Voltage-dependent Block of Charge Movement Components by Nifedipine in Frog Skeletal Muscle

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ABSTRACT Potential-dependent inhibition of charge movement components by nifedipine was studied in intact, voltage-clamped, frog skeletal muscle fibers. Available charge was reduced by small shifts in holding potential (from −100 mV to −70 mV) in 2 μM nifedipine, without changes in the capacitance deduced from control (−120 mV to −100 mV) voltage steps made at a fully polarized (−100 mV) holding potential. These voltage-dependent effects did not occur in lower (0–0.5 μM) nifedipine concentrations. The voltage dependence of membrane capacitance at higher (10 μM) nifedipine concentrations was reduced even in fully polarized fibers, but shifting the holding voltage produced no further block. Voltage-dependent inhibition by nifedipine was associated with a fall in available charge, and a reduction in the charge and capacitance–voltage relationships and of late (qₐ) charging transients. It thus separated a membrane capacitance with a distinct and steep steady-state voltage dependence. Tetracaine (2 mM) reduced voltage-dependent membrane capacitance and nonlinear charge more than did nifedipine. However, nifedipine did not exert voltage-dependent effects on charging currents, membrane capacitance, or inactivation of tetracaine-resistant (qₐ) charge. This excludes participation of qₐ or the membrane charge as a whole, from the voltage-dependent effects of nifedipine. Rather, the findings suggest that the charge susceptible to potential-dependent block by nifedipine falls within the tetracaine-sensitive (qₐ) category of intramembrane charge.

INTRODUCTION

There has been recent interest in receptors for 1,4-dihydropyridine calcium channel antagonists in skeletal muscle membrane in connection with possible roles as voltage sensors regulating excitation–contraction coupling. The latter process may depend on an interaction between the transverse tubular membrane, which produces the primary triggering electrical change, and junctional sarcoplasmic reticulum, where Ca²⁺ is stored and released. It is likely that such coupling occurs at “triad” or other specialized junctional regions where the two membrane systems are closely opposed (Franzini-Armstrong, 1970; Franzini-Armstrong and Nunzi, 1983; Ferguson et al., 1984). Thus, through its specific ryanodine binding, a Ca²⁺ release channel was
localized to the junctional feet linking the transverse tubules to terminal cisternal regions of sarcoplasmic reticulum. (Fleischer et al., 1985; Campbell et al., 1987; Imagawa et al., 1987; Inui et al., 1987; Lai et al., 1987; Lai et al., 1988). Conversely, dihydropyridine receptors are abundant in transverse tubular membrane (Fosset et al., 1983), and have been localized to the triad structures (Leung et al., 1987, 1988), from which they have been isolated as proteins (Curtis and Caterall, 1984; Borsotto et al., 1985; Flockerzi et al., 1986). Ca$^{2+}$ antagonists modified contractile activation in a manner parallel to changes produced by Ca$^{2+}$ deprivation (Luttgau and Spiecker, 1979; Luttgau et al., 1986, 1987). Taken with ultrastructural evidence suggesting direct interactions between transverse tubular and junctional sarcoplasmic reticular elements (Kawamoto et al., 1986; Block et al., 1988), these findings make a mechanical coupling hypothesis at the triadic junction (Chandler et al., 1976), involving dihydropyridine receptors as voltage sensors, an attractive possibility (see also Schwartz et al., 1985).

It has been suggested that intramembrane charge movements in skeletal muscle could reflect the voltage sensing step in contractile activation (Schneider and Chandler, 1973). Subsequently, studies in intact muscle fibers resolved this nonlinear charge into distinct components (Adrian and Peres, 1979). Of these, the tetracaine-sensitive ($q_\text{t}$) species had a voltage dependence, and kinetic and pharmacological properties consistent with a regulatory role in contractile activation (Huang, 1981, 1982, 1983; Vergara and Caputo, 1982; Hui, 1983; Adrian and Huang, 1984a). Additionally, recent reports suggested that $q_\text{t}$ charge movements occur independently of transitions in the $q_\text{a}$ charge (Adrian and Huang, 1984b; Huang, 1987) and can be exclusively localized to transverse tubule membranes (Huang and Peachey, 1989). The latter finding strongly suggests that the $q_\text{t}$ charge is causally related to initiation of contractile activation, although it need not exclude that fraction of $q_\text{a}$ existing in the tubules from this process.

Recently it has been reported that dihydropyridines also influence nonlinear charge (Lamb, 1986; Rios and Brum, 1987). These agents modify both contractile activation and calcium channel gating (Almers and Palade, 1981), but under different conditions (cf. McCleskey, 1985). Charge movements influenced by such agents might therefore be implicated in either or both of these processes. However, of particular interest was that fraction of the intramembrane charge inhibited by shifting the holding voltage from $-100$ mV to $-70$ mV in the presence of nifedipine in cut fibers. This inhibition was accompanied by a reduction, even if not abolition of the $\Delta[Ca^{2+}]$ signal (Rios and Brum, 1987). At least this portion of the nonlinear charge, inhibited when the double conditions of shifted holding voltage and the presence of nifedipine was satisfied, could accordingly be related to contractile activation. The earlier report did not discriminate whether the charge concerned corresponded to particular $q_\beta$ or $q_\gamma$ components or was drawn from the nonlinear charge as a whole. The experiments here accordingly first separate the voltage-dependent effects of nifedipine in intact fibers, and then examine for correspondence between such nifedipine and voltage-sensitive charge, and the earlier resolution of charge movement components (Adrian and Peres, 1979; Huang, 1981; Hui, 1983). Some of the experiments accordingly used a pulse procedure using small voltage steps (Adrian and Peres, 1979) to yield a description of membrane capaci-
tance rather than total charge, as a function of voltage. This approach emphasized steeply voltage-dependent effects and so was used, in combination with a voltage clamp of intact fibers, to separate and emphasize the features of $q_r$, charge on earlier occasions (Huang, 1982).

METHODS

Frog (*Rana temporaria*) sartorius muscles were dissected in Ringer solution at 4°C and mounted in a temperature-controlled recording chamber. The bathing solution was then altered to a hypertonic, tetraethylammonium-containing solution (see below). The muscle was stretched such that the fibers had a center sarcomere length of 2.2–2.4 μm measured with an eyepiece graticule with a ×40 water immersion objective (Zeiss, Oberkochen, FRO), and studied at 3.5–4.2°C. The pelvic end of the muscle fibers was subject to a three-microelectrode voltage clamp procedure described more fully elsewhere (Adrian and Almers, 1976a, b; Adrian, 1978; Adrian and Rakowski, 1978). Only superficial fibers on the inner surface of the muscle directly accessible to the bathing solution were studied. Conventional glass microelectrodes, of 4–10 MOhm resistance, were positioned at standard distances of $k = 375$ μm (voltage control electrode $V_1$), $2k = 750$ μm (second voltage electrode, $V_2$) and $5k/2 = 875$ μm (current injection electrode $I_0$), respectively, from the pelvic end of the fiber, unless otherwise stated. The bathing solution was then again replaced by one of the test solutions containing nifedipine, before electrode insertion. The voltage electrodes contained 3 M KCl, and the current electrode contained 2 M K citrate.

Membrane potential was first held at −100 mV, and linear membrane and cable constants determined using 20-mV depolarizing control steps of 124 ms duration, imposed 500 ms after a prepulse from the −100-mV holding potential to a −120-mV conditioning potential. In the course of each experiment, when obtaining repeat control records, holding potential was returned to −100 mV and the same control pulse procedure adopted whatever the test protocol. This ensured constant control conditions and avoided discrepancies that could arise in the amounts of control charge owing to possible charge interconversion (Brum and Rios, 1987). In addition, a set of control experiments were performed expressly to investigate for variations in the control capacitance under the different conditions. First, the capacitance as determined by a +20-mV voltage step to −100 mV was investigated in 2 μM nifedipine before and during charge inactivation by shifts in holding voltage, as well as after restoration of the holding voltage to −100 mV. Second, successive values of control capacitance that were used to bracket records of test transients were followed to examine for any systematic change in these values. These findings are considered in the Results.

The length constant, $\lambda$, internal longitudinal resistance, $r_p$, and membrane resistance of unit fiber length $\rho_m$, were calculated from steady values of $V_1(0)$, $V_2(0)$ and injected current $I_0(0)$ at the end of the 20-mV step. Fiber diameter and specific membrane resistance $R_m$ were determined using a value of the internal sarcoplasmic resistivity $R_i$ of 391 Ohm cm in 2.5× hypertonic solution at 2°C, assuming a $Q_{10}$ of 0.73 (Hodgkin and Nakajima, 1972). Fibers studied were selected to have diameters between 60 and 110 μm. Fiber cable constants accordingly were comparable between experimental groups and are summarized in the figure legends. The membrane current through unit area of fiber surface, $I_m(t)$ was computed as:

$$I_m(t) = \frac{[V_1(t) - V_2(t)]d}{6(\lambda^2 R_i)},$$

where $t$ is time. The capacitative charge moved by the applied voltage step $\Delta V(t)$ was computed by integrating the current transient at the beginning and after the end of each
voltage step using Simpson's rule:

$$ \int \left[ I_m(t) - \left(1/R_m\right) \Delta V(t) \right] dt $$

(Adrian and Almers, 1974; Adrian, 1978). In control steps, these integrals and calculated values of the linear cable constants were used to calculate the effective linear electrical capacity, referred to unit apparent lateral fiber surface area $C_c$ (μF/cm²), from both "on" and "off" transients. The control transients, scaled for the relative sizes of test and control voltage steps, were subtracted from test transients to obtain the nonlinear charge movement. Any significant baseline remaining after this subtraction resulting from extra ionic current in the test pulse, was corrected by fitting and subtracting a straight line to this baseline.

The above computations were obtained from values representing $V_1(t)$, $V_2(t) - V_2(0)$, and $I_0(t)$ obtained by 12-bit analogue-to-digital conversion at a sampling interval of 200 μs after filtering through 3-pole Butterworth filters set to a cut-off frequency of 1 kHz, and sampled using a PDP 11/23 computer (Digital Equipment Corporation, Maynard, MA) with a model 502 interface (Cambridge Electronic Design, Cambridge, UK). Five sweeps were averaged in each record, whether test or control, in the course of any given pulse procedure when obtaining steady-state quantities of capacitative charge. When obtaining records for display of transients, 8–10 sweeps were averaged into each record. Sets of five determinations of test records were bracketed by determinations of control records. This additionally monitored fiber stability and condition. Successive bracketing control records were used as controls for the intervening test pulses when deducing nonlinear charge movements. In addition, the first test record in the following bracket was obtained using the same pulse procedure as the last test pulse in the bracket before. This made it possible to monitor the stability of nonlinear as well as linear membrane properties in the course of each experiment.

Electrical recordings were made at 3–4°C in tetraethylammonium-containing solutions similar to one used in earlier work (e.g., Adrian and Peres, 1979; Huang, 1982), consisting of 80 mM tetraethylammonium sulphate, 15 mM tetraethylammonium chloride, 2.5 mM Rb₂SO₄, 8 mM CaSO₄, 350 mM sucrose, and 3 mM HEPES buffered to pH 7. Nifedipine stock solutions were freshly made in absolute ethanol for each experiment to a concentration adjusted for addition to test solution to result in a constant dilution of ethanol, whatever the intended nifedipine concentration (including bathing solutions not containing nifedipine), and protected from light. The test solution was introduced after electrode positioning over any given fiber but immediately before impalement.

**RESULTS**

*Control Membrane Capacitances*

When deriving charge movements, test transients were compared with those obtained under control pulses, which used voltage steps between the fixed membrane potentials of −120 and −100 mV. Where the holding voltage was varied in preceding test procedures, it was then returned to a level of −100 mV before imposing the control pulses. This was to allow any charge interconversion that may have occurred to reverse (Brum and Rios, 1987), in order to ensure a consistent reference record with which to compare test transients to obtain the relative charge movements. This latter point was empirically confirmed by a set of experiments that additionally investigated for variations in control capacitance at the altered holding voltages where charge inactivation had actually taken place.
First, capacitances were deduced from transients in response to control voltage steps at a holding voltage of \(-100\) mV, in 2 \(\mu\)M nifedipine, the concentration at which voltage-dependent inactivation was demonstrated as described below. This result was compared with similar determinations of control capacitance using pulses involving the same voltage excursion, when charge was inactivated by altering the holding voltage from \(-100\) mV to \(-70\) mV, and then to \(-50\) mV, respectively, as well as after returning the holding level to \(-100\) mV. Charging records were obtained at both 60 and 90 s after each change in holding potential, to confirm that stable readings were obtained. 

**Figure 1.** Investigation of membrane capacitance deduced by voltage steps from a prepulse voltage of \(-120\) mV to \(-100\) mV, in fibers held at \(-100\) mV, showing the total capacity transient (A). This was used in subtractions to give difference traces 90 s after the holding voltage was shifted to \(-70\) mV (B), \(-50\) mV (C), and finally back to \(-100\) mV in the same fiber (D). The difference traces are shown at a high gain similar to that used in the remaining figures. E, Steady-state capacitances (mean \(\pm\) SEM) at the different holding voltages, normalized to the initial result in the same fiber as obtained in A. Remaining cable constants: temperature = 4.9\(^\circ\)C, \(R_i = 325.32\, \Omega\) cm, \(\lambda = 2.2 \pm 0.41\) mm, \(r_i = 11.905 \pm 1.899\) k\(\Omega\) cm, diameter = 61.4 \(\pm\) 4.9 \(\mu\)m, \(r_m = 547.4 \pm 130.4\) k\(\Omega\) cm, \(R_m = 11.09 \pm 3.4\) k\(\Omega\) cm\(^2\). 

Initially at a holding potential of \(-100\) mV in one such experiment (A). This was subtracted from transients obtained at holding potentials of \(-70\) mV (B), \(-50\) mV (C), and after return of the holding voltage back to \(-100\) mV, respectively (D). The subtraction records are displayed (Fig. 1, B–D) at a high magnification similar to that used in the later figures. Such traces would demonstrate alterations in the response of the membrane capacitance to a control step with changes in holding potential or with a history of such changes. However, subtractions were flat not only after the final return of the holding voltage but at the altered holding levels as well.
Fig. 1 E goes on to plot steady-state membrane capacitances obtained by integrating the transients (Adrian and Almers, 1974). These are normalized to the initial capacitances at the outset of the experiment in each of four fibers. This confirms little change in steady-state capacitance with mean (± SE of the mean) ratios close to 1.0 throughout. Thus records from a control step at −100 mV offered consistent control comparisons for both transients as well as steady-state results obtained at test potentials.

Second, as indicated in the Methods, in all experiments, including those that explored the effect of holding potential, sets of test records were bracketed at regular intervals by control records obtained with the holding potential returned to its initial (−100 mV) holding value. It was therefore possible to follow, and so to detect, any systematic changes in successive sets of control records that might be the result of any interconversion processes failing to reverse. The ratios of capacitances obtained at the end of each experiment to that at the outset of each experiment before holding voltage was altered (and whose detailed cable constants are given in the legend to Fig. 4) are given below:

(a) Fibers in 0 uM nifedipine: 1.023 ± 0.066 (n = 5 fibers).
(b) Fibers in 0.5 uM nifedipine: 0.994 ± 0.189 (n = 4).
(c) Fibers in 2 uM nifedipine: 1.007 ± 0.088 (n = 6).
(d) Fibers in 10 uM nifedipine: 0.973 ± 0.154 (n = 4).

They indicate no significant change (P >> 5% on t testing) in the values of control capacitance through an experiment in the fibers discussed here.

Thus, at any given nifedipine concentration, it is possible to provide a consistent control steady-state capacitance for comparison of results even through varying holding levels. This made it possible to investigate the voltage-dependent effects of nifedipine, to which attention here is primarily directed, even without requiring that control capacitances obtained in different pharmacological conditions are comparable.

Voltage-dependent Effects of Nifedipine in Intact Fibers

The experiments here sought to investigate potential-dependent block by nifedipine in intact muscle fibers, using the technique used earlier to demonstrate different charge movement components (Adrian and Peres, 1979; Huang, 1982). This complemented the earlier studies, which used cut fibers (Lamb, 1986; Rios and Brum, 1987) but which did not examine for effects on individual charge species. Accordingly, the experiments first simply attempted to demonstrate such potential-dependent effects of nifedipine in intact fibers (Fig. 2). They applied single test steps to a range of membrane potentials chosen in 10-mV increments from the −100-mV holding voltage in parallel with the cut fiber study.

It was possible to demonstrate charge susceptibility to voltage-dependent block in intact fibers, but this required nifedipine concentrations of 2 μM, in contrast to the considerably lower levels (0.5 μM) used in cut fibers. Effects of varying drug concentrations are discussed further below. Nonlinear charge in 2 μM nifedipine increased with depolarization at a holding potential of −100 mV, as described on earlier occasions (Schneider and Chandler, 1973). For example, in Fig. 2 A, large depolarizations to test potentials of −20 mV moved over 30 nC/μF of charge.
Charge movements (Fig. 2 B) were monotonic decays showing no clear kinetic evidence of hump components even in “on” responses. This is in agreement with earlier contrasts made between cut and intact fibers. Thus earlier work (e.g., Adrian and Peres, 1979) reports that $q_\tau$ gives delayed currents only at a defined voltage range near the mechanical threshold, and these transients merge with the rest of the decay with further depolarization, and even then were easiest demonstrated with a pulse procedure using small (see below) rather than large steps, first adopted here.

The test pulses were then repeated at a holding voltage of $-70$ mV with repeat control records still taken at a holding voltage returned to $-100$ mV (see Methods).

Charge movement records were then obtained in the same fiber. This applies not
only to Fig. 2 but all the figures to follow, which display charge movement records obtained before and after voltage-dependent inactivation in nifedipine in the same fiber. Maximum available charge was now reduced: the charge moved by a pulse from -100 mV to -20 mV fell from 25.1 ± 0.4 to 19.8 ± 1.3 nC/µF (mean ± SEM) in five fibers. Altering holding voltage not only modified the amount of charge but also altered the form of the charge-voltage curve. For example, steady-state charge changed little with small voltage steps; the major reduction occurred with larger pulses beyond approximately -50 mV. The amount of inactivated charge was deduced by comparing data points at holding voltages of -100 mV (circles) and -70 mV (triangles; Fig. 2 A), respectively. This yielded a voltage dependence (squares) considerably steeper than either of its originating functions, rising sharply at -50 mV and approaching saturation close to -30 mV. These steady-state findings were reflected in the charge movement being reduced in comparison to the transients obtained in fully polarized fibers. This occurred particularly at large depolarizations beyond approximately -50 mV, but not at smaller voltage steps. Again, charge movements were relatively simple decays: delayed (q) components were not distinct with the single test pulses applied here.

Higher (10 µM) nifedipine concentrations caused partial charge inactivation even in fully polarized fibers in agreement with findings in mammalian fibers (Lamb, 1986). Charge movements in response to large (-100 to -20 mV) steps in voltage-clamped fibers using a reduced electrode spacing (d = 250 µm) were obtained before and after the same fiber was subject to a solution change to one containing 10 µM nifedipine. Control capacitances before and after adding drug were similar (within 0.03 of the value before adding drug). Fig 3 E, which uses as control the transient obtained before drug was added, shows that 10 µM nifedipine reduced the size of the charging transients.

Nifedipine Modifies Charge Inactivation

The voltage-dependent effects of nifedipine in intact fibers appeared to reflect a modification of charge inactivation. Such a notion would predict a relative fall in available charge as holding voltage was altered, at the effective concentrations of nifedipine. The experiments here sought primarily to examine that fraction of nonlinear charge that was inhibited when the double conditions of altered holding potential and presence of nifedipine were met. It was therefore important to examine the effect of varying the holding potential at different concentrations of nifedipine.

In such experiments the test steps were made from a constant prepulse voltage of -100 mV to a fixed test potential of -20 mV; this covers the range through which the phenomena outlined in Fig. 2 reached saturation. The pulse procedure was applied at least 30 s after each shift in holding voltage (cf. Brum et al., 1988). Holding voltages were examined particularly through -100 mV and -70 mV, where the voltage-dependent inhibition was observed.

Fig. 3, A-D plots charge so obtained, normalized to available linear membrane capacitance (means ± SEM) in the same fiber against holding potential, on separate ordinates. This was primarily to illustrate relative changes in available charge, using control voltage steps to -100 mV, which provided a consistent reference charge as
described in the control experiments above, provided comparisons of absolute values were not made between pharmacological groups of fibers. The fiber sets had comparable cable constants (Fig. 3, legend), and external solutions were similar apart from the different nifedipine concentrations.

When nifedipine was absent (Fig. 3 A), or at low concentrations (0.5 μM; Fig. 3 B), a change in holding voltage from −100 mV to −70 mV did not produce a significant alteration in relative available charge; the values fell ~26 nC/μF, in agreement with earlier reports (e.g., Huang, 1982; Hui, 1983). However, maximum charge did fall significantly when the holding voltage was depolarized beyond −50 mV or −40 mV.

In contrast, with 2 μM nifedipine even small changes in holding potential noticeably inactivated available charge. For example, a shift from −100 mV to −70 mV reduced overall charge by ~5 nC/μF (Fig. 3 C). This was in contrast to Fig. 3, A and B. Further inactivation took place at larger depolarizations. However, charge inactivation also took place in the lower nifedipine concentrations at these latter holding voltages, at which it would consequently be more difficult to attribute charge inactivation solely to a combination of nifedipine and holding potential change. Finally, further increasing nifedipine concentration fivefold (to 10 μM) reduced available charge even in fully polarized fibers, but shifting the holding potential (from −100 mV to −70 mV) now did not produce further inactivation (Fig. 3 D). This would suggest that an increased nifedipine concentration might substitute for a combination of nifedipine treatment and altered holding potential. However, this would then mask attempts to investigate the properties of charge whose inactivation by nifedipine was voltage dependent.

As the experiments here were particularly concerned with holding potential shifts between −100 mV and −70 mV in nifedipine, more heavily averaged transients were obtained at these voltages. Their features corroborated the steady-state results. Fig. 3, F–H shows pairs of records from one fiber in each experimental group. Charge movements were simple decays, as expected at such test depolarizations (Rios and Brum, 1987). At 0.5 μM nifedipine the transient at a holding potential of −100 mV (Fig. 3 F[a]) was similar to that at −70 mV (Fig. 3 F[b]). However, in 2.0 μM nifedipine, changing the holding potential reduced the size of the charge movement (Fig. 3, G[a and b], respectively). In contrast, transients were smaller in size in 10 μM nifedipine even at a holding potential of −100 mV (Fig. 3 E[a and b] and H[a]), but altering the holding potential to −70 mV (Fig. 3 H[b]) now did not further alter the charging currents.

These findings might be interpreted most simply in terms of the presence or absence of relative changes in available charge under each pharmacological condition. At low (0.5 μM) or zero nifedipine, there was little or no significant charge inactivation when holding voltage was altered from −100 mV to −70 mV. This agrees with earlier findings in intact fibers (Huang, 1981; Hui, 1983), although cut fibers do show inactivation under such conditions (Rios and Brum, 1987). Therefore, the present findings suggest that the contrasting changes in 2 μM nifedipine, with a −100 mV to −70 mV shift in holding potential was entirely due to charge susceptible to a combination of nifedipine treatment and a shift in holding potential. Under such conditions the degree of charge inactivation was comparable to the effect shown in Fig. 2. Larger depolarization caused further inactivation in 2 μM
FIGURE 3.

- Panel A: Graph showing voltage (V) vs. current density (I) with DHP concentrations.
- Panel B: Graph showing voltage (V) vs. current density (I) with DHP concentrations.
- Panel C: Graph showing voltage (V) vs. current density (I) with DHP concentrations.
- Panel D: Graph showing voltage (V) vs. current density (I) with DHP concentrations.
nifedipine, but did so in 0 μM nifedipine as well. Therefore, it would not be possible to exclude an influence of charge inactivation alone. Similarly, increasing the dose of nifedipine to 10 μM reduced available charge, but there was no further charge inactivation with small shifts in holding potential.

**Nifedipine-inactivated Charge Is Steeply Voltage Sensitive**

The findings above suggested that a combination of 2 μM nifedipine and a -100 mV to -70 mV shift in holding voltage would sample a component of the nonlinear charge whose inactivation would require both nifedipine treatment and a shift in holding voltage. It may not inactivate all this charge, for reasons given in the previous section, but nevertheless it would remain possible to explore its voltage dependence. These conditions were therefore adopted when examining the voltage dependence of such charge. This employed a procedure using small 10-mV test steps to a range of voltages superimposed 500 ms after large conditioning prepulses, as used on earlier occasions to demonstrate charge movement components (Huang, 1981). To corroborate the experiments above, nifedipine was employed at the same three concentrations (0.5, 2, and 10 μM) while examining the effect of shifts in holding voltage. Steady-state membrane capacitances at each test voltage were normalized to control values obtained at the -100-mV reference voltage, $C_T/C_C$ (plotted mean ± SEM). This approach gives a function more sensitive to changes in the dependence of nonlinear charge on membrane potential than charge-voltage ($Q(V)$) curves (Adrian and Peres, 1979). As indicated above, values of $C_C$ were consistent at any given pharmacological condition, but quantitative comparisons were not made between fibers where the nifedipine concentrations were different.

**FIGURE 3 (opposite).** Available charge $Q_{\text{ave}}$ assessed from the charge transferred by large voltage steps from -100 mV to -20 mV, at different holding voltages $V_h$ and nifedipine concentrations of 0 μM (A), 0.5 μM (B), 2 μM (C), and 10 μM (D). Value of zero charge obtained in each group arrowed with the corresponding symbol on the ordinate. In this and subsequent figures points with error bars indicate mean ± SEM. $E$. Charge movements in response to a step from -100 mV holding potential to -20 mV before and after adding 10 μM nifedipine. Control capacitances before and after the solution changes were 5.08 and 5.02 μF/cm$^2$, respectively. $F$–$H$. Pairs of charge movements obtained, in the same fiber in each case, in response to large voltage steps at holding potentials of -100 mV (a) and -70 mV (b) compared in examples of fibers in 0.5 μM (F), 2 μM (G), and 10 μM (H) nifedipine, respectively. Inactivation curves were assessed at nifedipine concentrations of: (A) 0 μM (tilted triangles, five fibers; temperature = 3.9 ± 0.07°C, $R = 336 ± 0.8 \Omega$ cm, $\lambda = 2.5 ± 0.11$ mm, $r_i = 8.471 ± 1.178 \Omega$ cm, diameter = 74.4 ± 5.3 μm, $r_m = 519.7 ± 58.4 \Omega$ cm, $R_m = 11.72 ± 0.63 \Omega$ cm$^2$, $C = 13.8 ± 1.52 \mu F/cm^2$); (B) 0.5 μM (open circles; four fibers; four fibers; temperature = 3.7°C, $R = 337.9 \Omega$ cm, $\lambda = 3.4 ± 0.64$ mm, $r_i = 12.946 ± 5.756 \Omega$ kcm, diameter = 74.0 ± 12.7 μm, $r_m = 887 ± 50.4 \Omega$ kcm, $R_m = 20.68 ± 4.38 \kappa$ cm$^2$, $C_m = 9.06 ± 1.01 \mu F/cm^2$); (C) 2.0 μM (filled circles; six fibers; temperature = 4.05 ± 0.27°C, $R = 332.8 ± 3.9 \Omega$ cm, $\lambda = 3.76 ± 0.17$ mm, $r_i = 5754.3 ± 1.0828 \kappa$ kcm, diameter = 90.8 ± 6.8 μm, $r_m = 792.3 ± 116.1 \kappa$ kcm, $R_m = 21.31 ± 1.81 \kappa$ kcm$^2$, $C_m = 9.19 ± 0.398 \mu F/cm^2$); and (D) 10 μM (inverted triangles, four fibers; temperature = 4.7 ± 0.09°C, $R = 327.6 ± 0.9 \Omega$ cm, $\lambda = 2.81 ± 0.09$ mm, $r_i = 8.165 ± 1.481 \kappa$ kcm, diameter = 77.05 ± 9.84 μm, $r_m = 508.4 ± 100.7 \kappa$ kcm, $R_m = 11.52 ± 1.54 \kappa$ kcm$^2$, $C_m = 11.72 ± 1.14 \mu F/cm^2$), respectively.
At low (0.5 μM) nifedipine concentrations (Fig. 4 A) capacitance increased gradually with depolarization to around -60 mV, ~20% above the reference value at -100 mV. It then increased steeply to >1.6 times the control value, at a test voltage of -40 mV, before falling with charge saturation. However, altering the holding voltage to -70 mV and repeating measurements in the same fiber (but continuing to take control records at a holding voltage of -100 mV) did not significantly alter the capacitance-voltage relationship. Thus, at such concentrations there is no contribution by voltage-dependent block to the influence of nifedipine. This complements the inactivation results described above.

At 2 μM nifedipine there remained the steep dependence of membrane capacitance on voltage, and this was similar to that obtained in 0.5 μM nifedipine at a holding potential of -100 mV (Fig. 4 B). However, obtaining test steps at a holding voltage of -70 mV (but still taking control steps at a -100 mV holding potential) now reduced the maximum capacitance, and altered not only the magnitude but also the form of the capacitance curve. Thus, the reduced dependence of capacitance on test voltage at a holding potential of -70 mV did not merely represent a simple scaling down of the corresponding function in fully polarized fibers. Shifting the holding potential had little effect on membrane capacitance between test voltages of -100 mV and -60 mV. However, capacitance was substantially reduced at larger depolarizations giving a more gradual capacitance-voltage relationship, rising to not more than 1.4 times the control value before falling with charge saturation.

Finally, 10 μM nifedipine (Fig. 4 C) reduced membrane capacitance. Even in fully polarized fibers, this rose to not more than 1.4 times its reference value. However, shifting holding voltage now caused no further change in capacitance, in parallel with results from the previous procedures.

The corresponding changes in charge movement records preferentially affected delayed (qt) currents rather than reducing the currents over their entire time course. For example, slow charge movement components (Fig. 4 D; horizontal bars) could be demonstrated in 2 μM nifedipine at a -100 mV holding voltage. In agreement with earlier reports (e.g., Adrian and Peres, 1979), they were readily visible using the pulse procedure employing small 10-mV steps, but over only a narrow voltage range (-40 to -30 mV) closely incremented about the normal contractile threshold. Their time course was initially prolonged but then became considerably more rapid and merged with the earlier decay even with 10–15 mV further depolarization. In contrast, altering the holding potential to -70 mV in the same fiber (Fig. 4 E) inhibited such slow components, suggesting a relative preservation of rapid qt charge movement (Adrian and Peres, 1979; Huang, 1982; Hui, 1983). Certainly, the major change in transients was not a simple scaling-down of the transients at -100 mV.

These findings demonstrate the existence and properties of nonlinear charge inactivated by a combination of nifedipine treatment and altered holding voltage. This charge had a steep and distinct voltage dependence. Thus it was only sensitive to depolarization beyond about -60 mV, but its steep voltage dependence beyond this was responsible for the greater part of the pronounced peak in membrane capacitance at 1.6 times the control. It contributed more to late qt transients than to the early qt currents (Adrian and Peres, 1979; Huang, 1982).
Charge susceptible to voltage dependent nifedipine block thus showed similarities with the $q_a$ charge described on earlier occasions (Huang, 1982). The $q_r$ charge was defined in terms of its sensitivity to tetracaine (Huang, 1981, 1982), which conserved the more rapid $q_a$ transient. It was accordingly of interest to determine whether the charging components subject to voltage-dependent block by nifedipine demonstrated here fell into the category of $q_a$ or $q_r$ charge, or were drawn from both $q_a$ and $q_r$ components, i.e., from the charge as a whole.

The experimental procedures were similar to those described above, but were performed on tetracaine-treated fibers. Fig. 5 displays the results of using large single pulses as used in Fig. 2 for a fiber in the presence of both 2 $\mu$M nifedipine and 2 mM tetracaine, studied at holding voltages of $-100$ mV and $-70$ mV, respectively. Tetracaine reduced charge transfer as expected from earlier reports (Huang, 1981; Hui, 1983). However, shifting the holding potential now did not further reduce available steady-state charge (Fig. 5 A). In addition, there was no major difference between charging transients before (Fig. 5 B) and after (Fig. 5 C) shifting the holding voltage in the same fiber. Thus tetracaine-resistant ($q_{tr}$) charge does not show voltage-dependent block by nifedipine.

**Nifedipine Does Not Modify Inactivation of $q_a$ Charge**

Charge inactivation was next investigated in fibers using large test pulses from fixed prepulse levels of $-100$ mV to a test voltage of $-20$ mV. The holding voltages examined were altered as described above, in successive 10-mV increments beginning with $-100$ mV. Fig. 6 A plots available charge against holding voltage in the presence of 2 mM tetracaine in fibers in both the presence and absence of 2 $\mu$M nifedipine. Tetracaine reduced charge movement even in fully polarized fibers to 15–18 nC/µF. This represents a larger reduction of available charge than accomplished by nifedipine alone under the conditions used here, but is in agreement with earlier reports (Huang, 1984). However, adding nifedipine (2 $\mu$M) did not influence the inactivation of such tetracaine-resistant ($q_{tr}$) charge. Furthermore, the same shifts in holding voltage that modified charge inactivation in the previous case (from $-100$ mV to $-70$ mV) produced little inactivation of the $q_a$ system. Fig. 6 B displays examples of the corresponding charge movements in the presence of both nifedipine and tetracaine. In agreement with the steady-state results, they remained similar in size and form at both holding potentials. They were monotonic delays, and slow ($q_a$) charging components were absent.

These findings contrast with the charge inactivation by nifedipine when tetracaine was absent. They appear to exclude tetracaine-resistant charge from participating in the effect of nifedipine and implicate the tetracaine-sensitive ($q_r$) charge.

**Nifedipine Does Not Affect the Voltage Dependence of $q_a$ Charge**

A final procedure to exclude $q_a$ charge from significantly participating in nifedipine action compared the voltage dependence of its contribution to membrane capacitance in 2 $\mu$M nifedipine at holding potentials of $-100$ mV and $-70$ mV,
Figure 4.

A. $C_T/C_C$

B. $V(mV)$

C. $DHP (\mu M)$: 0.5 $\bigcirc$, 2.0 $\bullet$, 10.0 $\nabla$

D. $V_h = -100 \text{ mV}$

E. $V_h = -70 \text{ mV}$

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respectively. Control capacitance–voltage curves were also obtained in 2 mM tetracaine but in the absence of nifedipine. As in the corresponding experiments above, small 10-mV steps to a series of test voltages were superimposed 500 ms after a prepulse step (Adrian and Peres, 1979). Tetracaine reduced the dependence of capacitance on voltage in agreement with earlier work (Fig. 7 A). This effect was more marked than the voltage-dependent block by nifedipine alone. Thus capacitance now did not exceed 1.3 times the control value. However, 2 μM nifedipine now had no further influence on the capacitance–voltage function, nor did shifting the holding potential from −100 mV to −70 mV in the same fiber produce any significant change. Charge movements (Fig. 7 B) were simple monotonic decays, did not show delayed (q_v) components, and were not significantly altered by changing holding potential from −100 mV (Fig. 7 B) to −70 mV (Fig. 7 C). Therefore, these findings also indicate that voltage-dependent block by nifedipine does not involve tetracaine-resistant (q_phi) charge.

**DISCUSSION**

The experiments here explored the effects of varying nifedipine concentrations on charge movements in intact amphibian muscle fibers with changes in holding potential. Rios and Brum (1987) reported that nifedipine reduced nonlinear charge even in fully polarized, cut fibers. However, a subsequent shift in holding potential from −100 mV to −70 mV further reduced available charge to a degree greater than achieved by such shifts in the absence of drug. However, the influence of nifedipine in fully polarized fibers was not associated with a reduction in Ca^{2+} signals. Even high nifedipine (150 μM) concentrations ample to abolish calcium currents, did not influence contractile activation in fully polarized fibers (McCleskey, 1985). It is therefore possible that intramembrane charge, affected in fully polarized

**FIGURE 4 (opposite).** Capacitance, normalized to values obtained at a reference voltage of −100 mV, C_Co/C_Co plotted against test voltage V at holding potentials of V_H = −100 mV and −70 mV, respectively, and measured in each case in the same fiber. Reference capacitances for each fiber group marked by arrows. Three different nifedipine concentrations (0.5[A], 2[B], and 10 μM[C], respectively) were explored. Charge movements in response to 10-mV steps to the test voltage V applied after a 500-ms prepulse from heavier signal averaging from the same fiber in 2 μM nifedipine before (D) and after shifting the holding voltage from −100 mV to −70 mV (E). Fibers subject to nifedipine concentrations of (A) 0.5 μM (open circles, V_H = −100 mV; open triangles, V_H = −70 mV), (B) 2.0 μM (solid circles, V_H = −100 mV; solid triangles, V_H = −70 mV), and (C) 10 μM (inverted triangles, V_H = −100 mV; rotated triangles, V_H = −70 mV), respectively. Fiber cable constants: in 0.5 μM nifedipine (A); four fibers; temperature = 3.4 ± 0.08°C, R_i = 341 ± 0.9 Ω cm, λ = 2.0 ± 0.2 mm, r_i = 6,581 ± 902 kΩ/cm, diameter = 82.8 ± 4.8 μm, r_m = 253 ± 41.8 kΩ cm, R_m = 6.69 ± 1.37 kΩ cm², C_m = 7.02 ± 0.42 μF/cm². In 2.0 μM nifedipine (B); four fibers; temperature = 3.7 ± 0.14°C, R_i = 332.7 ± 4.3 Ω cm, λ = 3.56 ± 1.07 mm, r_i = 3,968 ± 591 kΩ/cm, diameter = 96.3 ± 13.82 μm, r_m = 516.2 ± 182.3 kΩ cm, R_m = 18.58 ± 9.2 kΩ cm², C_m = 10.3 ± 0.64 μF/cm². In 10 μM nifedipine (C); four fibers; temperature = 3.9°C, R_i = 335.73 Ω cm, λ = 3.35 ± 0.32 mm, r_i = 6,368 ± 887 kΩ/cm, diameter = 84.7 ± 6.68 μm, r_m = 698 ± 127 kΩ cm, R_m = 18.33 ± 3.19 kΩ cm², C_m = 12.2 ± 1.6 μF/cm².
fibers, is involved in calcium current gating rather than contractile activation as has been suggested for mammalian fibers (Lamb 1986; Lamb and Walsh, 1987).

In contrast, a combination of nifedipine treatment and shifts in holding potential did reduce (if not always abolish) Ca$^{2+}$ signals and contractile activation (Rakowski et al., 1987; Rios and Brum, 1987). Such a charge that was correspondingly inactivated then would be of interest in connection with possible associations with contractile activation. The experiments here separated a sample of the charge whose inactivation demanded a combination of both nifedipine treatment and depolarization in

![Graph](https://example.com/graph.png)

**Figure 5.** A, Intramembrane charge $Q(V)$ transferred by single voltage steps from $-100$ mV to a range of test voltages $V$ in the presence of 2 mM tetracaine and 2 μM nifedipine at holding voltages of $-100$ mV (circles) and $-70$ mV (triangles). The difference (squares) indicates little inactivated charge. B and C, Charge movements obtained in the same fiber at the two holding voltages of $-100$ mV (B) and $-70$ mV (C). Alternate traces are labeled; respective traces in B and C correspond to the same test voltage. Fiber R47: temperature = 3.9°C, $R_i = 335.7 \ \Omega$ cm, $\lambda = 2.59$ mm, $r_i = 5.949$ kΩ/cm, diameter = 84.8 μm, $r_m = 397.6$ kΩ cm, $R_m = 10.59$ kΩ cm$^2$, $C_m = 6.68$ μF/cm$^2$. 
intact fibers, and gained an indication of its voltage dependence and kinetic properties. It then went on to investigate whether this involved the charge as a whole, or fell within one or another capacity component. Its major conclusions arise from comparing steady-state capacitances, using pulse procedures adopted on earlier occasions (Adrian and Peres, 1979; Huang, 1981, 1982) to fractionate components of intramembrane charge. Such an approach enabled changes in tetracaine-sensitive ($q_a$) charge to be followed even through voltages when its transients were rapid and not distinguishable from the $q_B$ current.

The experiments compared test records with transients obtained by small voltage steps at a holding potential restored to $-100$ mV. It included control experiments to confirm that under any particular pharmacological condition this procedure gave consistent reference steady-state and transient records with which to make comparisons (see, for example, Adrian and Almers, 1976a; Rios and Brum, 1987). At all events, quantitative comparisons of total charge were only made within a given pharmacological condition, in which the effect of holding voltage had been investigated in the same fiber. Quantitative comparisons of values between fiber groups subject to different drug concentrations were avoided.

Intact fibers differed in capacitative properties from cut fibers in some respects. Even in the absence of nifedipine, Rios and Brum (1987) reported a degree of charge inactivation when holding potential was shifted from $-100$ mV to $-70$ mV.

**FIGURE 6.** A, Charge inactivation in fibers in 2 mM tetracaine in the absence (triangles) and presence (circles) of 2 $\mu$M nifedipine. B, Charge movements obtained in one such fiber at holding voltages varied at 10-mV intervals. In 2 mM tetracaine: four fibers, temperature $= 4.4 \pm 0.1^\circ$C, $R_i = 330.7 \pm 1.3 \Omega$ cm, $\lambda = 2.23 \pm 0.34$ mm, $r_i = 16,529 \pm 3,486$ k$\Omega$/cm, diameter $= 84.47 \pm 6.22$ $\mu$m, $r_m = 706.23 \pm 109.17$ k$\Omega$ cm, $R_m = 12.31 \pm 2.47$ k$\Omega$ cm$^{-2}$, $C_m = 6.04 \pm 0.71$ nF/cm$^2$. In 2 mM tetracaine and 2 $\mu$M nifedipine: four fibers, temperature $= 3.5^\circ$C, $R_i = 335.7 \Omega$ cm, $\lambda = 3.45 \pm 0.41$ mm, $r_i = 7,024 \pm 2,592$ k$\Omega$/cm, diameter $= 92.2 \pm 13.3$ $\mu$m, $r_m = 625.4 \pm 124.1$ k$\Omega$ cm, $R_m = 16.80 \pm 2.67$ k$\Omega$ cm$^{-2}$, $C_m = 6.88 \pm 0.90$ nF/cm$^2$. 

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Figure 7. A, Voltage dependence of the membrane capacitance in 2 mM tetracaine for fibers in the presence of 2 µM nifedipine when studied at holding voltages of -100 mV (solid circles) and -70 mV (solid triangles) in the same fiber. These are compared with fibers studied at 0 µM nifedipine (open triangles). Charge movements in 2 µM nifedipine obtained using a pulse procedure using small (10 mV) voltage steps before (B) and after (C) changing holding voltage in the same fiber were similar. Alternate traces are labeled; respective records in B and C correspond to the same test voltage. Four fibers studied in 2 µM nifedipine, 2 mM tetracaine. Cable constants: temperature = 3.7 ± 0.1°C, $R_i = 337.9 ± 1.06 \Omega$ cm, $\lambda = 3.22 ± 0.26$ mm, $\tau_i = 4.981 ± 296$ kΩ/cm, diameter = $93.4 ± 2.7 \mu$m, $r_m = 520.6 ± 76.2$ kΩ cm, $R_m = 15.3 ± 2.4$ kΩ cm$^2$, $C_m = 7.87 ± 0.58 \mu$F/cm$^2$. Six fibers studied in 0 µM nifedipine, 2 mM tetracaine: temperature = 4.4 ± 0.1°C, $R_i = 365 ± 5.1$ Ω cm, $\lambda = 2.2 ± 0.2$ mm, $\tau_i = 11.438 ± 1.449$ kΩ/cm, diameter = 67.01 ± 4.6 μm, $r_m = 576 ± 119$ kΩ cm, $R_m = 11.83 ± 2.2$ kΩ cm$^2$, $C_m = 8.0 ± 0.73 \mu$F/cm$^2$. 
The present findings indicated a sharper onset of inactivation beginning only at holding levels depolarized to −70 mV in agreement with earlier reports (Hui, 1983; Huang, 1984). Intact fibers also required considerably higher nifedipine concentrations (2.0 μM instead of 0.5 μM) to yield positive effects. In the latter respect, the findings resemble those reports by Lamb (1986) in cut mammalian muscle fibers. Alternatively, Csernoch et al. (1988) reported differences in effective pharmacological concentrations between amphibian preparations concerning tetracaine action.

Nevertheless, some of the features in intact fibers facilitated the study of charge susceptible to voltage-dependent effects of nifedipine. Thus, studies of both inactivation and membrane capacitance independently demonstrated partial charge inactivation with a −100 mV to −70 mV shift in holding voltage at the same (2 μM) nifedipine concentration within the same muscle fiber in contrast to the absence of such effects at lower or zero drug concentrations. However, capacitative properties in 0 or 0.5 μM nifedipine were not influenced by shifts in holding voltage between −100 mV and −70 mV, which then assumed the steeper form resembling results in fully polarized fibers in 2 μM nifedipine. Higher concentrations (10 μM) depressed the capacitance−voltage function and reduced available charge, but small shifts in holding potential were now without further effect.

This particular action of nifedipine appeared to selectively involve a distinct component of nonlinear charge, rather than the charge as a whole. First, the capacitative contribution concerned was substantially more steeply voltage dependent than either the overall membrane capacitance preceding inactivation, or the fraction remaining after such treatment. Second, the voltage-dependent block preferentially involved late charging transients (Adrian and Peres, 1979; Hui, 1983; Huang, 1987; Huang and Peachey, 1989) rather than reducing the charge movement uniformly through its time course. At the same time, one may emphasize that the steady-state criteria used to separate the tetracaine-sensitive (qₜ) charge leave it possible that qₜ may include early currents overlapping in time course with qₜₜ charge. Third, nifedipine did not influence inactivation of qₜₜ charge, isolated by removing qₜ with tetracaine (Huang, 1981, 1982; Vergara and Caputo, 1982; Hui, 1983). In tetracaine-treated fibers, small shifts in holding potential did not further reduce nonlinear charge even in the presence of nifedipine, nor did such conditions significantly influence steady-state capacitances or charging currents of the qₜₜ system. Thus, tetracaine obliterates the voltage-dependent block of intramembrane charge by nifedipine.

The above observations exclude a significant participation of qₜₜ (tetracaine-resistant) charge from voltage-dependent nifedipine action. This leaves the charge concerned within the category of tetracaine-sensitive (qₜ) charge, while not directly indicating whether the pharmacological species are exactly equivalent. Thus, the extent of tetracaine-induced block exceeded the voltage-dependent block by 2 μM nifedipine. On the other hand, the prolonged depolarizations used were confined to the range −100 mV to −70 mV as the control data suggested that only within this range would any inhibition exclusively reflect voltage-dependent pharmacological block. It is possible that larger depolarization might have extended the block by nifedipine. Alternatively, some of these differences may parallel variations in the physiological actions of these drugs. Both inhibit calcium currents (e.g., Almers and
However, voltage-dependent nifedipine treatment often reduced rather than completely removed contractile activation (Rakowski et al., 1987) and Δ[Ca^{2+}] signals (Rios and Brum, 1987) in amphibian muscle. It reduced, rather than abolished contractility, and then in only ~50% of mammalian fibers studied (Lamb, 1986). In contrast, tetracaine could fully abolish both calcium signals and contractile activation (Luttgau and Oefliker, 1968; Vergara and Caputo, 1982). This leaves it possible that even high concentrations of nifedipine, particularly in fully polarized fibers, may not abolish the entire charge fraction related to excitation–contraction coupling, resulting in persistent contractile activation under such conditions (McCleskey, 1985).

In any event, the findings here demonstrate a charge fraction inhibited by a combination of imposed depolarization and nifedipine, which belongs to the category of tetracaine-sensitive (q_v) rather than tetracaine-resistant (q_t) charge. Such q_v charge has a distinct sensitivity to test and holding potential (Adrian and Peres, 1979; Huang, 1981, 1982), tetracaine (Huang, 1981; Hui, 1983), and lyotropic agents (Huang, 1986), and appears to be selectively localized to transverse tubular membrane (Adrian and Huang, 1984b; Huang, 1987; Huang and Peachey, 1989). These parallels would be consistent with this charge fraction having a causal relationship with excitation–contraction coupling. This could be through offering an independent voltage-sensing transition as part of q_v (Huang and Peachey, 1989), or as an end result of a reaction sequence that could involve the remaining intramembrane charge (Rios and Brum, 1987) or even the calcium ultimately released (Horowicz and Schneider, 1981; Huang, 1981; Csernoch et al., 1989; Pizarro et al., 1990).

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REFERENCES

Adrian, R. H. 1978. Charge movement in the membrane of striated muscle. Annual Reviews of Biophysics and Bioengineering. 7:85–112.
Adrian, R. H., and W. Almers. 1974. Membrane capacity measurements on frog skeletal muscle in media of low ionic content. Journal of Physiology. 237:573–605.
Adrian, R. H., and W. Almers. 1976a. The voltage dependence of membrane capacity. Journal of Physiology. 254:317–338.
Adrian, R. H., and W. Almers. 1976b. Charge movement in the membrane of striated muscle. Journal of Physiology. 254:339–360.
Adrian, R. H., and C. L.-H. Huang. 1984a. Charge movements near the mechanical threshold in skeletal muscle of Rana temporaria. Journal of Physiology. 349:488–500.
Adrian, R. H., and C. L.-H. Huang. 1984b. Experimental analysis of the relationship between charge movement components in skeletal muscle of Rana temporaria. Journal of Physiology. 353:419–434.
Adrian, R. H., and A. Peres. 1979. Charge movement and membrane capacity in frog skeletal muscle. Journal of Physiology. 289:83–97.
Adrian, R. H., and R. F. Rakowski. 1978. Reactivation of membrane charge movement and delayed potassium conductance in skeletal muscle fibers. Journal of Physiology. 278:533–557.
Aimers, W., and P. T. Palade. 1981. Slow calcium and potassium currents across frog muscle membrane: measurements with a Vaseline gap technique. *Journal of Physiology*. 312:159–176.

Block, B. A., T. Imagawa, K. P. Campbell, and C. Franzini-Armstrong. 1988. Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *Journal of Cell Biology*. 107:2587–2600.

Borsotto, M., J. Barhanin, M. Fosset, and M. Lazdunsky. 1985. The 1,4-dihydropyridine receptor associated with the skeletal muscle voltage dependent Ca\(^{2+}\) channel: purification and subunit composition. *Journal of Biological Chemistry*. 260:14255–14263.

Brum, G., R. Fitz, G. Pizarro, and E. Rios. 1988. Voltage sensors of the frog skeletal muscle require calcium to function in excitation-contraction coupling. *Journal of Physiology*. 398:475–505.

Brum, G., and E. Rios. 1987. Intramembrane charge movement in skeletal muscle fibers: properties of charge 2. *Journal of Physiology*. 387:489–517.

Campbell, K. P., C. M. Knudson, T. Imagawa, A. I. Leung, J. L. Suito, S. D. Kahl, C. R. Raub, and L. Madson. 1987. Identification and characterization of the high affinity \(^{3}H\)ryanodine receptor of the junctional sarcoplasmic reticulum Ca\(^{2+}\) release channel. *Journal of Biological Chemistry*. 262:6460–6463.

Chandler, W. K., R. F. Rakowski, and M. F. Schneider. 1976. A non-linear voltage-dependent charge movement in frog skeletal muscle. *Journal of Physiology*. 254:243–283.

Csernoch, L., C. L.-H. Huang, G. Szucs, and L. Kovacs. 1988. Differential effects of tetracaine on charge movements and Ca\(^{2+}\) signals in frog skeletal muscle. *Journal of General Physiology*. 92:601–612.

Csernoch, L., I. Uribe, M. Rodriguez, G. Pizarro, and E. Rios. 1989. Q, and calcium release flux in skeletal muscle fibers. *Biophysical Journal*. 55:88a. (Abstr.)

Curtis, B. M., and W. A. Catterall. 1984. Purification of the calcium antagonist receptor of the voltage sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry*. 23:2113–2118.

Ferguson, D. G., H. W. Schwartz, and C. Franzini-Armstrong. 1984. Subunit structure of junctional feet in triads of skeletal muscle: A freeze-drying rotary-shadowing study. *Journal of Cell Biology*. 99:1735–1742.

Fleischer, S., E. M. Oggunbunni, M. C. Dixon, and E. A. M. Fleer. 1985. Localization of Ca\(^{2+}\) release channels with ryanodine receptors of fast skeletal muscle. *Proceedings of the National Academy of Sciences USA*. 82:7256–7259.

Flockerzi, V., H. J. Ocken, and F. Hofman. 1986. Purification of a functional receptor for calcium-channel blockers from rabbit skeletal muscle microsomes. *European Journal of Biochemistry*. 161:217–224.

Fosset, M., E. Jaimovich, E. Delpont, and M. Lazdunski. 1983. \(^{3}H\) Nitrendipine receptors in skeletal muscle: properties and preferential localization in transverse tubules. *Journal of Biological Chemistry*. 258:6086–6092.

Franzini-Armstrong, C. 1970. Studies of the triad. I. Structure of the junction in frog twitch fibers. *Journal of Cell Biology*. 47:488–499.

Franzini-Armstrong, C., and G. Nunzi. 1983. Junctional feet and particles in the triads of fast twitch muscle fibers. *Journal of Muscle Research and Cell Motility*. 4:233–252.

Hodgkin, A. L., and S. Nakajima. 1972. The effects of diameter on the electrical constants of frog skeletal muscle fibers. *Journal of Physiology*. 221:105–120.

Horowicz, P. A., and M. F. Schneider. 1981. Membrane charge movement at contraction thresholds in skeletal muscle fibers. *Journal of Physiology*. 314:565–593.

Huang, C. L.-H. 1981. Dielectric components of charge movements in skeletal muscle. *Journal of Physiology*. 313:187–205.
Huang, C. L.-H. 1982. Pharmacological separation of charge movement components in frog skeletal muscle. *Journal of Physiology.* 324:375–387.

Huang, C. L.-H. 1983. Time domain spectroscopy of the membrane capacitance in frog skeletal muscle. *Journal of Physiology.* 341:1–24.

Huang, C. L.-H. 1984. Analysis of 'off' tails of intramembrane charge movements in skeletal muscle of *Rana temporaria.* *Journal of Physiology.* 356:375–390.

Huang, C. L.-H. 1986. The differential effects of twitch potentiators on charge movements in frog skeletal muscle. *Journal of Physiology.* 380:17–33.

Huang, C. L.-H. 1987. 'Off' tails of intramembrane charge movements in frog skeletal muscle in perchlorate containing solutions. *Journal of Physiology.* 384:491–510.

Huang, C. L.-H., and L. D. Peachey. 1989. The anatomical localization of charge movement components in frog skeletal muscle. *Journal of General Physiology.* 93:565–584.

Hui, C. S. 1983. Pharmacological studies of charge movements in frog skeletal muscle. *Journal of Physiology.* 337:509–529.

Imagawa, T., J. S. Smith, R. Coronado, and K. P. Campbell. 1987. Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca$^{2+}$ permeable pore of the calcium release channel. *Journal of Biological Chemistry.* 262:16636–16643.

Inui, M., A. Saito, and S. Fleischer. 1987. Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle. *Journal of Biological Chemistry.* 262:1740–1747.

Kawamoto, R. M., J. P. Brunschwig, K. C. Kim, and A. H. Caswell. 1986. Isolation, localization and characterization of the spanning protein of the skeletal muscle triad. *Journal of Cell Biology.* 103:1405–1414.

Lai, F. A., H. P. Erickson, B. A. Block, and G. Meissner. 1987. Evidence for a junctional feet-ryanodine receptor complex from sarcoplasmic reticulum. *Biochemical and Biophysical Research Communications.* 143:704–709.

Lai, F. A., H. P. Erickson, E. Rousseau, Q. Y. Liu, and G. Meissner. 1988. Purification and reconstitution of the calcium release channel from skeletal muscle. *Nature.* 331:315–320.

Lamb, G. D. 1986. Components of charge movement in rabbit skeletal muscle: the effect of tetracaine and nifedipine. *Journal of Physiology.* 376:85–100.

Lamb, G. D., and T. Walsh. 1987. Calcium currents, charge movements and dihydropyridine binding in fast and slow-twitch muscles of rat and rabbit. *Journal of Physiology.* 393:595–618.

Leung, A. T., T. Imagawa, B. Block, C. Franzini-Armstrong, and K. P. Campbell. 1988. Biochemical and ultrastructural characterization of the dihydropyridine receptor from rabbit skeletal muscle: evidence for a 52 kilodalton subunit. *Journal of Biological Chemistry.* 263:994–1001.

Leung, A. T., T. Imagawa, and K. P. Campbell. 1987. Structural characterization of the 1,4 dihydropyridine receptor of the voltage-dependent Ca$^{2+}$ channel from rabbit skeletal muscle. *Journal of Biological Chemistry.* 262:7843–7846.

Luttgau, H. C., G. Gottschalk, and D. Berwe. 1986. The role of Ca$^{2+}$ in inactivation and paralysis of excitation-contraction coupling in skeletal muscle. *Fortschritte der Zoologie.* 33:193–203.

Luttgau, H. C., G. Gottschalk, and D. Berwe. 1987. The effect of calcium and Ca antagonists upon excitation-contraction coupling. *Canadian Journal of Physiology and Pharmacology.* 65:717–723.

Luttgau, H. C., and H. Oetliker. 1968. The action of caffeine on the activation of the contractile mechanism in striated muscle fibers. *Journal of Physiology.* 194:57–74.

Luttgau, H. C., and W. Spiecker. 1979. The effects of calcium deprivation upon mechanical and electrophysiological parameters in skeletal muscle fibers of the frog. *Journal of Physiology.* 296:411–429.
McCleskey, E. W. 1985. Calcium channels and intracellular calcium release are pharmacologically different in frog skeletal muscle. Journal of Physiology. 361:231-249.
Pizarro, G., L. Csernoch, and E. Rios. 1990. An inward phase in intramembrane charge movement during a depolarizing pulse. Biophysical Journal. 57:341a.(Abstr.)
Rakowski, R. F., E. Olszewksa, and C. Paxson. 1987. High affinity effect of nifedipine on K contracture in skeletal muscle suggest a role for Ca channels in excitation-contraction coupling. Biophysical Journal. 51:550a.(Abstr.)
Rios, E., and G. Brum 1987. Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. Nature. 325:717-720.
Schneider, M. F., and W. K. Chandler. 1973. Voltage-dependent charge in skeletal muscle: a possible step in excitation-contraction coupling. Nature. 242:244-246.
Schwartz, L. M., E. W. McCleskey, and W. Almers. 1985. Dihydropyridine receptors in muscle are voltage-dependent but most are not functional calcium channels. Nature.314:747-751.
Vergara, J., and C. Caputo. 1982. Effects of tetracaine on charge movements and calcium signals in frog skeletal muscle. Proceedings of the National Academy of Sciences, USA. 80:1477-1481.