 mM $[Ca^{2+}]_o$ (left column) or at 10 mM $[Ca^{2+}]_o$ (right column). The numbers between the traces indicate the corresponding membrane potential for both conditions. The traces were corrected with a sloping baseline fitted during the second half of the depolarizations as there was not a prominent I_{Ca} in this fibre. The amount of charge moved was calculated as the integral of the area below the initial transient deflection at the beginning of the depolarizations. Charge movement at the beginning of the pulses may be underestimated for potentials where the I_{Ca} was activated and when the extracellular solution contained 10 mM $[Ca^{2+}]_o$. Since charge movement at the end of the pulses may be contaminated with an inward Ca^{2+} tail current it was not quantified.

In the majority of the fibres, charge movement was recorded at membrane potentials as negative as −80 mV in 2 mM $[Ca^{2+}]_o$, and saturated at about −10 mV. Charge movement was increased after raising external Ca^{2+} and the maximum amount of charge moved saturated between 0 and 10 mV. Figure 5*B* shows the voltage–charge movement relationship at either 2 or 10 mM $[Ca^{2+}]_o$. The average values of different fibres were fitted to the Boltzmann relation

$$Q = Q_{max}/(1+\exp(V_{q\frac{1}{2}}-V/k)), \quad (3)$$

where Q_{max} is the maximum charge moved, $V_{q\frac{1}{2}}$ is the mid-point potential of the curve, and k is the steepness of the curve. The best fit of the data gave the following values for each parameter: 2 mM $[Ca^{2+}]_o$, $Q_{max} = 5{\cdot}2 \pm 0{\cdot}9$ nC/μF, $V_{q\frac{1}{2}} = -45{\cdot}9 \pm 4$ mV and $k = 12{\cdot}9 \pm 1$ mV (13); 10 mM $[Ca^{2+}]_o$, $Q_{max} = 9{\cdot}8 \pm 1{\cdot}8$ nC/μF, $V_{q\frac{1}{2}} = -28{\cdot}8 \pm 5$ mV and $k = 15{\cdot}2 \pm 2$ mV (11). Q_{max} in human muscle fibres is smaller than in skeletal muscle from other species. In frog muscle, the values reported for Q_{max} range between 20 and 40 nC/μF under comparable recording conditions (Horowicz & Schneider, 1981; Melzer, Schneider, Simon & Szücs, 1986; Brum, Fitts, Pizarro & Ríos, 1988; Hui & Chandler, 1990). In rat and rabbit fast-twitch muscle, Q_{max} ranges between 15 nC/μF and 26 nC/μF (Hollingworth & Marshall, 1981; Dulhunty & Gage, 1983; Simon & Beam, 1985; Lamb, 1986; Lamb & Walsh, 1987). The increase of Q_{max} after raising the $[Ca^{2+}]_o$ is in agreement with results obtained in frog muscle. This has been attributed to the transformation of charge 2 into charge 1 (Brum & Ríos, 1987). The steepness of the curve obtained from cut fibres, k, falls within the range reported for both frog and rat muscles between 10 and 20 mV (Horowicz & Schneider, 1981; Hollingworth & Marshall, 1981; Dulhunty & Gage, 1983; Simon & Beam, 1985; Melzer *et al.* 1986; Lamb, 1986; Lamb & Walsh, 1987; Brum *et al.* 1988; Hui & Chandler, 1990).

DISCUSSION

Passive properties

The membrane parameters for human muscle (vastus lateralis and biceps) obtained with the Vaseline-gap technique are reported for the first time in this study. Previous measurements from intercostal muscles have been reported with the use of

intracellular microelectrodes (Kwiecinski *et al.* 1984). The specific membrane capacitance measured in those fibres was between 4·7 and 5·2 μF/cm^2 which is lower than our value of 8·9 μF/cm^2. This difference may be due to the contribution of the muscle membrane under the Vaseline seals, since in these portions there is a spatial variation of the potential compared to the middle-pool membrane (Hui & Chandler, 1990). Our value of R_m (1·176 kΩ cm^2) is about 3–5 times smaller than the ones reported by Kwiecinski *et al.* (1984) (5·970 kΩ cm^2). If the intercostal muscle and the vastus lateralis were of the same type, then we would expect a higher value of R_m since we blocked or maximally reduced the conductances to Na^+, K^+, and Cl^-. One possibility to explain this difference is that a fraction of current may pass under the Vaseline seals. However, the resistance under the seals, r_e, was about 100 times larger than the internal resistance per unit length and about 10 times larger than the membrane resistance for unit length. Thus, our measured R_m value of the vastus lateralis is representative of the specific membrane resistance of this muscle measured with this technique.

The inward current is carried by Ca^{2+}

The presence of an inward current carried by Ca^{2+} in adult cut fibres of human skeletal muscle is demonstrated with the use of the Vaseline-gap technique. The external $[Ca^{2+}]_o$ (2–10 mM) we used is close to the free Ca^{2+} concentration in plasma and the interstitial fluid. Even at $[Ca^{2+}]_o$ as low as 2 mM and at 17 °C, we were able to detect the I_{Ca} (Fig. 1*A*). The inward current was carried by Ca^{2+} ions since: (a) it was increased when the external Ca^{2+} concentration was raised from 2 to 10 mM (Fig. 1*A*); (b) the extracellular solution always contained TTX; (c) the external Na^+ was replaced by a non-impermeant cation such as TEA, and (d) the current was reduced (0·2–0·5 μM) or completely blocked by 2 μM-nifedipine.

Comparison with previous work

Two distinct types of Ca^{2+} channels have been described in skeletal muscle of the adult frog (Cota & Stefani, 1986). The main current resembles the L-type current in its voltage dependency and sensitivity to nifedipine and differs in its activation, which is very slow. In adult rat and rabbit muscle, the I_{Ca} is L type and is also blocked by nifedipine (Donaldson & Beam, 1983; Lamb & Walsh, 1987). I_{Ca} from mammalian muscle cells in culture displays two components. Embryonic or neonatal muscle cells have a T-type current very similar to cardiac and smooth muscles and a slow, DHP-sensitive one that appears later (Beam, Knudson & Powell, 1986; Cognard *et al.* 1986; Gonoi & Hasegawa, 1988). However, in newborn rats the relative amplitude of the two currents varied with development; in older animals the slow current is increased while the fast one becomes too small to be recorded (Beam & Knudson, 1988). In this study, we found only a I_{Ca} that resembles the L-type current. Human muscle may behave like that of other mammals in that it has at least two calcium currents in culture but only one is detected in the adult muscle.

The density of I_{Ca} in human muscle was much lower than in frog and rat skeletal muscles. The maximum I_{Ca} in human muscle was $-3{\cdot}04$ μA/μF in 10 mM $[Ca^{2+}]_o$ and at 27 °C, which corresponds to about -27 μA/cm^2 (Fig. 1*B*). The corresponding value for rat muscle is -226 μA/cm^2 at temperatures of 26–37 °C, and -139 μA/cm^2

at 20–26 °C (Donaldson & Beam, 1983). For frog muscle the maximum amplitude of I_{Ca} is $-80\ \mu A/cm^2$ at 22–26 °C (Sánchez & Stefani, 1983). In 2 mM $[Ca^{2+}]_o$ at 17 °C the I_{Ca} in human muscle was $-0{\cdot}42\ \mu A/\mu F$ or $-3{\cdot}74\ \mu A/cm^2$. The smaller I_{Ca} amplitude in these experiments can be related to a mixed population of fibre types present in vastus lateralis muscle. The activation and the time to peak of the I_{Ca} is slower in human muscle (see Figs 1*A* and 3). The time constant of activation reached a minimum value of about 100 ms at 17 °C while in muscle from rat, τ_m is about 50 ms (Mejía-Alvarez *et al.* 1990; Delbono *et al.* 1991). In frog muscle, τ_m is 40 ms for the slow I_{Ca} (Sánchez & Stefani, 1983). The slower value of τ_m in human muscle may represent an actual property of this muscle.

An interesting correlation between the amplitude and the time course of activation of the I_{Ca} can be pursued in skeletal muscle. In frog muscle, where the density of I_{Ca} is much greater, the activation of the I_{Ca} could be better described by using an exponent of 3 in a m^3h Hodgkin–Huxley relationship (Sánchez & Stefani, 1983). In rat muscle, with a large I_{Ca}, fitting of the current required an exponent of 1 (Mejía-Alvarez *et al.* 1991). The fitting performed by Sánche & Stefani (1983) in frog muscle was probably due to a loss of voltage clamp control. This indicates that the clamp control of the tubular membrane was better attained in human muscle, resulting in a more uniform opening of Ca^{2+} channels.

Effect of temperature

Raising the temperature caused several modifications of the I_{Ca}: (a) increase in amplitude; (b) reduction of τ_m during depolarizing pulses at all the membrane potentials; and (c) increase of the deactivation time constant. These data indicate that the Ca^{2+} channel from human muscle stays in an open state for longer times and over a wider range of membrane potentials. In order to achieve such a condition, there must be an increase of the rate constant from the closed to the open state and/or a decrease of the rate constant from the opposite direction. Evidence indicating that the latter rate constant was decreased comes from the deactivation of the tail current curve (Fig. 4).

Non-linear charge movement

The maximum amount of charge movement in human muscle is several times smaller than in other species. However, the mid-point potential and the steepness of the curve for the voltage–charge relationship (Fig. 5) are within the range of the values reported for frog and rat muscles (Horowicz & Schneider, 1981; Hollingworth & Marshall, 1981; Dulhunty & Gage, 1983; Simon & Beam, 1985; Melzer *et al.* 1986; Lamb, 1986; Lamb & Walsh, 1987; Brum *et al.* 1988; Hui & Chandler, 1990). Furthermore, the charge movement is sensitive to the $[Ca^{2+}]_o$ as in the case of the frog muscle, in which the charge increases with the elevation of the $[Ca^{2+}]_o$ (Brum & Ríos, 1987). Measurement of the charge movement at higher temperatures was not possible due to the activation of the I_{Ca}.

As seen from the activation curve for the I_{Ca} (Fig. 2) and the voltage–charge relationship (Fig. 5*B*), the mid-point potentials are very different. At 17 °C and 2 mM $[Ca^{2+}]_o$ the former has a mid-point close to 0 mV while the mid-point of the activation curve for the charge movement was −46 mV. This large difference in the half-

activation potentials for I_{Ca} and charge movement suggests the presence of many voltage-dependent closed states prior to channel opening. In addition it is possible that only a fraction of the charge movement may correspond to the gating of Ca^{2+} channels and that the rest of the charge may function as the voltage sensor of excitation–contraction coupling (Lamb & Walsh, 1987; Ríos & Brum, 1987; Tanabe, Beam, Powell & Numa, 1988).

This study demonstrates the electrophysiological properties of the I_{Ca} and charge movement in normal mature human muscle. These techniques and experiments open new exciting possibilities for the study of human muscle physiology in both health and disease.

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