J. Physiol. (1984), **353**, *pp.* 419–434
With 10 *text-figures*
Printed in Great Britain

EXPERIMENTAL ANALYSIS OF THE RELATIONSHIP BETWEEN CHARGE MOVEMENT COMPONENTS IN SKELETAL MUSCLE OF *RANA TEMPORARIA*

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(*Received 24 January 1984*)

SUMMARY

1. Experiments were performed to ascertain whether the monotonic (q_β) and delayed (q_γ) components of non-linear charge in skeletal muscle membranes form a sequential system, or are the result of separate, independent processes.

2. The non-linear capacitance studied in a large number of fibres increased with fibre diameter. This dependence was attributable to tetracaine-sensitive (q_γ) but not to tetracaine-resistant (q_β and q_α) charge.

3. The kinetics and total quantity of q_γ charge moving in reponse to voltage steps from varying pre-pulse potentials to a fixed probe potential remained constant despite variations in the size of the early q_β decay.

4. The kinetics of the delayed (q_γ) charging current obtained from a single 20 mV depolarizing step were compared with the sum of the responses to two 10 mV steps adding to the same voltage excursion. The respective transients superimposed only if one of the 10 mV steps did not reach the voltage at which q_γ first appears.

5. In the two preceding experiments, total charge was conserved.

6. These results are consistent with separate and functionally independent q_β and q_γ systems of potential-dependent charge, with q_γ residing in the transverse tubules and q_β on surface membrane. The findings can be discussed in terms of a contractile 'activator' with a steep sensitivity to voltage that begins only with depolarization beyond a level close to the actual mechanical threshold.

INTRODUCTION

There has been increasing evidence that the intramembrane charge movement first described by Schneider & Chandler (1973) in skeletal muscle includes at least three components of non-linear charge (Adrian & Peres, 1979; Huang, 1980, 1982; Hui, 1983). One of these, the q_α current, is best recorded after prolonged depolarization; its significance is unknown. The remaining monotonic (q_β) and delayed (q_γ) components can be recorded in response to depolarizing steps from the resting potential. Of these, it has been suggested that the q_γ charge is the one most likely to be involved in contractile activation (Adrian & Peres, 1979; Huang, 1982). However, in experimental records derived from such work, transients reflecting both q_β and q_γ appear in the same traces. Near the mechanical rheobase, the q_β charging current is a

relatively rapid monotonic decay, and the q_γ component appears as a delayed 'hump' which however becomes more rapid, so merging with the q_β transient with further depolarization.

The question therefore arises whether the empirical division of the charge that moves in the depolarizing voltage range into q_β and q_γ components reflects successive stages in a single, though complicated sequential process, initiated by a relatively rapid redistribution of q_β charge (cf. Horowicz & Schneider, 1981). Alternatively, the q_β and q_γ components might reflect separate systems, independent of each other, but with different rate constants, activated in parallel by an appropriate voltage change.

This paper describes a series of experiments attempting to distinguish between the two major possibilities. The rationale for each kind of experiment is described separately under the Results. The findings suggest that the q_β and q_γ charge form functionally separate systems with q_γ residing in transverse tubular, and q_β on surface membrane. These findings are discussed in terms of a hypothesis of contractile activation that assumes an involvement of the q_γ charge movement component in excitation–contraction coupling.

METHODS

A three-micro-electrode voltage clamp of the pelvic end of frog (*Rana temporaria*) sartorius muscle fibres employed experimental apparatus fully described elsewhere (Adrian & Rakowski, 1978). What follows is therefore only a summary of the experimental method, except where procedures are modified or optimized. Glass micro-electrodes, resistance 4–10 MΩ, were inserted 375 (voltage control electrode V_1), 750 (second voltage electrode V_2) and 875 μm (current injection electrode I_0) from the pelvic end. Potential recording electrodes were filled with 3 M-KCl, and the current injection electrode with 2 M-K citrate. The membrane current i_m for various imposed potential steps was examined:

$$i_m(t) = \frac{d}{6l^2 R_i}(V_2(t) - V_1(t)).$$

The linear membrane and cable constants were measured by 10 mV depolarizing steps applied from the holding potential of −90 mV. The values of the length constant, λ, the internal longitudinal resistance, r_i, and the resistance of unit length of membrane, r_m, were calculated from the steady values of the potentials V_1 and V_2, and the injected current I_0, at the end of the 10 mV step. The diameter, d, of the fibre studied, and its specific membrane constants R_m and C_m were computed employing a value of the internal sarcoplasmic resistivity $R_i = 391$ Ω cm in hypertonic solution at 2 °C and a Q_{10} of 1·37 (Hodgkin & Nakajima, 1972). The capacitative charge moved in response to the applied potential steps was computed as the Simpson's Rule integral of the transient part of the current at the beginning and at the end of each potential step, $\int (i_m(t) - g_m \Delta V_1(t))\,dt$ (Adrian & Almers, 1974) currents due to the leak admittance g_m having been corrected for. The above computations were performed on arrays representing $V_1(t)$, $V_2(t) - V_1(t)$ and $I_0(t)$, obtained by 12-bit analog-to-digital conversion, after filtering through three-pole Butterworth filters set at a corner frequency of 1·0 kHz, and sampled using a PDP 11/10E computer (Digital Equipment Corporation, Maynard, MA) with a Model 502 interface (Cambridge Electronic Design, Cambridge), at a sampling interval of 200 μs. Four to six sweeps were averaged in each record. Repeated control sweeps in the course of the experiments checked the condition and stability of the fibre. During analysis, and in particular before integration, the arrays were smoothed using the algorithm described by Adrian & Rakowski (1978): this approximates a sinc (f) function with a vertical cut-off frequency of 1·0 kHz.

Fibres were studied at 3–4 °C in the following solution at neutral pH: Rb_2SO_4, 2·5 mM; tetraethylammonium $(TEA)_2$ sulphate, 80 mM; (TEA) Cl, 15 mM; $CaSO_4$, 8 mM; tetrodotoxin (TTX), 2×10^{-7} M; Tris buffer, 3 mM; sucrose, 350 mM. Where the pulse procedure entailed depolarization of the fibre to −30 mV and beyond, 20 mM-$CoSO_4$ was added to suppress Ca^{2+} currents. In this case, the concentration of sucrose was adjusted to maintain constant hyperosmolarity.

RESULTS

In the course of analysis of the membrane capacitance of a number of fibres, we observed that the dependence of capacitance on fibre diameter differed between controls obtained at −90 mV, and the additional non-linear capacitance measured at membrane potentials between the resting potential (−90 mV) and 0 mV.

Hodgkin & Nakajima (1972) showed that when the membrane capacity is measured

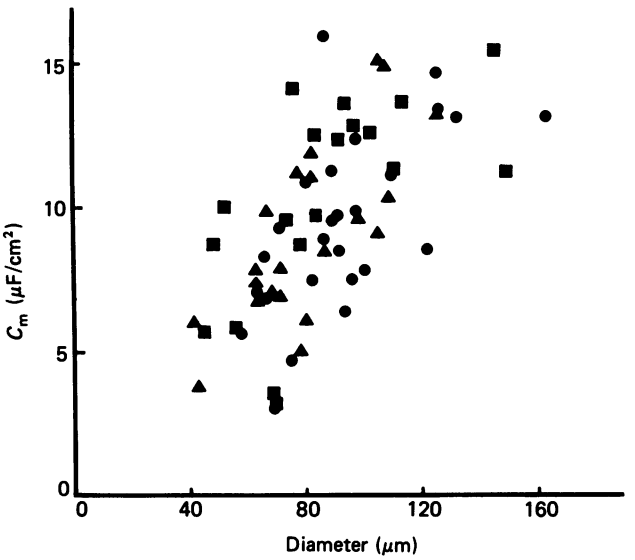


Fig. 1. Linear capacitance (measured at −90 mV) measured in $\mu F/cm^2$ of fibre surface area as a function of the diameter in the absence of local anaesthetics, the presence of 2 mM-tetracaine and the presence of 10 mM-lidocaine. The slopes of lines fitted to the three groups of points are: (circles) controls ($n = 26$): 0·80 ± 0·04 F/l; (triangles) 2 mM-tetracaine ($n = 21$): 0·83 ± 0·05 F/l; (squares) 10 mM-lidocaine ($n = 21$): 0·80 ± 0·05 F/l.

at the resting potential, larger fibres have larger capacitances when expressed as $\mu F/cm^2$ of fibre surface. They concluded that this dependence was what would be expected if a substantial fraction of the measured capacitance arose from the membranes of the transverse tubular system. Fig. 1 collects measurements of control capacitance at the resting potential for fibres in the Rb/TEA solutions described in the Methods section, and in this solution in the presence of either tetracaine or lidocaine. Fig. 1 confirms that under such conditions the control capacity behaves in the same way as described by Hodgkin & Nakajima (1972), and so one can conclude that neither the capacitance in the membrane of the transverse tubules nor its accessibility to measurement is affected by the local anaesthetics.

If one considers now the maximum value of the additional, voltage-dependent capacitance which can be measured by imposing a 10 mV step from a testing potential in the membrane potential range between −50 and −30 mV, tetracaine and lidocaine substantially affect the dependence of this additional capacitance on fibre diameter. The additional capacitance depends upon diameter *only* in the absence of local anaesthetic. In lidocaine, there is essentially no non-linear additional capacitance;

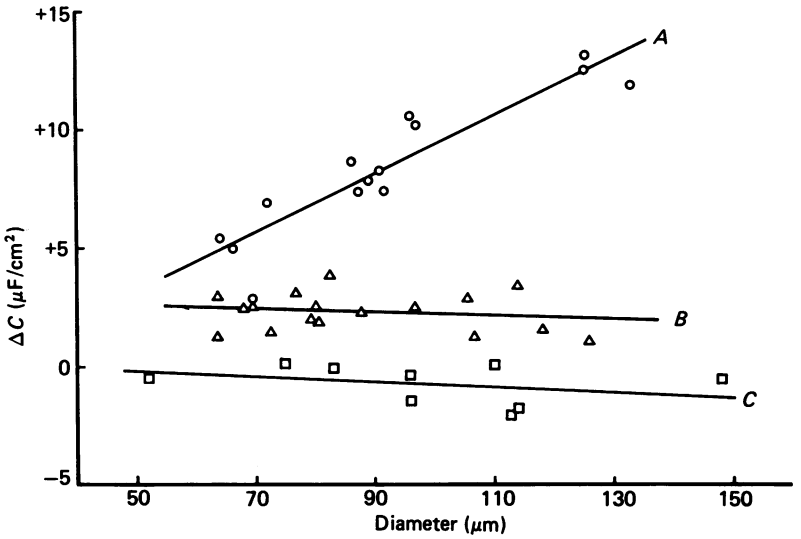


Fig. 2. Non-linear capacitance (μF/cm^2) as a function of diameter: *A*, in the absence of any local anaesthetics, *B*, in 2 mM-tetracaine and *C*, in the presence of 10 mM-lidocaine. The slopes of lines fitted to the three groups of points are: *A*, controls ($n = 14$): $1{\cdot}23 \pm 0{\cdot}04$ F/l; *B*, 2 mM-tetracaine ($n = 17$): $-0{\cdot}08 \pm 0{\cdot}024$ F/l; *C*, 10 mM-lidocaine ($n = 9$): $-0{\cdot}11 \pm 0{\cdot}036$ F/l. The slopes of *B* and *C* do not differ significantly from zero ($P \gg 5\%$). The slope of *A* does differ significantly from zero ($P \ll 0{\cdot}1\%$). The points on the plots were obtained by taking the difference between total capacitance and control capacitance as measured at −90 mV.

in tetracaine, the additional capacitance is only about 2 μF/cm^2, and it is constant for all observed fibre diameters (see Fig. 2).

The effect of tetracaine and lidocaine on the non-linear charging current has been described on earlier occasions. Tetracaine appears to remove the humped component q_γ, leaving an early and exponential component q_β (Huang, 1981, 1982; Hui, 1983). Lidocaine removes both q_β and q_γ (Huang, 1982). If this is the case, then the results of Fig. 2 suggest that the quantity of q_γ, but not the quantity of q_β, depends on fibre diameter, and this dependence in turn argues that q_γ is a charge movement arising from the polarization of a membrane component located in the transverse tubular system as well as possibly in the surface membrane. In contrast, the non-dependence of the quantity of q_β on fibre diameter suggests that it arises from the polarization of a different membrane component and one that is not found in the transverse tubular system.

If the membrane components giving rise to q_β and q_γ are located in different parts of the membrane, and in particular if q_γ can take place in a membrane without the q_β component, it becomes difficult to suppose that these two charge movements represent successive stages of one complex polarization process. It is much simpler to consider them as arising in parallel, one from a component that polarizes rapidly (q_β), and one from a component which, at least over a range of membrane potentials, polarizes much more slowly (Adrian & Huang, 1984).

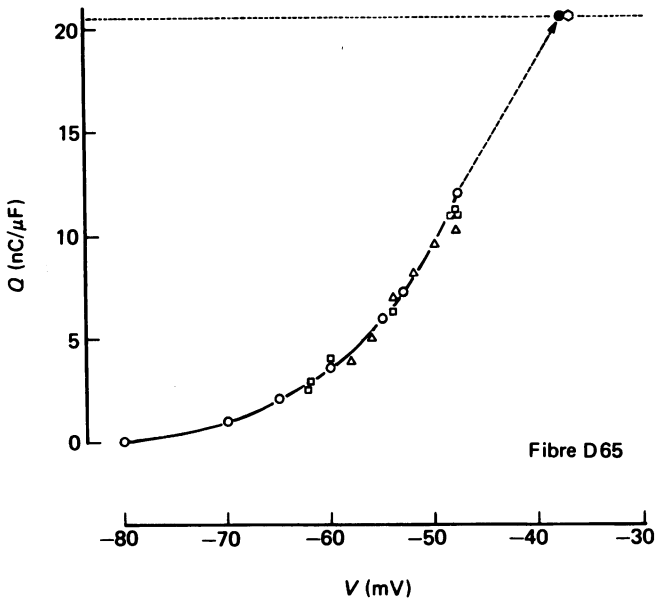


Fig. 3. Conservation of charge during a step to −38 mV in the pre-pulse experiments. The dependence of non-linear capacitance on voltage predicts a Q–V curve (open circles) that yields a charge at −38 mV (filled circle) in agreement with the measurement obtained using a large step (hexagon). This value, together with the charge moved on going from a pre-pulse voltage to the test level gave Q–V curves (triangles and squares) coincident with the line drawn through the clear circles, implying that all these pulses measure a conserved charge. Fibre cable constants: $R_i = 344\ \Omega$ cm; temperature = 3·1 °C; $\lambda = 2{\cdot}72$ mm; $r_i = 6042\ \text{k}\Omega/\text{cm}$; $d = 85\ \mu$m; $r_m = 480{\cdot}7\ \text{k}\Omega$ cm; $R_m = 13{\cdot}12\ \text{k}\Omega\ \text{cm}^2$; $C_m = 9{\cdot}5\ \mu\text{F/cm}^2$.

Voltage-clamp steps to a fixed potential from varying initial potentials

If q_β and q_γ represent the polarization of independent membrane components it should be possible to alter their magnitude independently. Since the potential dependence of the charge moved for q_β and q_γ differs, we attempted to find conditions where the slowly moving q_γ was readily identifiable and constant, but where the exponential and initial q_β component varied. This was achieved by stepping the membrane potential from −90 mV with a pre-pulse to a range of values and examining the charge movement during a 125 ms step to a fixed potential. This test step, which varied in size depending on the initial level, was imposed 500 ms from the beginning of the pre-pulse so that charge movement during the pre-pulses could relax fully.

The responses to the test pulses were sampled, with five sweeps averaged for each record. Groups of four or five such averages were bracketed by taking control responses to 10 mV steps at the −90 mV holding potential. The non-linear transients from each test step were calculated as the difference between the test responses and the control responses scaled by the ratio of the test to the control voltage step size. Each run began and ended with records of currents from 10 mV steps to the test voltage.

In order to use this approach to examine the relationship between the initial (q_β)

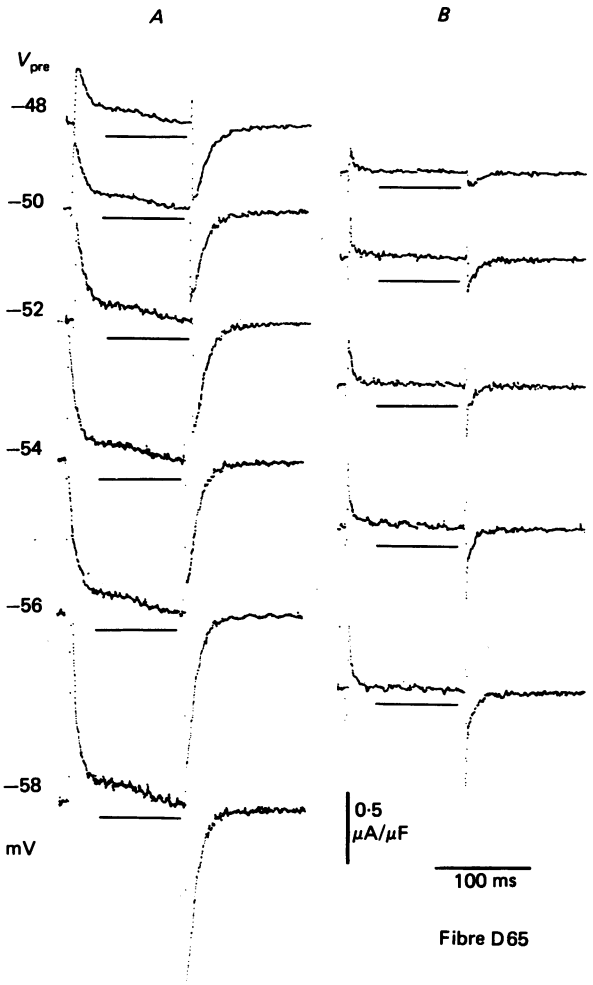


Fig. 4. *A*, charge movements obtained in response to voltage-clamp steps from closely spaced pre-pulse levels to a fixed −38 mV test level, just beyond the 'threshold' of distinct q_γ transients (marked by horizontal bars). *B*, the differences between each successive pair of transients imply that the early q_β becomes successively larger, but the delayed q_γ (horizontal bars) show little or no change.

and delayed (q_γ) components of the charge movement, the chosen test voltage was a depolarization of moderate amplitude where q_γ is a delayed transient distinct in time from the relatively more rapid q_β decay.

Accordingly, before each procedure, the membrane capacitance was explored using 10 mV steps through successively varied, closely spaced, pre-pulse levels (cf. Adrian & Peres, 1979; Huang, 1982). From the currents derived from such a procedure it was possible to choose a pulse voltage at which the two components of the transient response were distinct. In addition, the charge–voltage (Q–V) curve for the non-linear part of the charge was derived by integrating the values of the capacitance obtained as a function of potential (Fig. 3: open circles), up to the chosen pulse voltage. The

amount of charge at this level (Fig. 3: filled circle), was compared with the amount of non-linear charge measured on imposition of a large voltage excursion from the −90 mV to the chosen pulse voltage (Fig. 3: hexagon), and these values agreed. Therefore the Q–V curve obtained by an analysis of results from small steps gives the same total charge as obtained from the response to a large step, and the measurements are a valid indication of the quantities of a conserved charge at the different voltages. The next step was to observe the non-linear charge redistributing in response to potential changes from the range of pre-pulse voltages to the chosen potential as defined above. With these values, as well as that of the total non-linear charge measured in response to the large step (Fig. 3: hexagon), by assuming that charge is conserved, it was possible to deduce the amount of non-linear charge expected at each pre-pulse potential (Fig. 3: triangles and squares). These points fell on the same function as the previous Q–V curve (Fig. 3: open circles). Therefore the latter pulse procedure also observes a conserved (capacitative) charge (Huang, 1983*a*); this latter point is a necessary condition for interpretation of the results to follow.

Fig. 4 shows charge movements for potential steps from a range of closely spaced pre-pulse levels, V_{pre}, to a constant, −38 mV, test potential. Records are shown at high gain to give a clear indication of the time course of the delayed (q_γ) component, but sometimes at the expense of fully representing the larger (q_β) initial decay. Earlier work (Huang, 1982; Duane & Huang, 1982) has shown that the q_β charge is significantly potential dependent over both the narrow (Fig. 4) and most of the wide (Fig. 5) range of pre-pulse potentials examined. The initial distribution of q_β charge would therefore vary with each trace as the pre-pulse was altered. Consequently, the initial exponential decays (q_β) would be expected to vary as the charge moved from its varying initial to a constant final distribution. This was confirmed in the experimental records (Figs. 4*A* and 5*A*), in which the initial decays became larger as the pre-pulse step was made to less depolarized levels.

In contrast, the delayed q_γ transients, marked by horizontal lines in Figs. 4*A* and 5*A*, assumed the same form despite the varying pre-pulse levels. This was borne out in the successive differences between pairs of charge movement traces (Figs. 4*B* and 5*B*). These records clearly show differences in q_β between successive pairs of records, but show little or no difference at times when q_γ is moving. One can conclude that although q_β moves over most of the pre-pulse potential range between −64 and −38 mV, little or no q_γ is moved between −64 and −48 mV. Almost all the q_γ visible in the records of Figs. 4 and 5 is moved between −48 and −38 mV.

Had the initial decay (q_β) and the q_γ transient been the result of a sequence of reactions in series, the varying initial distributions of q_β, varying because the pre-pulse potential varies, should in turn have influenced the distribution of q_γ at the beginning of the test step, and this should have affected the q_γ transients. The experiments here are not in accord with this prediction; the q_γ transient is substantially constant despite demonstrable variations in the q_β decays. Rather, such results suggest that q_β and q_γ are what one would expect of separate systems independently influenced by voltage. If this is so, earlier results (Adrian & Peres, 1979; Huang, 1982) can be interpreted in terms of an independent q_γ system steeply dependent upon membrane potential, but only from depolarizations beyond around −50 mV. The

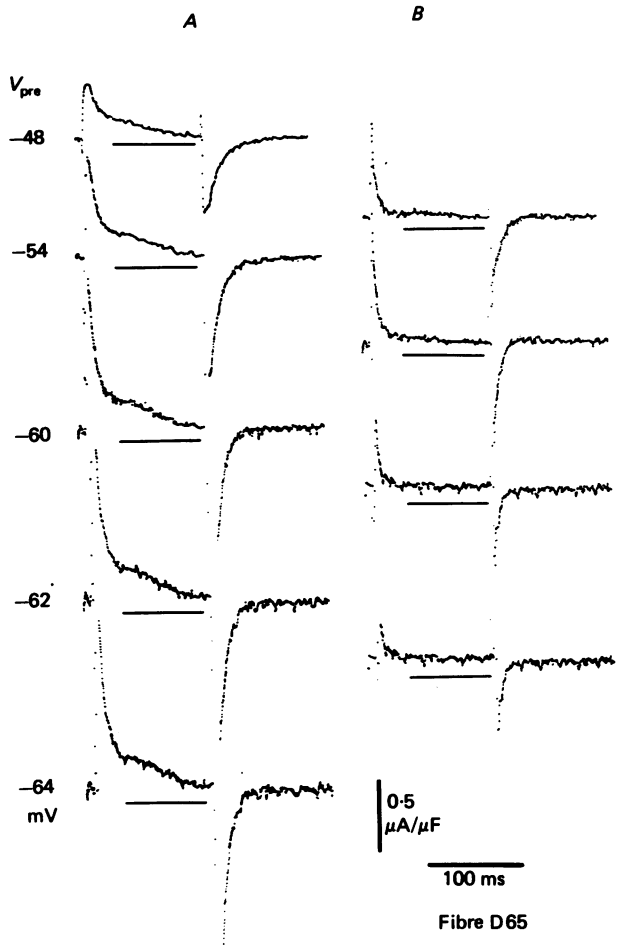


Fig. 5. Experiment described as in Fig. 4, but using a run incorporating a wider voltage range.

particular pre-pulses adopted here then would leave the q_γ system in the same, fully saturated initial state, as all the test voltage steps investigated were from levels close, or hyperpolarized to, around −50 mV. The test steps to a fixed potential (of −38 mV) should then result in a constant q_γ transient despite variations in the other, earlier, q_β component: this is in agreement with the experimental results.

For each imposed test step, the total potential-dependent charge was obtained by integrating the transient part of the non-linear current. This fell as the pre-pulse potentials were moved to more depolarized levels (Fig. 6*A*), as would be expected as the voltage excursion of the test step was reduced. An estimate of the amounts of q_β and q_γ moving during the test step was made in the following way. The 'on' transients were plotted on a semilogarithmic scale. The initial decay then approximated a straight line. The initial amplitude A_0 and the time constant τ accordingly gave the charge $A_0\tau$, attributable to q_β (Fig. 6*B*). This also fell with depolarization of

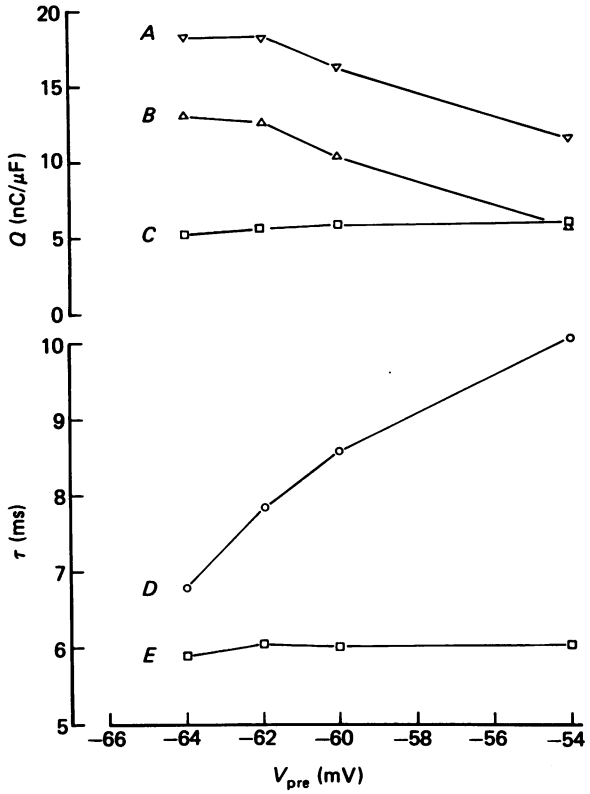


Fig. 6. Pre-pulse experiments measuring the non-linear charge resulting from steps from varying pre-pulse voltages V_{pre}, to a fixed −38 mV probe level. Both *A*, the total charge and *B*, the portion of charge due to q_β fall with depolarization. However, despite this, *C*, the q_γ charge, estimated as the difference *A*–*B*, remains constant. Whereas *D*, the single time constant of the 'off' response varies with the voltage, V_{pre}, to which the system returns, *E*, that of the q_β charge in the 'on' response remains constant.

pre-pulse level, as would be expected of a process that was voltage-dependent through the explored pre-pulse potential range. The steady-state (delayed) q_γ charge was estimated as the difference (*A*–*B*) between total and q_β charge (Fig. 6*C*). This quantity remained constant in the face of changes in q_β, a result that reinforces the conclusions from the subtractions of pairs of transients shown in Figs. 4 and 5.

A straightforward separation of components was not possible in the 'off' currents. These approximated single exponentials whose time constants (Fig. 6*D*) varied with the potential, V_{pre}, to which the membrane was returned from the test pulse. In contrast, the initial decays of the 'on' records all showed the same time constant independent of the pre-pulse potential (Fig. 6*E*). This behaviour would be expected of a first-order system with potential-dependent rate constants, in which the changes with pre-pulse in the quantity of q_β moved appear as simple scaling of its transient current.

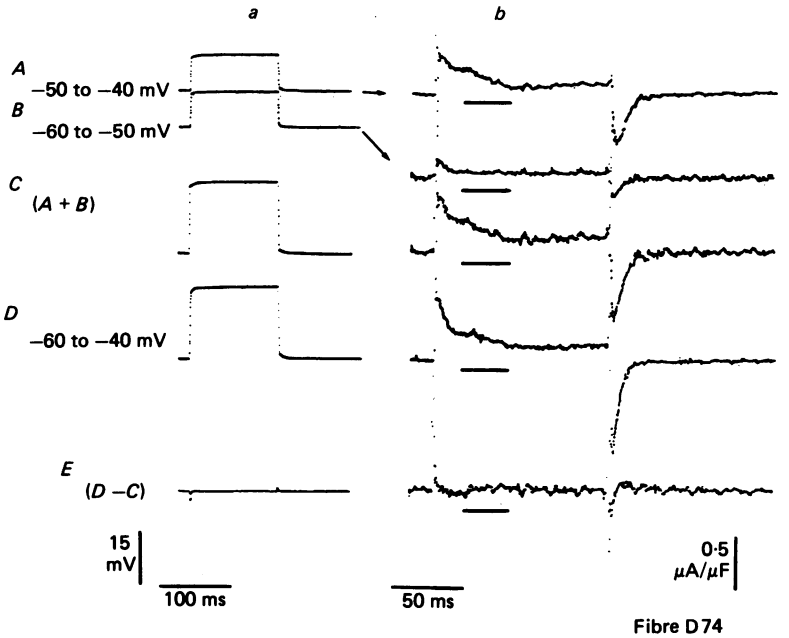


Fig. 7. Voltage superposition experiment in which only one of the applied 10 mV steps moves obvious delayed charge: *a*, records of voltage-clamp steps; *b*, non-linear transients. *A*, a voltage step from −50 to −40 mV results in a distinct q_γ charge movement (horizontal bar), but *B*, one from −60 to −50 mV does not. The sum (*C*) of the steps *A* and *B*, amounts to a 20 mV step. The current arrays so produced are compared with the effect of *D*, a single 20 mV step from −60 to −40 mV. The transient (*b*) has a faster q_β decay, but *E*, the difference *D*−*C* gives a virtually flat current trace at times corresponding to relaxation of q_γ (horizontal bar). Fibre cable constants: R_i = 333·6 Ω cm; temperature = 4·1 °C; λ = 3·32 mm; r_i = 6509 kΩ/cm; d = 81 μm; r_m = 718·4 kΩ cm; R_m = 18·23 kΩ cm²; C_m = 8·0 μF/cm².

Small and large steps of potential

A variant of the experimental approach in the previous section confirms the conclusion that q_γ is moved only when the membrane is depolarized to values positive to around −50 mV and that its movement is independent of the initial distribution of q_β.

Measurements were made on fibres in the sulphate solution described in Methods to which 20 mM-Co^{2+} had been added to minimize Ca^{2+}-current transients. Each experiment began by exploring different pre-pulse potentials at close intervals (2–3 mV) from which 10 mV test steps (duration 125 ms) were imposed. The object was to locate a pre-pulse voltage where the transient current during the test pulse showed a clear and prolonged q_γ component.

In the experiment shown in Fig. 7, two 10 mV test steps were applied from pre-pulse voltages of −50 and −60 mV respectively. The pre-pulse voltage was maintained for 500 ms before the test pulse was applied. The voltage records of the test pulses are shown in Fig. 7*Aa* and *Ba*; the transient non-linear currents in Fig. 7*Ab* and *Bb*. These records have been summed in Fig. 7*C*. Fig. 7*D* shows experimental traces (*Da*,

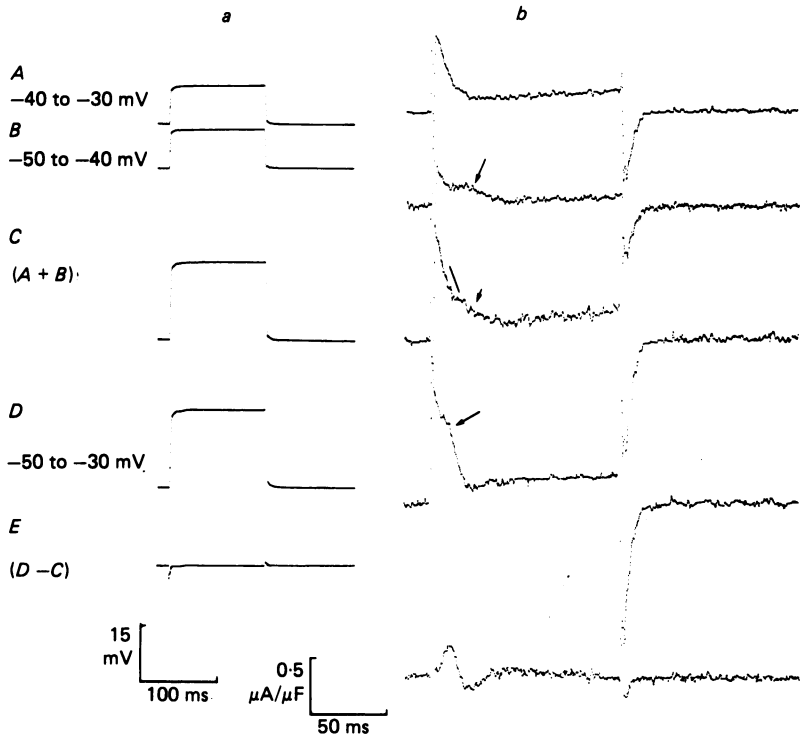


Fig. 8. Records of *a*, voltage steps and *b*, non-linear transients in a voltage superposition experiment performed at a more depolarized level than in Fig. 7. In *A*, the voltage step between −40 and −30 mV, q_β and q_γ components have merged (cf. Huang, 1981); the q_γ component is nevertheless distinguishable (arrow) on applying *B*, the voltage step from −50 to −40 mV. *C*, both the voltage and the current records are added together to give $A+B$, and compared with the effect of *D*, an actual 20 mV step between −50 and −30 mV. In *D*, the q_γ kinetics are noticeably faster. In the difference trace *E*, which is $D-C$, whereas *a*, the voltage-clamp traces superpose, *b*, the kinetics of the non-linear transients do not. Fibre cable constants: $R_i = 342\ \Omega$ cm; temperature = 3·3 °C; $\lambda = 3{\cdot}43$ mm; $r_i = 6796$ kΩ/cm; $d = 80{\cdot}2\ \mu$m; $r_m = 773{\cdot}7$ kΩ cm; $R_m = 20{\cdot}69$ kΩ cm²; $C_m = 6{\cdot}8\ \mu$F/cm².

voltage trace; *Db*, transient current) in response to a 20 mV step imposed from a pre-pulse potential of −60 mV. Records *C* and *D* should therefore be identical except in so far as in one, all the charge has moved at a test potential of −40 mV, and in the other, part of the charge has moved at −50 mV, and the rest at −40 mV. The same total quantity of charge should have moved at the end of both Figs. 7*Cb* and *Db*. Fig. 7*E* gives the difference between traces *D* and *C* (*D*−*C*). The voltage excursions are virtually identical but the current transients only show any difference early in the test step. This strongly suggests that q_β *only* is affected in its distribution by membrane potentials between −60 and −50 mV.

The experiment shown in Fig. 8 compares two 10 mV test steps from respective pre-pulse potentials of −50 and −40 mV. As before, there are clearly separate q_β and

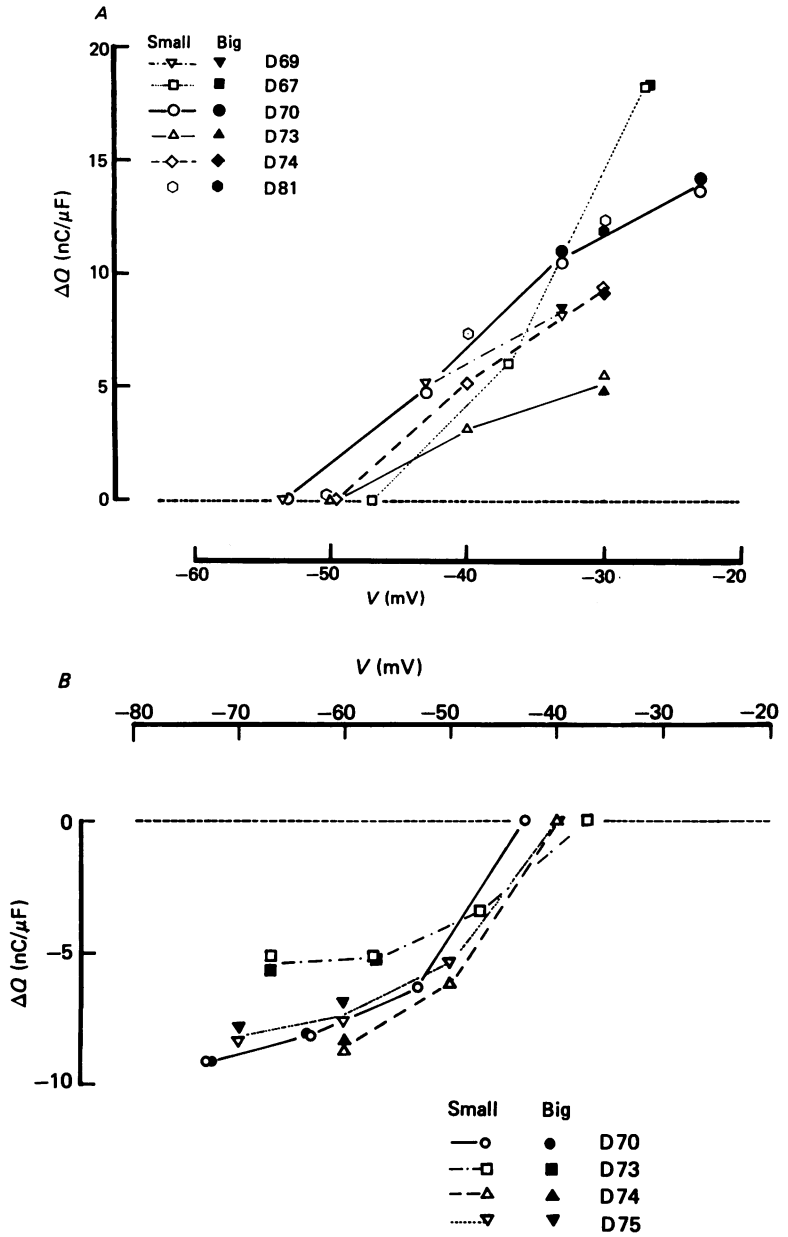
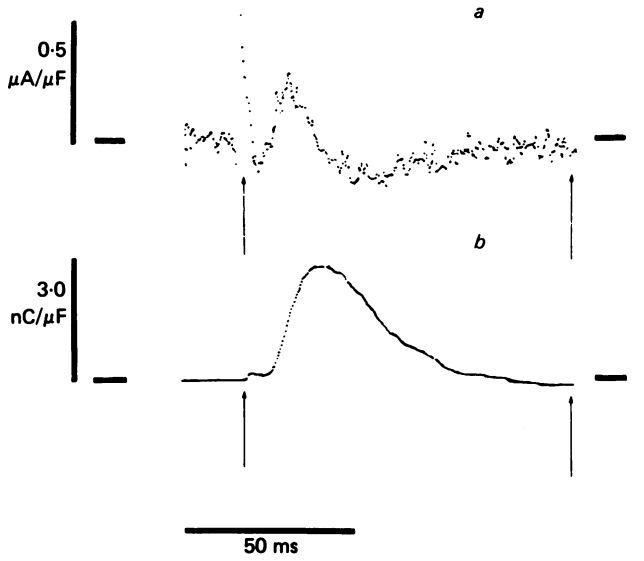


Fig. 9. The total non-linear charge moved by successive small (10 mV) steps (open symbols) compared with that moved by single large steps directly to the voltages concerned (filled symbols). In both *A* and *B*, the reference charge was at that pre-pulse level from which a 10 mV step first elicited a clear delayed q_γ component (Figs. 7 and 8). In *A*, the voltage steps in both cases moved appreciable q_γ; in *B*, only one of the 10 mV steps moved appreciable q_γ charge. In both kinds of experiments filled and open symbols coincided, i.e. charge was conserved.

q_γ transients in the non-linear transient current for the test step from −50 mV. In the 10 mV test step from −40 mV, the transient non-linear currents are much less clearly separated into two parts. Fig. 8*C* shows the records in *A* and *B* added, and Fig. 8*D* gives experimental records for a 20 mV step from −50 to −30 mV. It is clear that the transient non-linear currents have a different configuration in Fig. 8*C*



Fibre D81

Fig. 10. *a*, difference trace, obtained as described in Fig. 8, at high gain. The rapid upward deflexion gives an indication of q_γ kinetics at the maximal depolarization of −30 mV, the negative part of the wave, of q_γ kinetics at −40 mV, in the absence of the q_β transient. *b*, the running integral obtained between the cursors (arrows) suggests a q_γ charge moved of at least 3·0 nC/μF, in this 20 mV interval. The integral eventually ends close to zero, implying an over-all conservation of charge. Fibre cable constants: R_i = 336 Ω cm; temperature = 3·9 °C; λ = 3·77 mm; r_i = 10030 kΩ/cm; d = 66 μm; r_m = 1416 kΩ cm; R_m = 29·37 kΩ cm²; C_m = 4·2 μF/cm².

and *D*; this reflects the fact that q_γ is moved in *both* test steps and that in this potential range its rate of movement is steeply dependent on potential.

Once again, Fig. 8*E* shows the difference between the actual 20 mV test step and the 20 mV step synthesized from two 10 mV steps. In this case there is a clear biphasic transient in the region of the trace associated with q_γ movement. This is in contrast to the results in the previous kind of experiment (Fig. 7).

For reasons given earlier, interpretation of the above experiments assumes an over-all conservation of charge through the different voltage excursions studied. Fig. 9 shows the non-linear charge derived from adding together the areas under the transients obtained in response to 10 mV voltage steps imposed at levels depolarized successively by 10 mV (open symbols). These are compared with the charge obtained when large voltage steps were imposed directly to the potentials concerned (filled symbols). Results are shown for both (*A*) experiments where both the 10 mV steps moved a significant amount of q_γ charge, and (*B*) where only one of the two 10 mV steps moved any q_γ

charge. In the latter case the zero charge line corresponds to the charge displacement obtained at the more depolarized of the voltage steps. In both cases conservation of charge is confirmed in that results from using small steps (open symbols) agree with those when larger steps were imposed (closed symbols).

Difference traces such as Fig. 8*E* give a limited, but useful insight into the dependence of q_γ kinetics upon membrane potential. For example, since the subtraction is the difference between responses obtained from the large step, and the sum of the responses obtained from the small steps, the initial upward deflexion gives the minimum value of the q_γ transient at the larger depolarization. At such voltages, this shows (Figs. 8*E* and 10*a*) that the kinetics of the q_γ transient so isolated consist of a rising phase succeeded by a fall, and the form of this current is in close agreement with the kinetics for q_γ deduced by Hui (1983), who used a different and independent procedure. The running integral (Fig. 10*b*) indicates a minimum amount of 3 nC/μF of q_γ charge moved through just the 20 mV excursion being investigated. Conversely, the prolonged negative deflexion in the difference trace is a consequence of slow movement of q_γ charge near the foot of its dependence upon voltage. Finally, Fig. 10*b* show the integral of this transient and the fact that at 75 ms from the beginning of the pulse the integral is zero. This is to be expected if the observed transients are movements of a conserved charge. There is also a suggestion of biphasic transients in record *E* of Fig. 7, but the noise level makes it hard to substantiate. The small and rapid transients at the beginning and end of the test-pulse period could be due to different rates of movement of q_β at -50, -40 and -30 mV, or, if they do not have a zero integral, to a small degree of inactivation during the pre-pulse to the more depolarized potential.

DISCUSSION

Previous work (Adrian & Peres, 1979; Huang, 1981, 1982, 1983*b*; Hui, 1983) has supported the suggestion that non-linear charge movement in frog striated muscle can be separated into three components q_α, q_β and q_γ. The experiments in this paper are concerned with the question of whether q_β and q_γ move independently in response to a change of membrane potential or whether the movement of q_γ depends in some way on the movement of q_β. The strongest evidence for the independence of q_γ from q_β is their respective dependence on fibre diameter. Linear capacitance increases with increasing fibre diameter (Hodgkin & Nakajima, 1972); likewise non-linear capacitance increases with fibre diameter, but this dependence arises entirely from the tetracaine-sensitive fraction of the non-linear polarization currents. Huang (1980, 1982) and Hui (1983) have shown specifically that tetracaine interferes with movement of q_γ. The simplest interpretation of these findings is that q_α and q_β are not present in the membrane of the transverse tubular system and that therefore in the tubular membrane at least, movement of q_γ can take place in the absence of any preceding movement of either q_α or q_β.

The experiments which examined the movements of q_β and q_γ in response to test steps of potential were based on a superimposition criterion for the analysis of non-linear systems that presupposes that the measured transients result from the redistribution of a finite amount of conserved charge. This latter conservation requirement was established empirically for each of the experimental procedures. The experimental confirmation of charge conservation in these circumstances reinforces earlier arguments for regarding the transient non-linear currents as capacitative as opposed to resistive (Chandler, Rakowski & Schneider, 1976; Adrian & Almers, 1976; Huang, 1983*a*). The membrane potentials selected for investigation were near the mechanical threshold: at such levels the q_γ relaxation was easily recognized as a delayed transient with a time course very sensitive to voltage changes.

One set of experiments imposed potential steps to a fixed membrane potential from a range of pre-pulse voltages. As would be expected from a potential dependence of q_β extending well into the pre-pulse levels studied, the initial decay of the transient non-linear current varied in size but maintained the same approximately exponential shape as the pre-pulse potential changed. In contrast, both the shape and total quantity of displaced q_γ remained constant, suggesting a process which did not depend on the initial distribution of q_β and which was independent of the pre-pulse voltage within the range studied (-65 to -50 mV).

The second set of experiments compared the transient non-linear currents for a 20 mV step and for 10 mV steps over the identical voltage range. Currents for the two steps were summed and compared to that for the 20 mV step. As might be expected, the summed and single current transients were not identical though the integral of the transients was equal, indicating that the single and divided pulses had moved the same quantity of charge, though with differing time courses. When one of the divided pulses covered the voltage range -60 to -50 mV, there were no differences in the summed and single current traces in the range of times associated with the movement of q_γ. Again, this confirms that the movement of q_γ is not affected by the initial distribution of q_β.

The findings here bear directly upon a possible role for the non-linear charge in initiating contraction. In this connexion, it has been argued that the delayed (q_γ) component of charge is the more directly involved. The separation of the q_γ fraction of charge has been accomplished by a variety of independent methods, including its removal by local anaesthetics (Huang, 1982), and analysis of its kinetics in the frequency domain (Huang, 1981, 1983*b*). Additionally, a separate kinetic criterion assuming a particular form for the shape of the q_γ current using both tetracaine and Dantrolene sodium as means of suppressing q_γ has been described by Hui (1983), and the q_γ transients so obtained closely resembled the currents deduced here (Fig. 10). *All* these approaches *independently* gave results in general agreement, and a steady-state distribution of the q_γ charge upon potential that closely paralleled the voltage dependence of the production of tension (Caputo & de Bolanos, 1979), and of the optical signals thought to reflect the release of sarcoplasmic Ca^{2+} (Vergara & Caputo, 1983). Additionally, there is the agreement between the slow transfers of q_γ charge and the kinetics of the build-up of 'activator' (Adrian, Chandler & Hodgkin, 1969) with prolonged depolarizations close to rheobase (Adrian & Huang, 1984). One might therefore assume that movements of q_γ, and the kinetics of the 'activator' can be equated, and that the rest (q_α and q_β) of the non-linear charge may play little or no part in the process of contractile activation.

In terms of such a hypothesis, the production of 'activator' possesses higher-order kinetics steeply dependent upon voltage, and is appreciable only beyond depolarizations close to levels corresponding to the actual mechanical threshold. At such potentials, the build-up of 'activator' is slow, with a time course that may exceed 200–300 ms at temperatures close to 4 °C, but the relaxations become an order of magnitude more rapid with even relatively little (5–10 mV) further depolarizations (cf. Adrian & Huang, 1984). In contrast, smaller depolarizations would not perturb the activator system, although they may affect the distribution of the (unrelated) q_β system. In any case, even in the absence of these admittedly simplified parallels,

the behaviour of q_γ is of interest as being the result of a steeply potential-dependent dielectric process, whose behaviour in response to the field cannot be explained in straightforward terms.

We thank Mr W. Smith for skilled assistance.

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