

Intramembrane Charge Movements in Frog Skeletal Muscle in Strongly Hypertonic Solutions

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ABSTRACT Intramembrane charge movements were studied in intact, voltage-clamped frog (*Rana temporaria*) skeletal muscle fibers in external solutions made increasingly hypertonic by addition of sucrose. The marked dependence of membrane capacitance on test potential persisted with increases in extracellular sucrose concentration between 350 and 500 mM. Charge movements continued to show distinguishable early monotonic (q_b) decays and the strongly voltage-dependent delayed (q_r) charging phases reported on earlier occasions. In contrast, a further increase to 600 mM sucrose abolished the most steeply voltage-sensitive part of the membrane capacitance. It left a more gradual variation with potential that closely resembled the function that resulted when q_r charge was abolished by tetracaine in the presence of 500 mM sucrose. Charging transients were now simple monotonic (q_b) decays and lacked delayed (q_r) transients. Furthermore, tetracaine (2 mM) altered neither the kinetic nor the steady-state features of the charge left in 600 mM sucrose. However, Ca^{2+} current activation in the same fibers persisted through such tonicity increases under identical conditions of temperature, external solution, and holding voltage. Tonicity changes thus accomplish an independent separation of q_r and q_b charge as defined hitherto through their tetracaine sensitivity. Their effects on q_r charge correlate with earlier observations of osmotic conditions on $\Delta[Ca^{2+}]$ signals (1987. *J. Physiol. (Lond.)* 383:615–627.) and the parallel effects of other agents on excitation–contraction coupling and q_r charge. In contrast, they suggest that Ca^{2+} current activation does not require q_r charge transfer whether by itself or as part of the excitation–contraction coupling process.

INTRODUCTION

An increasing amount of evidence has implicated receptors for 1,4 dihydropyridine calcium channel antagonists in voltage-sensing processes in skeletal muscle. Nifedipine partially blocks both intramembrane charge and intracellular Ca^{2+} signals elicited by depolarizing voltage clamp steps, particularly after small shifts in holding potential (Rios and Brum, 1987). Charge movements in turn have been resolved into several components on the basis of their voltage and pharmacological sensitivities

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(Adrian and Peres, 1979; Huang, 1981a; Hui, 1983). Of these, the q_v charge (Huang, 1982; Hui, 1983; Hui and Chandler, 1990) showed a distinctly steeper voltage sensitivity which closely resembled that of Ca^{2+} release at voltages around contractile threshold (Maylie, Irving, Sizto, and Chandler, 1987). Furthermore, q_v but not q_β charge was implicated in voltage-dependent block by nifedipine (Huang, 1990). This suggested parallels between q_v charge transfers and excitation–contraction coupling processes.

However, dihydropyridine calcium channel antagonists influence Ca^{2+} current gating as well as intracellular Ca^{2+} release during excitation–contraction coupling, albeit under slightly different conditions (Almers and Palade, 1981; McCleskey, 1985). It has been suggested on a number of occasions that the voltage sensor for excitation–contraction coupling may also be the Ca^{2+} channel (Pizarro, Fitts, Uribe, and Rios, 1989; for reviews, see Huang, 1988; Rios, Ma, and Gonzalez, 1991). The experiments here applied changes in extracellular osmolarity through a range earlier reported specifically to abolish Ca^{2+} release in intact fibers (Parker and Zhu, 1987). They explore empirically the extent to which this procedure dissociates charge movement from Ca^{2+} current activation in skeletal muscle.

METHODS

Frog (*Rana temporaria*) sartorius muscles were dissected in Ringer solution cooled to 4°C and mounted in a temperature-controlled recording chamber. Each muscle then was stretched over a 1.5-mm-high perspex ramp mounted close to 1 cm from its origin. It was then secured at origin and insertion, respectively, to the bottom of the bath by spring clips. Fibers were stretched to a center sarcomere length of 2.2–2.4 μm . This was measured using an eyepiece graticule with a $\times 40$ water immersion objective (Zeiss, Oberkochen, Germany). These precautions gave preparations that were mechanically stable even when voltage steps prolonged beyond 4 s were applied in some of the experiments. The bathing solution was then altered to an isotonic tetraethylammonium-containing solution (see below). After 5 min this was substituted for an identical solution to which 350 mM sucrose was added. In fibers where the effect of such conditions was being examined this was replaced after a further 5 min by a solution containing 500 or 600 mM sucrose. All solutions used were cooled to 4°C before these substitutions.

The pelvic end of the superficial muscle fibers directly accessible to the bathing solution was subject to a three-microelectrode voltage clamp (Adrian and Almers, 1976a, b; Adrian, 1978; Adrian and Rakowski, 1978). A few additional details of experimental method differed from the procedures adopted in earlier work (Huang and Peachey, 1989; Huang, 1990). Experiments were performed at 6–7°C. Conventional glass microelectrodes of resistance 3–6 M Ω were used. These were positioned at closer distances than used hitherto: $\ell = 250 \mu\text{m}$ (voltage control electrode, V_1), $2\ell = 500 \mu\text{m}$ (second voltage electrode, V_2), and $5\ell/2 = 750 \mu\text{m}$ (current injection electrode, I_0), respectively, from the pelvic end of the fiber. The voltage recording electrodes contained 6 M CsCl rather than 3 M KCl, and the current injection electrode contained 2 M K citrate. Fibers were held at a voltage of -80 mV between pulses.

Values of linear cable constants were deduced from the steady values of the voltages $V_1(t)$ and $V_2(t)$ and the injected current, $I_0(t)$, at the end of the 10-mV control steps. These were imposed 500 ms after the fiber was subjected to a prepulse level of -100 mV . Values of the length constant, λ , internal longitudinal resistance, r_i , and membrane resistance of unit fiber length, r_m , were followed both in test steps and in successive control steps that were interposed between sets of test steps. This gave an indication of fiber condition and stability. The membrane

current through unit length of fiber surface, $i_m(t)$ was computed as:

$$i_m(t) = [V_1(t) - V_2(t)]2/(3\ell^2r_i)$$

where t is time.

The leak admittances were deduced from the baseline currents to which the charging currents had decayed. A pedestal was obtained from the best-fit straight line to this current and then subtracted from the record. It was only very rarely necessary to use a sloping baseline for this subtraction (see Huang and Peachey, 1989). The capacitive charge, Q , moved by the applied voltage step, $\Delta V_1(t)$, was determined by integration of the on and off transients of the resulting record using Simpson's rule (Adrian and Almers, 1974):

$$Q = \int [i_m(t) - (1/r_m)\Delta V_1(t)] dt$$

On and off integrals were compared to check for charge conservation. Their mean values and computed values of the linear cable constants were then used to calculate the effective linear electrical capacity. This was referred to unit fiber length (in $\mu\text{F}/\text{cm}$). Control records were compared with currents obtained in the test pulse procedures described individually in Results. The control transients were first scaled by the relative magnitudes of the applied test and control voltage steps. The resulting arrays were then subtracted from the test transients to derive the nonlinear charge movement.

The above numerical procedures were performed on records of $V_1(t)$, $V_1(t)-V_2(t)$, and $I_0(t)$ during pulse procedures designed to observe the charge movement. These were obtained at a 12-bit analog-to-digital conversion interval of 200 μs . The originating signals were filtered through three-pole Butterworth filters set to a cut-off frequency of 1 kHz. They were sampled using a PDP 11/23 computer (Digital Equipment Corp., Maynard, MA) with a model 502 interface (Cambridge Electronic Design, Cambridge, UK). Five sweeps were averaged in each test or control record when measuring steady-state charge. Sets of five determinations of test charge were bracketed by the control protocol. This precaution additionally enabled fiber stability and condition to be monitored.

Calcium current activation was studied in the same fibers in which charge movements were examined. The pulse procedure now applied single steps of 4-s duration from the holding potential, and traces were sampled at 5-ms intervals. Traces were obtained at 2-min intervals and each record was the average of four sweeps.

The electrical recordings were made at 6–7°C in bathing solutions that consisted of 80 mM tetraethylammonium sulfate, 15 mM tetraethylammonium chloride, 2.5 mM Rb_2SO_4 , 8 mM CaSO_4 , and 3 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffered to pH 7.0 (Huang, 1990). To this, 350, 500, or 600 mM sucrose was added as a means of varying solution tonicity and experiments conducted in the absence or presence of 2 mM tetracaine. All experiments were performed within 2 h of introducing either test or control solutions.

RESULTS

Stable Preparations Could Be Obtained at High Tonicities

The combination of experimental conditions chosen here made it possible to assess the effect of tonicity on charge movements on the one hand and on the activation of slow calcium current on the other, and both of these in the same fibers. This entailed applications of a pulse procedure which involved the use of small 10-mV test steps superimposed upon prepulse potential excursions of varying magnitudes to intact fibers. Such an approach offered an investigation of membrane capacitance attributable to nonlinear charge, and has been adopted in earlier investigations of steeply

voltage-dependent phenomena, in particular of the q_v system, which nevertheless might account for relatively small absolute quantities of total charge (Adrian and Peres, 1979; Huang, 1981a, 1982, 1990).

In other respects, the conditions differed from those used hitherto. Holding levels were less polarized, and temperatures slightly higher (6–7°C), to expedite demonstration of Ca^{2+} currents in the same preparation. Further increases in temperature (to 10–12°C) resulted in marked fiber deterioration in hypertonic solutions. Use of shorter electrode spacings reduced the applied current required to establish prolonged depolarizations that extended over 4 s. Finally, use of CsCl rather than KCl electrodes reduced the proportion of fibers that showed marked outward delayed rectifier currents.

Under the above circumstances, preparations remained stable though the duration of each experiment at all the tonicities and pharmacological conditions examined. This was monitored through a comparison of membrane capacitances and resistances of unit fiber length through the successive bracketing control voltage steps obtained in the course of the pulse procedures. Ratios of these values at the end of each experiment to those determined at the outset are indicated in the figure legends. Such an approach has been used for similar purposes on earlier occasions (Huang, 1990, 1991).

Similar Charge Movements in 350 and 500 mM Sucrose

Both the time courses and the steady-state properties of intramembrane charge in the presence of 350 mM sucrose remained in agreement with earlier reports (Adrian and Peres, 1979; Huang, 1982). Fig. 1 also plots the voltage dependence of the membrane capacitance with an increase in extracellular sucrose concentration from 350 to 500 mM. Values are normalized to control magnitudes derived from application of a 10-mV voltage step at a potential of –90 mV. At all voltages examined, capacitances determined at the two sucrose concentrations were in agreement. Thus, under both conditions there was a gradual increase in membrane capacitance from 1.0 to ~1.2 as the test potential was altered from –90 to –50 mV. However, stronger depolarizations resulted in a sharp increase in the magnitude of membrane capacitance, and this reached a maximum value over 1.6 at a transition potential close to –40 mV in both sets of fibers. Capacitance then declined with further depolarization, reflecting charge saturation. Thus capacitance voltage functions under both sucrose concentrations assumed similar forms.

The kinetic features of the intramembrane charge, in particular the presence of distinguishable early (q_b) and delayed (q_v) currents at some voltages, were retained in the face of the presence of 500 mM sucrose in the extracellular solution. The prolonged q_v currents appeared at similar voltages and assumed similar features, including a steep dependence of time course upon voltage, as observed at the lower tonicities (Fig. 2). Thus small depolarizations to levels positive to –50 mV elicited only simple monotonic, q_b , decays. The q_v currents appeared at around –40 mV, and at this level their time courses extended over 50–100 ms. However, with even slight further depolarization, the current decays became more rapid. Thus with even 10 mV further depolarization the initial and delayed kinetic components merged and became indistinguishable.

Higher Tonicities Reduce Nonlinear Charge

In contrast to the preceding description, still further increases in sucrose concentration to 600 mM produced marked but reversible changes in properties of the intramembrane charge. The changes were consistent with a selective action of tonicity on the q_v charge while sparing transitions in the q_b system. Thus, there remained the increase in membrane capacitance with small depolarizations between -90 and -50 mV. Such values were similar to those observed at the lower tonicities (Fig. 3, circles). However, the higher tonicity abolished the sharp increase in membrane capacitance with larger voltage steps. The result was a capacitance voltage curve of a more gradual form and not merely a quantitative scaling-down of this dependence upon membrane potential. The function did not exceed values of 1.2–1.3 at a transition potential around -30 mV.

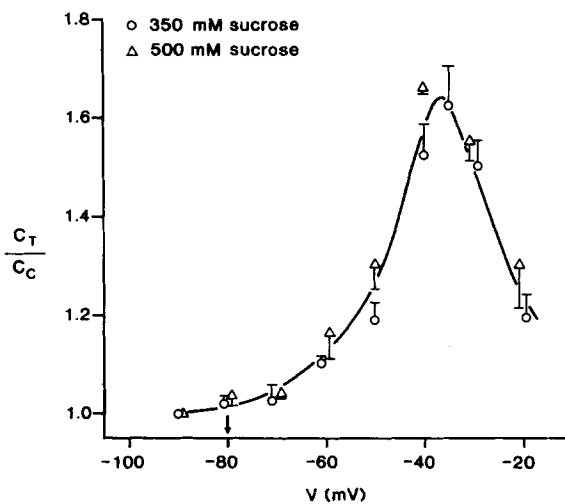


FIGURE 1. Dependence of membrane capacitance on test voltage, V , in intact fibers in the presence of 350 mM (circles: fibers S43, S46, S48, and S49; initial cable constants: length constant = 1.65 ± 0.3 mm, $r_m = 299 \pm 71.8$ k Ω cm, $c_m = 0.166 \pm 0.029$ μ F/cm) and 500 mM (triangles: fibers S27, S28, S29, and S30; initial length constant = 1.86 ± 0.15 mm, $r_m = 345 \pm 74.1$ k Ω cm, $c_m = 0.153 \pm 0.0164$ μ F/cm) sucrose in the bathing media. Capacitance C_T normalized to control value C_C obtained at -90 mV.

Control capacitances and resistance of unit fiber length at the end of each run were 1.089 ± 0.034 and 0.947 ± 0.061 (mean \pm SEM; 4 fibers) when normalized to the values at the beginning of each run in 350 mM sucrose. The corresponding ratios were 1.011 ± 0.038 and 1.060 ± 0.035 ($n = 4$ fibers), respectively, in 500 mM sucrose. The arrow designates the holding voltage. Line drawn through points by eye.

Second, the capacitance–voltage function now resembled the results expected when q_b charge was separated through the use of 2 mM tetracaine on earlier occasions (Huang, 1981a, 1982). Fig. 3 (squares) plots the variation of membrane capacitance in fibers in the lower concentration of 500 mM sucrose in the presence of 2 mM tetracaine. The lower tonicity would have permitted the marked variation in membrane capacitance with testing potential illustrated in Fig. 1 in the absence of tetracaine. However, addition of tetracaine reduced the voltage dependence of the membrane capacitance and the resulting function closely resembled both earlier findings using tetracaine as a means of charge separation (Huang, 1982, 1990; Huang and Peachey, 1989), and the findings here in 600 mM sucrose.

Third, the introduction of 600 mM sucrose abolished delayed q_v currents (Fig. 4). Charge movements were reduced in size and consisted solely of early monotonic q_B decays in response to 10-mV test steps. These effects of tonicity were reversed within 10 min of returning the sucrose concentration from 600 to 350 mM. Thus, in three fibers maximum capacitance returned to 1.66 ± 0.035 even after the muscle had been exposed to 600 mM sucrose for 2 h.

The Persistent Charge Is Tetracaine Resistant

The final test on the charge movements that persisted in the presence of 600 sucrose investigated the effect of superimposed treatment with 2 mM tetracaine. The findings

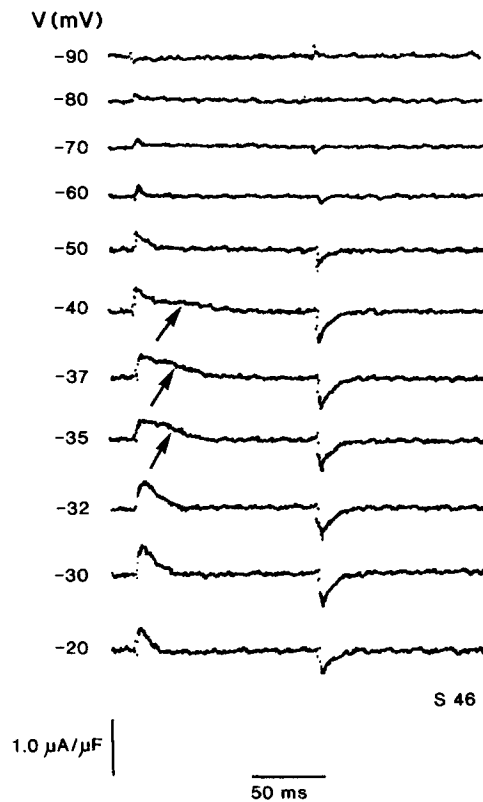


FIGURE 2. Charge movement components in response to 10-mV test steps to a range of test voltages superimposed 500 ms after a conditioning pulse from a -80 -mV holding potential. Hypertonic bathing solution containing 500 mM sucrose. Delayed (q_v) components between test levels of -40 and -30 mV (arrows).

were consistent with the hypothesis that such a charge was entirely tetracaine resistant. This in turn suggested that tonicity and tetracaine affected selectively the same species of intramembrane charge. Thus, inclusion of 2 mM tetracaine did not further alter the voltage dependence of the membrane capacitance in 600 mM sucrose. The function retained its broad form with a maximal value of 1.2 at testing levels around -30 mV (Fig. 3, *triangles*). Furthermore, Fig. 3 indicates that experiments which influenced the charge movement through increases in tonicity, the addition of tetracaine, or a combination of both gave identical steady-state results (cf.

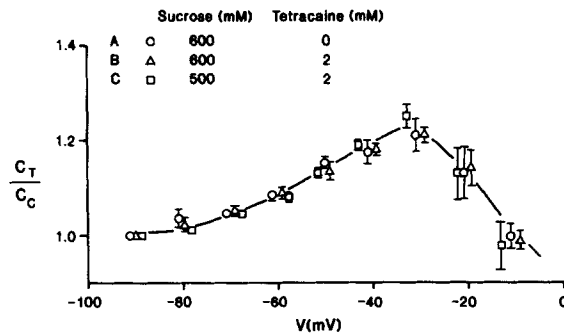


FIGURE 3. Dependence of membrane capacitance, C_T/C_C , on test voltage, V , in solutions containing 600 mM sucrose in which tetracaine was absent (circles: fibers S33, S34, S36, and S55; initial length constant = 2.4 ± 0.15 mm, $r_m = 581 \pm 108.2$ k Ω cm, $c_m = 0.188 \pm 0.024$ μ F/cm) or present (triangles: fibers S37, S45, S52, S53, and S56; initial length constant

2.17 ± 0.27 mm, $r_m = 313 \pm 59.8$ k Ω cm, $c_m = 0.178 \pm 0.077$ μ F/cm) and in solutions containing 500 mM sucrose in the presence of 2 mM tetracaine (squares: fibers S67, S68, S69, S70, and S71; initial length constant 1.96 ± 0.30 mm, $r_m = 491 \pm 58.28$ k Ω cm, $c_m = 0.195 \pm 0.017$ μ F/cm). Control capacitances and resistances of unit fiber length at the end of each run normalized to respective values at the outset (mean \pm SEM) were (circles) 1.040 ± 0.011 and 0.969 ± 0.035 ($n = 4$), (triangles) 1.062 ± 0.036 and 1.003 ± 0.213 ($n = 5$), and (squares) 0.975 ± 0.018 and 0.965 ± 0.034 ($n = 5$). Line drawn through points by eye.

Fig. 3, circles, triangles, and squares). Numerical integration of the capacitance–voltage functions to give total charge in the -90 - to -20 -mV voltage range further suggested that all these procedures produced a similar reduction in available charge. The charge assumed a value of 23.8 ± 0.98 nC/ μ F ($n = 4$ fibers) in 500 mM sucrose, and this was reduced to levels of 8.8 ± 0.29 nC/ μ F ($n = 4$) in 600 mM sucrose, 9.16 ± 0.33 nC/ μ F ($n = 5$) in 600 mM sucrose and 2 mM tetracaine, and 9.06 ± 0.78 nC/ μ F ($n = 5$) in 500 mM sucrose and 2 mM tetracaine (means \pm SEM), respectively. These

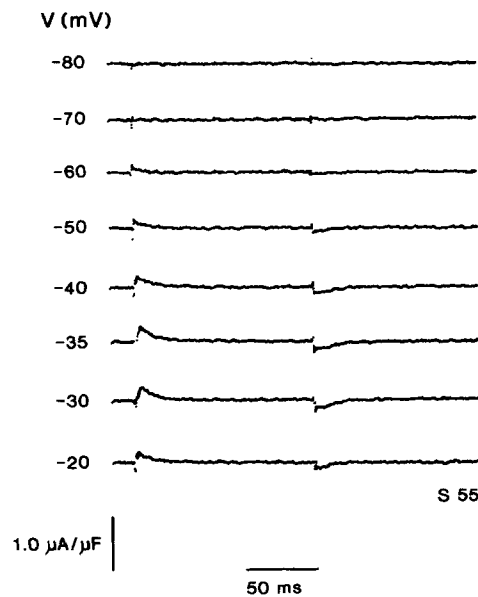


FIGURE 4. Nonlinear charge movements in response to 10-mV steps to a range of test voltages (V) superimposed 500 ms after conditioning pulses from a -80 -mV holding level, in a fiber in a solution containing 600 mM sucrose. Delayed (q_d) currents, were not observed.

values are similar to the results of earlier integrations of capacitances from identical pulse procedures between the similar limits (Huang, 1982).

Finally, charge movements obtained in the presence of 600 mM sucrose and 2 mM tetracaine were similar in form to those obtained under high tonicity alone. They consisted of monotonic q_b decays in which delayed q_v charge transfers were absent (Fig. 5). These findings accordingly suggest a selective effect of strong tonicities on q_v charge. This is in common with the influence of other agents that affect excitation-contraction coupling, such as perchlorate, tetracaine, nifedipine, and dantrolene Na (Huang, 1981a, 1986, 1987, 1990; Hui, 1983; Vergara and Caputo, 1983).

Activation of Slow Ca^{2+} Currents Persists at High Tonicities

Stanfield (1977) reported that slow Ca^{2+} current activation persisted despite progressive increases in extracellular tonicity in intact amphibian muscle fibers studied in a

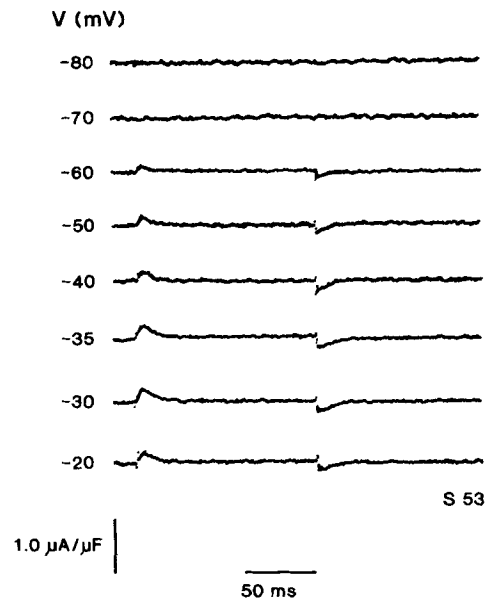


FIGURE 5. Nonlinear charge movements in response to 10-mV steps to a range of test voltages (V) superimposed 500 ms after conditioning pulses from a -80 -mV holding level. Fiber in 600 mM sucrose in the presence of 2 mM tetracaine.

three-electrode voltage clamp at room temperature. The present experiments observed Ca^{2+} currents under the same conditions of tonicity and a lower temperature as those under which charge movements were studied. The relevant pulse procedures were imposed on the same fibers preceding the charge movement studies in half the fibers, and following them in the remainder. This necessitated modifications in pulse procedure to allow for their prolonged kinetics at these temperatures. First, simple steps were applied from the holding potential of -80 mV rather than the small steps used above. Second, transients were sampled at 5-ms rather than 200- μ s intervals, which enabled use of an extended pulse duration of 4 s. Each record was an average of four experimental traces. The latter were obtained at 2-min

intervals. The results of obtaining individual sweeps were followed to ensure consistency before signal averaging.

As reported in earlier papers in fibers at room temperature (Sanchez and Stefani, 1978, 1983), contraction was not completely abolished even in the presence of 350 mM sucrose. The sustained pulses used here gave rise to contraction artefacts in the experimental traces. Nevertheless, recordings could be obtained at some voltages, free of such artefacts, and comparisons accordingly made, at the higher external sucrose concentrations of 500 and 600 mM. In any case, it was the shift between these sucrose concentrations that led to the marked alterations in intramembrane charge movement described above.

Fig. 6 illustrates records from a fiber in which electrode impalement was preserved through a solution change in which the external sucrose concentration altered from 500 mM (A) to 600 mM (B). They illustrate a number of points. Ca^{2+} currents first appeared at depolarizations close to -20 to -30 mV, in agreement with the earlier

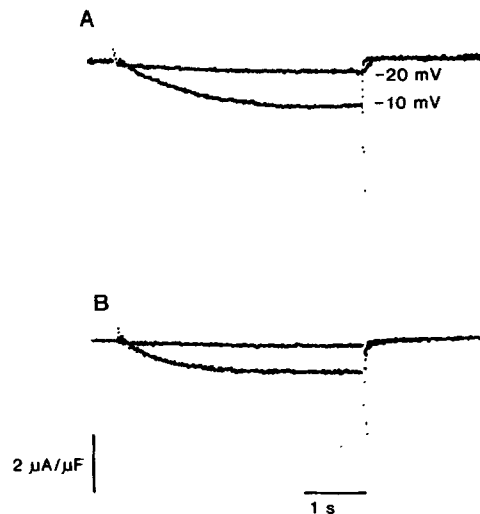


FIGURE 6. Ca^{2+} current activation in a fiber before (A) and after (B) a change of sucrose concentration in the bathing solution from 500 to 600 mM. Temperature 6°C .

findings at higher temperatures. However, they developed over a considerably longer time course: around 3–4 s at a test potential of -10 mV (cf. around 200 ms at -30 mV at 22 – 26°C ; Sanchez and Stefani, 1978). Additionally, they were considerably smaller in magnitude. Nevertheless, they were present at both sucrose concentrations (500 and 600 mM). Thus at the end of a 4-s step they accounted for 1.5 ± 0.19 $\mu\text{A}/\mu\text{F}$ of membrane current in three fibers in 500 mM sucrose, and 1.32 ± 0.18 $\mu\text{A}/\mu\text{F}$ in three fibers in 600 mM sucrose, at a test voltage of -10 mV. They were absent in the fibers in 2 mM tetracaine, or in the presence of 10 mM CoSO_4 .

These currents were considerably smaller than reported in studies made at higher temperatures (cf. around 12 – 15 $\mu\text{A}/\mu\text{F}$ at a test potential of -30 mV assuming a membrane capacitance of 5.9 ± 0.5 $\mu\text{F}/\text{cm}^2$; Sanchez and Stefani, 1978). Nevertheless, the findings indicated an increase in sucrose concentration from 500 to 600 mM was compatible with continued Ca^{2+} current activation insofar as it was possible to examine Ca^{2+} currents under such conditions. It was not possible to examine larger

depolarizations owing to residual contractile activation that persisted even at the higher tonicities, with the prolonged voltage steps that were required.

DISCUSSION

The experiments here explored the effects of progressive increases of extracellular tonicity on intramembrane charge in intact, voltage-clamped amphibian skeletal muscle, and compared these with effects on Ca^{2+} current activation. They were prompted by two sets of earlier observations. First, fibers in strongly hypertonic solutions lacked the delayed charging phases, subsequently attributed to a particular (q_v) species of nonlinear charge (see below), reported in solutions of more moderate tonicity (Schneider and Chandler, 1973; Chandler, Rakowski, and Schneider, 1976*a*, *b*; Adrian and Peres, 1979). Second, excitation–contraction coupling processes have been monitored through aequorin (Taylor, Rudel, and Blinks, 1975) or arsenazo III signals (Parker and Zhu, 1987) in response to large voltage clamp steps applied to intact amphibian fibers in solutions of different tonicities. These were made at conditions of temperature (9–11°C) and holding potential (–70 mV) similar to those used here. The optical signals did not alter with increases in sucrose concentration up to 310 mM but were abolished above 560 mM sucrose (Parker and Zhu, 1987). The present experiments utilize such effects of tonicity to demonstrate a strong parallel between q_v charge and excitation coupling. Such a parallel might exist in the form of schemes in which the charge is either a cause (Huang, 1981*b*, 1983) or a consequence (Pizarro, Csernoch, Uribe, Rodriguez, and Rios, 1991) of Ca^{2+} release.

In addition, the selective effects arising from such tonicity changes achieved a charge fractionation that was in quantitative agreement with earlier independent separations based on the analysis both of steady-state charge–voltage curves and the maximal effects of tetracaine (Huang, 1981*a*; Hui and Chandler, 1990). Such earlier investigations first detected q_v charge through its prolonged kinetics close to the contractile rheobase of intact fibers (Adrian and Peres, 1979; Adrian and Huang, 1984; Huang, 1981*b*). Characterizations using its tetracaine sensitivity (Huang, 1981*a*; Hui, 1983), or steady-state descriptions through two fitted Boltzmann systems (Hui and Chandler, 1990) both suggested a steep voltage sensitivity that closely resembled the potential dependence of Ca^{2+} release around contractile threshold (Maylie et al., 1987) and distinct from that of the remaining (q_b) charge (Duane and Huang, 1982; Huang, 1982).

Delayed charging phases were not observed in some cut amphibian and mammalian fiber preparations. However, this need not exclude multiple charge contributions (see discussion by Melzer, Schneider, Simon, and Szues, 1986; Hollingworth and Marshall, 1981). Thus, minor variations in conditions exerted substantial effects on q_v kinetics that differed between cut and intact preparations. Delayed charging phases lost their distinct “hump” appearance with temperature reductions below 6°C in cut but not intact fibers. Yet steady-state charge distributions still fulfilled expectations for two distinguishable charge species (Hui, 1991). Additionally, cut fiber preparations where current flow beneath the Vaseline seals was monitored and corrected for yielded kinetic and steady-state results that strongly suggested distinct charge species (Hui and Chandler, 1990). Steady-state components were also separable through their tetracaine sensitivity in mammalian fibers (Hollingworth, Marshall, and Robson,

1990). Accordingly, q_v has been defined here in terms of its tetracaine sensitivity as first introduced in earlier work (Huang, 1981a).

The first major finding from the present study is that sucrose concentration changes from 500 to 600 mM, but not from 350 to 500 mM, selectively abolished the q_v component and spared the q_b component as defined by the above criteria. It inhibited the prolonged hump currents and so left exponential q_b decays over the studied voltage range. Furthermore, combinations of high tonicities alone, the addition of 2 mM tetracaine, or both, all reduced the steady-state voltage dependence of membrane capacitance. They resulted in identical functions whose more gradual form was as expected for the q_b charge.

The second major finding is that Ca^{2+} currents persisted in the absence of q_v charge movement in the same fibers. The Ca^{2+} currents were observed under the same conditions of solution tonicity, temperature, and electrode spacing as were the charge movements. At the lower temperatures examined here, the currents were smaller in size and exhibited substantially slower kinetics than those obtained at higher temperatures (Stanfield, 1977; Sanchez and Stefani, 1983). Nevertheless, they remained even when q_v charge movement was abolished by high tonicity.

The results taken together have a number of implications. First, they add tonicity change to the list of agents that simultaneously affect both excitation–contraction coupling and q_v charge movement selectively. These include administrations of tetracaine, dantrolene Na, Ca^{2+} deprivation, and nifedipine (Putney and Bianchi, 1974; Huang, 1981a, 1990, 1991; Hui, 1983; Brum, Fitts, Pizarro, and Rios, 1988; Rios and Brum, 1987). The physiological effects of changes in extracellular tonicity have been attributed to changes in intracellular ionic strength arising from the consequent fiber volume change (Howarth, 1958; Dydynska and Wilkie, 1963; Blinks, 1965; Caputo, 1968; Gordon and Godt, 1970). The mechanism for their influence on charge may therefore differ from those of the pharmacological agents. Nevertheless, the empirical result emerges that osmolarity changes reported to abolish excitation–contraction coupling selectively inhibited q_v charge. In contrast, smaller osmolarity changes did not affect nonlinear charge, in agreement also with earlier reports of intact q_v and q_b charge movements in 350 mM (Adrian and Peres, 1979; Huang, 1981a, 1982; Hui, 1983) and 500 mM sucrose, respectively (Huang and Peachey, 1989).

Second, the experiments demonstrate that Ca^{2+} current activation does not require q_v charge transfer. This complements earlier findings where perchlorate (8–10 mM) facilitated both q_v charge movement and excitation–contraction coupling (Gomolla, Gottschalk, and Luttgau, 1983; Luttgau, Gottschalk, Kovacs, and Fuxreiter, 1983; Huang, 1986, 1987), yet did not modify calcium current activation (Feldmeyer and Luttgau, 1988). Taken together, these findings make it unlikely that the q_v charge movement reflects a necessary step in calcium current activation, whether by itself or as part of some process that also involves excitation–contraction coupling. Any involvement of intramembrane charge in gating the Ca^{2+} channel (Pizarro et al., 1989; Rios et al., 1991) can then only implicate q_b and not q_v charge. Finally, they suggest that the influence of the calcium channel blocker nifedipine on q_v charge (Huang, 1990) reflects effects on excitation–contraction coupling rather than calcium channel activation.

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