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Charge movement associated with contraction threshold depolarizations in frog skeletal muscle fibers

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Fisiologia. — *Charge movement associated with contraction threshold depolarizations in frog skeletal muscle fibers* (*). Nota di ANTONIO PERES, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Sono stati eseguiti esperimenti che permettono l'osservazione simultanea del movimento di carica intramembrana e della contrazione meccanica di singole fibre di muscoli scheletrici di rana.

La quantità di carica associata ad impulsi che provocano una contrazione appena visibile risulta essere variabile rispetto al potenziale dell'impulso, con un valore minimo al potenziale di reobase di circa 4.75 nC/μF.

Esaminando i meccanismi per mezzo dei quali si pensa che il movimento di carica intramembrana possa regolare la concentrazione mioplasmica di calcio, si può arguire che le quantità di carica misurate con la presente procedura sperimentale non rappresentano solo quelle strettamente necessarie per innescare il rilascio del calcio da parte del reticolo sarcoplasmatico. Ciononostante i risultati ottenuti in questi esperimenti sono consistenti con l'ipotesi che il rilascio di calcio sia provocato dal movimento di una quantità critica di carica.

INTRODUCTION

It has been suggested that the rearrangement of charged particles in the membrane electrical field [1], [2] is involved in the excitation-contraction coupling of muscle fibers. Whatever the details of the actual mechanism might be, it seems plausible to think of charge movement as the first of a number of steps relating tubular membrane depolarization to calcium release from the sarcoplasmic reticulum [3]. From this point of view it is of great interest to perform experiments in which charge movement and mechanical contraction can be observed simultaneously. The recently introduced [4], [5] cut fiber voltage-clamp technique meets this requirement.

METHODS

The method used in the present experiments is essentially the same as the one developed by Kovacs and Schneider [4]. Single fibers isolated for a length of about 1 cm from semitendinosus muscles of Rana esculenta were immersed in a solution containing 120 mM K-glutammate, 2 mM $MgCl_2$, .01 mM EGTA and 5 mM Tris-maleate buffer at a pH of 7.0. The fiber was then cut and placed

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in an experimental chamber in which a partition made it possible to electrically isolate the cut end of the fiber from the intact end. The solution in the intact end compartment was changed to one containing 90 mM TEA_2SO_4, 10 mM RbCl, 8 mM $CaCl_2$, 5 mM Tris-maleate buffer at a pH of 7.0 and 2×10^{-7} g/ml TTX in order to minimize ionic currents through the membrane.

Extracellular electrodes connected the chamber to an electronic circuit which made it possible to take into account and compensate the effects of imperfect isolation and of the resistances in series with the membrane. After a preliminary phase of compensation the membrane potential was clamped at a holding potential of —100 mV over which voltage pulses were applied and the membrane current recorded.

The experiments were controlled by a Zilog MCZ 1/05 computer. Analog signals of membrane currents were sampled at a frequency of 4 KHz. The number of points in each record was reduced by a fourth order data decimation algorithm before storing them on the disk.

All experiments were done at temperatures between 2.2 and 5.4 °C.

RESULTS

In order to measure the charge movement associated with pulses giving rise to just detectable contractions, the following protocol was used. The duration of the depolarizing pulse was initially set and then the amplitude was changed millivolt by millivolt until a just visible movement occurred. Observations were made using a dissecting microscope at a magnification of 80×. This procedure appears to be quite reliable as often exactly the same duration and amplitude values were found for repeated measurements on the same fiber.

Once duration and amplitude values for threshold contraction were found, a computer controlled sequence of pulses was applied. This sequence included five 20 mV amplitude control pulses of the same duration followed by two test pulses to the pre-determined amplitude. Passive electrical properties were calculated from the currents elicited by the control pulses and also the control currents were appropriately subtracted from the test currents in order to obtain non-linear membrane currents. Another pulse duration was then chosen and all the previous steps repeated. This was done several times in order to obtain charge movement as well as the strength-duration curve for threshold contraction. This kind of curve is very similar to the curves obtained with the microelectrodes voltage-clamp [6], as already reported [4].

Fig. 1 shows a set of traces obtained with the procedure described above.

The control and test pulse protocol si designed to eliminate the linear components of membrane capacitance and membrane conductance. In most cases, however, non-linear conductance components are appreciably large in the subtracted traces. For high depolarizations and long durations these components might also be time-dependent. In the present experiments, since long pulses were given only at moderate depolarizations, subtraction of the control

currents from the test currents resulted in traces which apparently contained only a time independent, non linear component of the membrane conductance. This can be seen in Fig. 1 in the two uppermost traces. Elimination of this component was done by subtracting a horizontal baseline to all points for the duration of the pulse. The level of this baseline was the mean value of the points in an interval at the end of the pulse where the trace appeared by eye to be horizontal. When the duration was so short that no horizontal segment was detected

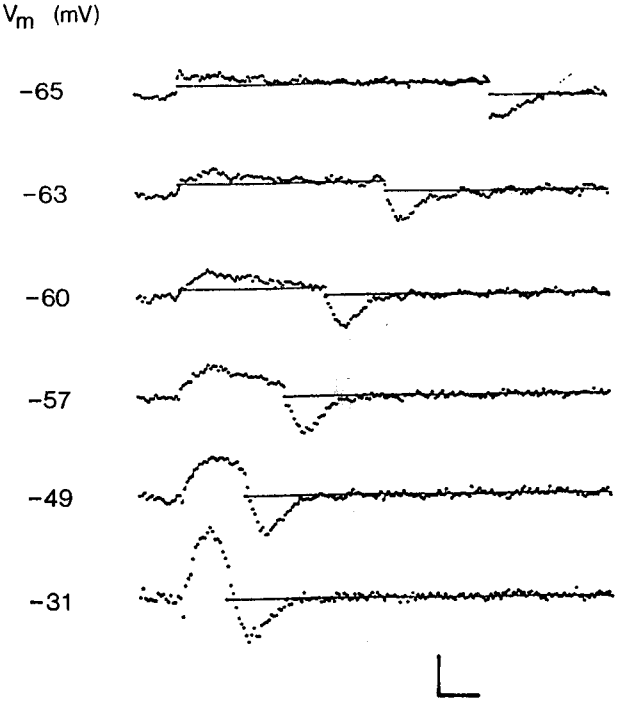

Fig. 1. – Transient currents associated with pulses causing just detectable contractions. Numbers on the left are membrane potential values during the pulses. The baselines substracted from the recording before integration of the transients are shown. Calibration bars represent 0.46 μA/μF and 20 msec. Fiber number 6, temperature between 2.7 and 3.6 °C, diameter 127.7 μm, length 696 μm.

the " on " transient was not used for the estimation of the charge. For the " off " part of the traces the baseline level to be substracted was estimated automatically as the mean value of the points from the forty-first millisecond after the end of the pulse to the last point of the recording. Fig. 1 gives an example of this baseline subtraction procedure. In this case the " on " transients were taken into account only for the three uppermost traces. Integration of the transients was then performed using Simpson's rule and the results were normalized to the membrane capacity measured from the control pulses.

Results of integration of the traces in Fig. 1 are shown in Fig. 2, where it is seen that the amount of charge associated with pulses causing a just detectable

contraction ($Q_{threshold}$) is smaller at rheobase than at more positive voltages. The rounding off of the voltage pulses, together with the baseline subtraction procedure outlined above, causes an underestimation of the amount of charge. This error will tend to be larger for increasing depolarizations, where the charge movement occurs faster. In view of this consideration, the non-equality of the charge, shown in Fig. 2, should be even greater.

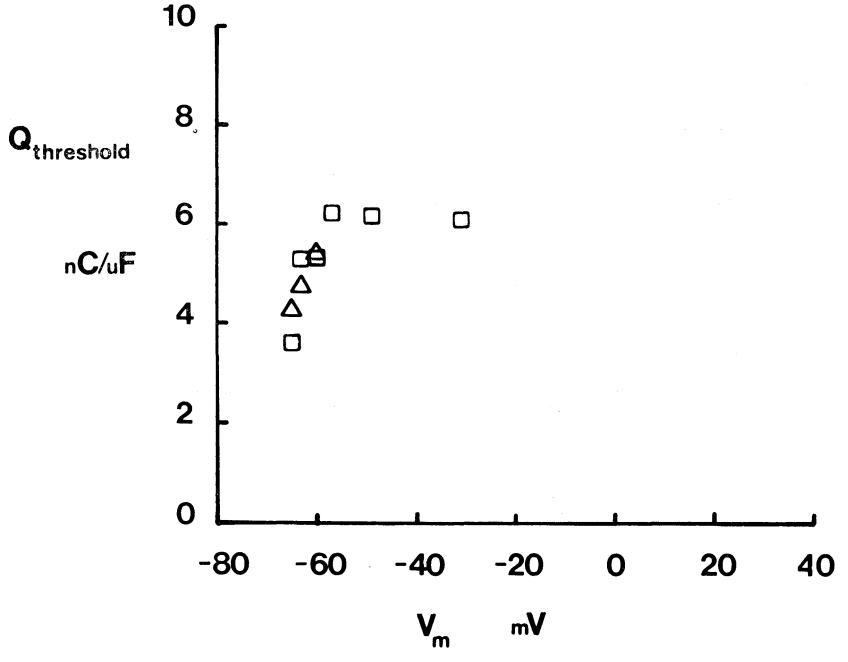

Fig. 2. – Results of integration of traces in Fig. 1. Squares are integrals of " off " transients only; triangles are mean values of " on " and " off " transients.

Fig. 3 shows pooled results from experiments of the same kind carried out on five different fibers. In order to normalize the results in this figure, the charge value corresponding to the voltage which was the nearest to —40 mV has been taken as equal to 1 for each fiber. These results confirm the finding that the amount of charge associated with pulses causing a just detectable contraction is not constant.

From similar experiments Horowicz and Schneider, [7], [8] reported that the amount of charge measured at contraction threshold was constant at the level of 11.5 nC/μF at all potentials. This value is much larger than the 4.75 nC/μF measured in the present experiments at rheobase potential. On the other hand the latter value is close to the estimates of Adrian *et al.* [9] and of Huang [10].

The reasons for these differences are not clear, but if it is true that $Q_{threshold}$ varies as shown in Fig. 3, it will be necessary to reconsider the model proposed by Horowicz and Schneider [8].

Indeed the results of the present experiments are to be expected on the basis of at least two different kinds of reasons. Firstly, if the total charge is made up of different species (Adrian and Almers, [11], [12]; Adrian and Peres [13], [14]), even if the one involved in the regulation of contraction moved in a constant amount, it is very unlikely that all the others would also do so. Secondly, though it might be true that a critical amount of charge has to be moved in order to trigger calcium release and the subsequent contraction, it should prove to be impossible to measure it directly following the procedure used in this work, as I will try to make clear in the following.

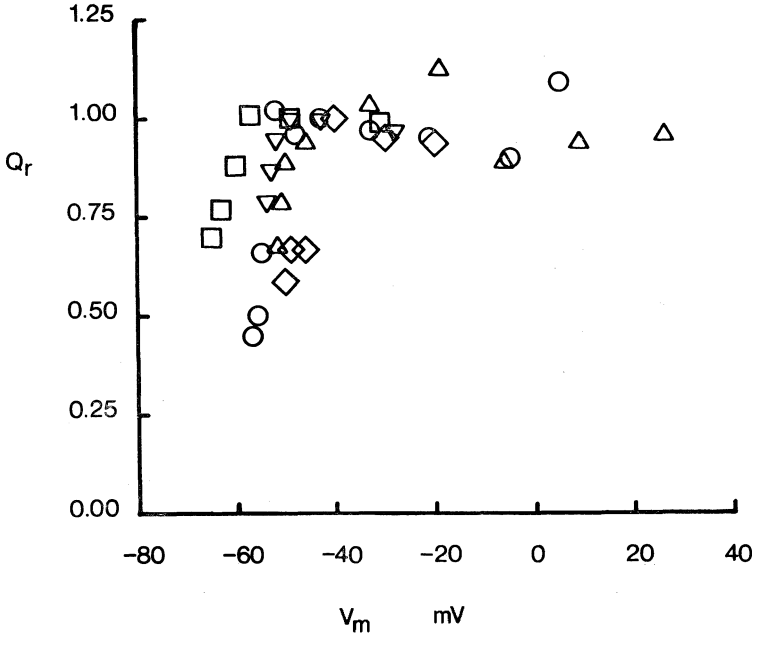

Fig. 3. – Data from experiments as in Fig. 1. performed on five different fibers. Q_r is the ratio of $Q_{threshold}$ (V) to $Q_{threshold}$ (V closest to —40 mV) for each fiber. Twenty-two of the thirty-eight points shown are mean values between " on " and " off "; the remaining sixteen are only " off " transients.

Kovacs *et al.* [3] showed that the time course of myoplasmic calcium concentration on depolarizaton might be interpreted as a redistribution of the ion among three compartments, which can be roughly identified as a release site of the sarcoplasmic reticulum, myoplasm and an uptake site of the sarcoplasmic reticulum. They showed that calcium follows a three compartments kinetics with constant transfer coefficients only after charge movement is finished (at least for the " on " transients). This seems to imply that the rate coefficients are dependent only on the amount of charge moved. If this is the case, calcium will begin to increase in the myoplasm only when the rate of its release exceeds the rate of uptake, i.e. when a critical amount of charge has been moved.

Consider a long pulse just above rheobase producing threshold contraction. The entire charge moving at this potential is needed to trigger calcium release. When the critical amount of charge has been reached calcium begins to increase. Some time must then pass before calcium reaches a concentration sufficient to produce contraction; during this time interval no additional charge moves so that the charge measured ($Q_{threshold}$) in this case is the true critical amount. This interpretation is consistent with the observation that at this potential, though the same amount of charge moves, the contraction is stronger when the pulse duration is increased (Peres, unpublished observations).

At larger depolarizations, when the total charge largely exceeds the critical amount, the time interval between the beginning of calcium release and the moment at which calcium reaches the critical concentration will decrease, but nevertheless during this time some other quantity of charge in addition to the critical amount will move. In this case the charge measured ($Q_{threshold}$) will be greater than the critical amount.

It is not possible to go further along this line of analysis without knowing a precise relationship between charge movement and calcium transfer rate coefficients, but it is clear that this schematization, together with the experimental procedure used in this work, will qualitatively predict the kind of results shown in Figs. 2 and 3.

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