

Differential Effects of Tetracaine on Charge Movements and Ca^{2+} Signals in Frog Skeletal Muscle

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ABSTRACT The effects of tetracaine on charge movements and on antipyrilazo III signals monitoring intracellular $\Delta[\text{Ca}^{2+}]$ were compared in cut frog semitendinosus muscle fibers in a single vaseline gap-voltage clamp. Low tetracaine concentrations (25–40 μM) markedly reduced $\Delta[\text{Ca}^{2+}]$ signals and shifted the rheobase. However, they neither influenced charge movement nor that peak $\Delta[\text{Ca}^{2+}]$ value associated with the contractile threshold. Higher tetracaine concentrations (100–200 μM) partly inhibited charge movements in cut fibers. They separated a steeply voltage-sensitive charge, some of whose features resembled ' q_7 ' reported in intact fibers, and whose movement preceded $\Delta[\text{Ca}^{2+}]$ signals at threshold. These findings: (a) directly confirm an earlier suggestion that tetracaine acts on steps in excitation-contraction coupling rather than myofilament activation; (b) show that tetracaine at low concentrations can directly interfere with sarcoplasmic reticular calcium release without modifying charge movement; (c) show that the tetracaine-sensitive charge, first found in intact fibers, also exists in cut fibers; and (d) make it unlikely that tetracaine-sensitive charge transfer is a consequence of Ca^{2+} release as suggested on earlier occasions.

INTRODUCTION

A number of apparently differing reports exist on the actions of tetracaine on skeletal muscle charge movements. Almers (1976) successfully suppressed contraction even when neither the nonlinear charge nor the contractile response of skinned fibers to applied Ca^{2+} was significantly altered (Almers and Best, 1976). This suggested that there were effects on excitation-contraction coupling stages that followed any required charge movement, but preceded myofilament activation. On this basis, tetracaine has been used to immobilize mammalian muscle when studying its charge movements, which, at that time, were assumed not to be affected by such pharmacological treatment (e.g., Dulhunty and Gage, 1983; Dulhunty et al., 1987). However, in contrasting findings, tetracaine removed as much as one-third of the nonlinear charge, and particularly reduced the delayed (' q_7 ') transients obtained near the contractile threshold (Huang, 1981). Indeed, use of tetracaine, dantrolene

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sodium, or prolonged depolarization all separated a capacitance whose steep dependence upon voltage resembled that of tension generation (Huang, 1982; Hui, 1983) or of $\Delta[\text{Ca}^{2+}]$ signals (Vergara and Caputo, 1983). However, the reasons for the apparent discrepancies between the two sets of results have not been investigated further.

The present experiments explore whether tetracaine could have two sites of action in the excitation-contraction coupling sequence. If Ca^{2+} release from the sarcoplasmic reticulum were the more sensitive step, then contractile activity could be suppressed before the charge movement was altered (cf., Almers, 1976). In contrast, greater drug concentrations could then influence both charge movement and calcium release. Charge movements, intracellular $\Delta[\text{Ca}^{2+}]$, and contractile activity were therefore simultaneously measured in cut fibers to which antipyrylazo III was added from the open end.

METHODS

Single fibers from frog (*Rana esculenta*) semitendinosus muscle were mounted in a single vaseline-gap voltage clamp (Kovacs and Schneider, 1978; Kovacs and Szucs, 1983). The closed segment in the pool that contained external solution was fixed by its tendon and stretched such that the length of its sarcomeres was between 2.4 and 3.0 μm . The length of this closed segment was between 355 and 543 μm and that under the vaseline seal was 200–600 μm . Fiber diameters ranged from 77 to 120 μm , and experiments were performed at 4–6°C. Fibers were dissected in a Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , 2 mM Tris maleate buffer) and cut in a relaxing solution (120 mM K glutamate, 2 mM MgCl_2 , 0.1 mM EGTA, 5 mM Tris sodium maleate buffer) before they were mounted in the experimental chamber. The external solution consisted of 75 mM tetraethylammonium sulfate, 10 mM Cs_2SO_4 , 8 mM CaSO_4 , 3×10^{-7} M tetrodotoxin; and 5 mM Tris sodium maleate buffer. The internal solution contained 105 mM Cs glutamate, 5.5 mM MgCl_2 , 0.1 mM EGTA, 0.0082 mM CaCl_2 , 4.5 mM Tris sodium maleate buffer, 13.2 mM Tris Cs maleate buffer, 5 mM ATP, and 5.6 mM glucose. Further details of experimental procedures are available elsewhere (Csernoch et al., 1987).

Antipyrylazo III, at a concentration of 0.5–1 mM in the open-end pool, was allowed to diffuse into the intracellular space of the voltage-clamped fiber segment. Changes in intracellular $[\text{Ca}^{2+}]$ that resulted from depolarizing pulses were calculated from dye concentrations and absorbances at wavelengths of 720 and 850 nm as described by Csernoch et al. (1987). A K_D of 1.76×10^{-8} M² at a $[\text{Mg}^{2+}]$ of 1 mM, and an extinction coefficient of 8.2×10^3 M⁻¹ cm⁻¹ at a 720-nm wavelength were assumed. When alternative values were assumed ($K_D = 35,100$ μM^2 , extinction coefficient = 1.5×10^4 M⁻¹ cm⁻¹) 2:1 dye: Ca^{2+} stoichiometry (20°C; Hollingworth et al., 1986) did not lead to large divergences. Thus, as seen in Fig. 1, using the latter values altered peak $\Delta[\text{Ca}^{2+}]$ by only 8.7% in controls, and 6.1 and 6.5% in 25 μM tetracaine, and then in the same positive direction.

Charge movement records were simultaneously obtained by comparing capacity currents resulting from depolarizing (test) pulses of 100- or 60-ms duration, with those from 30-mV hyperpolarizing (control) pulses from the -100-mV holding potential scaled by the ratio of the respective voltage excursions. Unless otherwise stated, each control or test record was the average of 12 sweeps. The control sweeps bracketed the test sweeps and, together with monitoring holding current, checked fiber stability and condition. Linear capacitances deduced from such pulses are provided in the figure legends. When investigating the effect of tetracaine, the above procedures were performed both before and after adding the drug. Tetracaine-sensitive charge movement was then isolated by comparing records obtained in the

presence and absence of pharmacological agent, after correction for linear transients. In such experiments, linear capacitances in runs before and after adding tetracaine were compared to assess preparation stability. In eight fibers, the ratio of linear capacitances before and after adding 200 μM tetracaine was 1.0 ± 0.03 (mean \pm SE). Contractile thresholds were determined by using 100-ms long depolarizing pulses of graded (1–2 mV) amplitude and by looking for mechanical activity through a 40 X water immersion objective (see Kovacs et al., 1987).

When investigating the quantity and late time course of tetracaine-sensitive charges to compare them with $\Delta[\text{Ca}^{2+}]$ signals (see Figs. 5 and 6), high-frequency noise was digitally filtered within each pulse interval. The computational procedure fitted successive sets of 10 data points to a second-order polynomial through a least-squares procedure, as described by Guest (1961). The method choice was checked by comparing the peak values and integrals derived from the filtering with those of the original data. Apart from a smoothing at early times, this gave a clearer representation of the time course of charge movements particularly around their peak values.

RESULTS

Selective Effects on $\Delta[\text{Ca}^{2+}]$ Signals

Fig. 1 A illustrates how different tetracaine concentrations affect $\Delta[\text{Ca}^{2+}]$ transients at the normal fiber threshold voltage, which was determined before local anesthetic was added. Calcium transients and charge movements were first obtained at the rheobase, which was determined in the absence of local anesthetic. Next, tetracaine (25 μM) was added to the extracellular pool and the new rheobase was determined. $\Delta[\text{Ca}^{2+}]$ transients were then obtained at voltages that corresponded to these respective old and new (Fig. 1 B) rheobases in the presence of 25, 40, and 200 μM tetracaine. Where possible, the contractile threshold was also evaluated at all these concentrations. In addition, charge movements were explored over a wide voltage range in the same fiber when tetracaine levels were 0 and 40 μM . This experimental

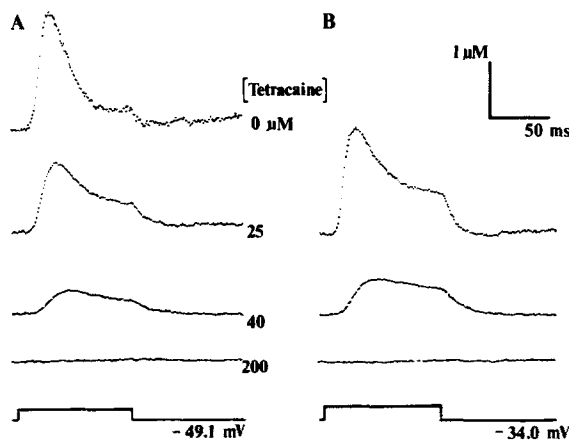


FIGURE 1. Ca^{2+} transients at (A) the normal rheobase (-49.1 mV) determined in the absence of drug, and (B) at the rheobase (-34 mV) as determined in 25 μM tetracaine at progressively increasing tetracaine concentrations (0, 25, 40 and 200 μM). Fiber 1212: fiber diameter, 120 μm ; linear capacitance, 6.48 nF; sarcomere spacing, 2.24 μm ; temperature, 5.5°C. Intracellular antipyrilazo III concentrations: control trace, 320 μM ; in 25 μM tetracaine, 470–545 μM ; in 40 μM tetracaine, 770–830 μM ; and in 200 μM tetracaine, 950 μM .

design gave us the following interrelated findings: (a) $\Delta[\text{Ca}^{2+}]$ transients at two voltages over a range of tetracaine concentrations; (b) the influence of tetracaine on the contraction threshold; (c) whether there was a tetracaine concentration at which $\Delta[\text{Ca}^{2+}]$ signals were reduced despite the nonlinear charge remaining intact; (d) $\Delta[\text{Ca}^{2+}]$ transients at voltages where mechanical activity was just observable, in both the presence and absence of tetracaine; (e) charge movements both at old ($0 \mu\text{M}$ tetracaine) and new ($25 \mu\text{M}$ tetracaine) thresholds; and (f) charge movements in the presence and absence of tetracaine over a wide voltage range.

Our use of the single gap at the end of the fiber thus allowed us to examine contractile threshold and charge movements simultaneously, the latter often over a wide voltage range. In the preparations reported, the contractile rheobase did not change more than 2–3 mV during an experimental run conducted in the absence of tetracaine. However, we could examine $\Delta[\text{Ca}^{2+}]$ transients only before or around rheobase, where contractile activity was weak and developed only towards the end of the pulse. Intrinsic ($\lambda = 850 \text{ nm}$) signals were then additionally monitored to exclude mechanical optical artifacts. Such artifacts occurred in experiments that demanded large depolarizations (see below), when, for this reason, only capacity transients could be investigated.

Changes in rheobase. Tetracaine ($25 \mu\text{M}$) significantly shifted the rheobase from $-42.4 \pm 3.32 \text{ mV}$ (mean \pm SE; seven fibers) to $-29.7 \pm 2.5 \text{ mV}$. Tetracaine at $40 \mu\text{M}$ prevented contraction even with voltage steps to positive potentials. This suggests a more profound effect on contractile activation in cut fibers than in intact muscle where millimolar concentrations were used (Almers and Best, 1976).

Changes in $\Delta[\text{Ca}^{2+}]$ transients. These were depressed with increasing tetracaine concentration at both voltages examined (Fig. 1 A and B). Peak $\Delta[\text{Ca}^{2+}]$ in the seven fibers studied at the normal threshold was $1.9 \pm 0.15 \mu\text{M}$ when tetracaine was absent. At the same testing voltage, even $25 \mu\text{M}$ tetracaine reduced this peak by $44 \pm 4.3\%$ and $40 \mu\text{M}$ reduced it by 80%. Finally, adding $200 \mu\text{M}$ tetracaine completely abolished $\Delta[\text{Ca}^{2+}]$ signals.

Peak $\Delta[\text{Ca}^{2+}]$ associated with the onset of contraction. This could be determined for tetracaine concentrations of 0 and $25 \mu\text{M}$ only; even at $40 \mu\text{M}$, mechanical activity was completely suppressed even with large ($>100 \text{ mV}$) voltage steps. A voltage step to the rheobase produced a peak $\Delta[\text{Ca}^{2+}]$ of $1.9 \pm 0.15 \mu\text{M}$ (seven fibers) before local anesthetic was added. Adding $25 \mu\text{M}$ tetracaine shifted the rheobase (see above), but the peak $\Delta[\text{Ca}^{2+}]$, once contractile threshold was reached with a larger voltage step, did not change more than $6.7 \pm 1.2\%$ in any given fiber. This suggests that the same value of peak $\Delta[\text{Ca}^{2+}]$ corresponded to the onset of contraction, which is in agreement with findings of Kovacs and Szucs (1983). Changes in intracellular antipyrylazo III concentrations through times before and after adding $25 \mu\text{M}$ tetracaine (between 470 and 545 μM in Figs. 1 and 2) would not affect our interpretation of free $\Delta[\text{Ca}^{2+}]$ from antipyrylazo III absorbances in these experiments (Kovacs et al., 1983).

Relation between nonlinear charge and $\Delta[\text{Ca}^{2+}]$. Fig. 2 A compares charge movement and $\Delta[\text{Ca}^{2+}]$ signals in response to threshold voltage steps before tetracaine was added, at early times immediately after the voltage steps were imposed. Such voltage steps moved $14.9 \pm 2.2 \text{ nC}/\mu\text{F}$ (four fibers) of nonlinear 'threshold' charge

(cf., Horowicz and Schneider, 1981). The same voltage steps moved similar charge (15.0 ± 2.05 nC/ μ F) in 25 μ M tetracaine, even though $\Delta[\text{Ca}^{2+}]$ signals were reduced (peak $\Delta[\text{Ca}^{2+}]$ changed from 2.0 to 1.25 μ M in Fig. 2 B), and contraction was suppressed. To elicit contraction in 25 μ M tetracaine required larger voltage steps. The latter then elicited a peak $\Delta[\text{Ca}^{2+}]$ similar to those obtained before the addition of tetracaine (1.9 μ M; compare Fig. 2 A and C). However, such larger depolarizations moved a larger nonlinear charge of 19.1 ± 1.9 nC/ μ F. Hence, although 25 μ M tetracaine did not alter charge movement, it increased the charge transfer required to achieve a particular peak $\Delta[\text{Ca}^{2+}]$.

Effects on charge movement. Noticeable effects on $\Delta[\text{Ca}^{2+}]$ signals were thus observed with 25 μ M tetracaine and there were large effects at 40 μ M. This was despite little or no significant change in the charge movement at the threshold volt-

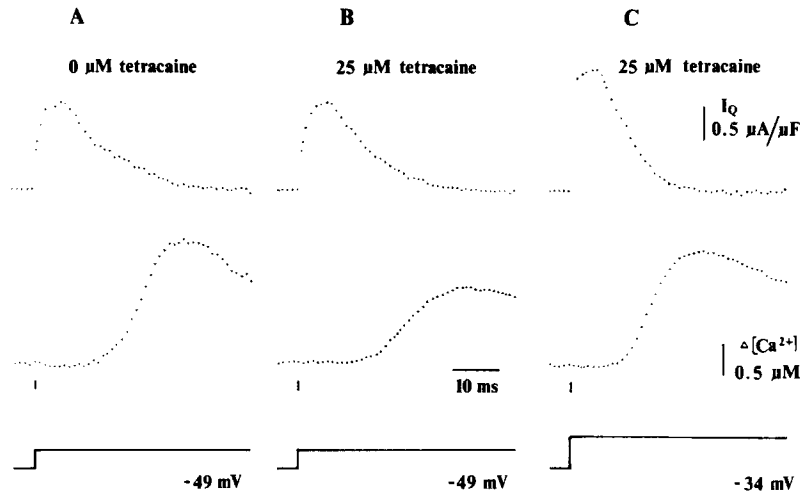


FIGURE 2. Comparison of charge movements and Ca^{2+} transients at (A) the normal rheobase (-49 mV), (B) at the same voltage, and (C) at the new threshold (-34 mV) in 25 μ M tetracaine. A faster timebase than in Fig. 1 was chosen to emphasize early events. (A and B) Charge movements whether in 0 or 25 μ M tetracaine similar at -49 mV despite reductions in peak $\Delta[\text{Ca}^{2+}]$. (C) Similar peak $\Delta[\text{Ca}^{2+}]$ associated with the onset of contractile activation at -34 mV in tetracaine despite increased nonlinear charge transfer. Fiber 1212, as in Fig. 1.

age, which was determined at the outset of the experiments. We then investigated charge movements over a wider voltage range in three fibers at both 0 and 40 μ M tetracaine. In these experiments, the pulse durations were shortened at larger depolarizations, the closed segment was left slack to minimize contraction artifacts in the electrical traces, and linear capacitance was monitored in runs before and after tetracaine was added. However, this arrangement permitted mechanical artifacts to appear in optical traces and so precluded the measurement of $\Delta[\text{Ca}^{2+}]$ signals. Fig. 4 (legend) indicates linear capacitance decreased by not >3% through the two sets of readings. In any case, values of maximum charge were expressed per unit linear membrane capacitance. The charge movements were similar under both conditions in both amplitude and time course (Fig. 3). Tetracaine did not alter the maximum

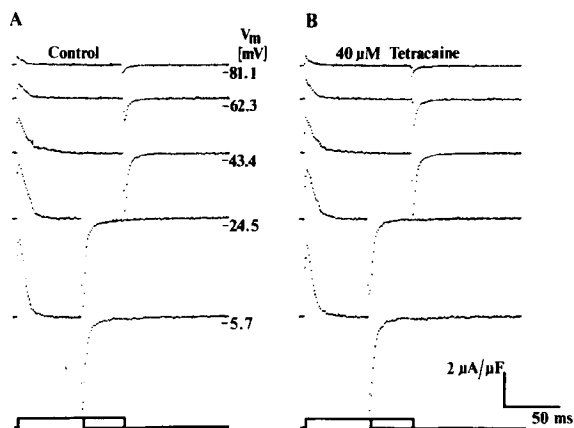
charge (Q_{\max}) by more than $-3.3 \pm 1.9\%$ and Q_{\max} values obtained by large (100 mV) depolarization (23.3 ± 4.42 nC/ μ F) agreed with earlier reports (Schneider and Chandler, 1973). Charge-voltage curves (Fig. 4) were normalized to Q_{\max} values and then described by a two-state Boltzmann function. These yielded similar steepness factors (k) (controls: $k = 13.6 \pm 1.1$ mV; tetracaine added: $k = 13.7 \pm 1.32$ mV) and transition voltages ($\bar{V} = -46.9 \pm 1.9$ and -49.9 ± 2.3 mV, respectively) in the presence (filled symbols) and absence (open symbols) of tetracaine.

Low tetracaine concentrations thus noticeably affected calcium signals and contractile activation. However, they did not significantly influence charge movements nor the relationship between peak $\Delta[\text{Ca}^{2+}]$ and the contractile threshold.

Tetracaine-sensitive Charge Does Exist in Cut Fibers

Tetracaine-sensitive (q_{γ}) charge is associated with delayed transients and 'hump' phases close to the contractile threshold in intact fibers (Huang, 1981, 1982; Hui,

FIGURE 3. Charge movements over a wide voltage range (A) in 0 μ M and (B) 40 μ M tetracaine compared in the same fiber. Fiber 1210; diameter, 81.4 μ m; sarcomere spacing, 2.5 μ m; temperature, 5.4°C. Currents normalized to the linear fiber capacitance, which was 6.56 ± 0.058 nF in the run before tetracaine was added, and 6.30 ± 0.036 nF after tetracaine was added. Values of Q_{\max} were 37.61 and 36.98 nC/ μ F (normalized to the respective linear capacitances), \bar{V} values were -43.85 and -46.58 mV, and k values were 14.62 and 14.66 mV before and after adding tetracaine, respectively.



1983; Adrian and Huang, 1984a, b). However, such slow currents were frequently less distinguishable or even absent both in the cut fibers observed here, and on earlier occasions (Melzer et al., 1986b). We therefore investigated whether such components exist, at least as tetracaine-sensitive charge in cut fibers, by trying higher tetracaine concentrations. However, the concentrations of the order of 2–4 mM used on intact fibers were rapidly toxic to cut fibers, which deteriorated even at 400- μ M levels. Similar findings have been reported in cut mammalian fibers (Lamb, 1986).

The effect of tetracaine was consequently studied only up to 100–200 μ M at voltages around the normal contraction threshold in seven fibers. It was therefore possible that the tetracaine-sensitive charge was not entirely suppressed.

Nevertheless, we could still estimate lower limits for its quantity and obtain indications of its time course and voltage sensitivity. Additionally (see below) the amount of charge abolished at the contractile threshold did not alter significantly when the

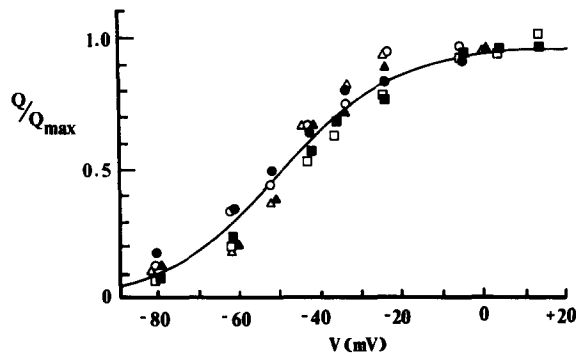


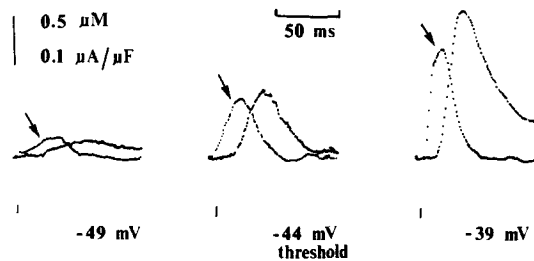
FIGURE 4. Steady state normalized charge-voltage curves in (A) $0 \mu\text{M}$ (open symbols) and (B) $40 \mu\text{M}$ tetracaine (filled symbols) studied in the same fiber for three fibers. The line corresponds to a two-state Boltzmann model fitted to data in the absence of tetracaine. Fiber capacitances: 7.3 ± 1.31 and 7.1 ± 1.22 nF before and after adding tetracaine, respectively.

tetracaine concentration was increased from 100 to $200 \mu\text{M}$, which suggests that a maximal effect was already reached at $100 \mu\text{M}$ tetracaine under the present conditions. The experiments first determined the contractile threshold. Voltage steps were then made to this membrane potential, and to levels 5 mV on either side of it. Tetracaine ($200 \mu\text{M}$) was then added and the procedure was repeated. Charge movements and $[\text{Ca}^{2+}]$ transients were recorded simultaneously. Tetracaine-sensitive charge movement was determined by comparing records before and after tetracaine was added. There was no significant change ($<1.6\%$, $P > 5\%$) in linear capacitances before (7.2 ± 0.84 nF) and after (7.08 ± 0.92 nF) the drug was added. This procedure suggested that 100 and $200 \mu\text{M}$ tetracaine isolated 2.77 ± 0.37 and 2.8 ± 0.98 nC/ μF (mean \pm SE; seven fibers studied) of 'threshold' nonlinear charge, respectively.

Fig. 5 compares tetracaine-sensitive 'on' currents (arrowed traces) with $\Delta[\text{Ca}^{2+}]$ transients. It confirms that tetracaine-sensitive charge exists in cut fibers as suggested in intact fibers (Adrian and Peres, 1979; Huang, 1982; Hui, 1983). This would make it less distinguishable if it was not isolated from the rest of the current in cut fibers by the subtraction procedure used here.

Nevertheless, it showed a 'hump' waveform and a steep voltage sensitivity both in the time to the peak stage (which was altered by 6 ms) and in the relative magnitude, which was comparable with changes in related parameters in $\Delta[\text{Ca}^{2+}]$ signals whose

FIGURE 5. Ca^{2+} signals in the absence of tetracaine compared with tetracaine-sensitive components of charge movements (arrowed) in response to depolarizing steps in 5 -mV increments through the contractile rheobase. Fiber 0318A: diameter, $110 \mu\text{m}$; sarcomere spacing, $2.34 \mu\text{m}$; temperature, 2.8°C ; and antipyrilazo III concentrations, 442 – $580 \mu\text{M}$. Linear capacitances before and after adding tetracaine were 9.89 and 9.79 nF, respectively. Estimated voltage excursion for an e-fold change in peak $\Delta[\text{Ca}^{2+}]$, 5.8 mV; in tetracaine-sensitive charge, 7.3 mV.

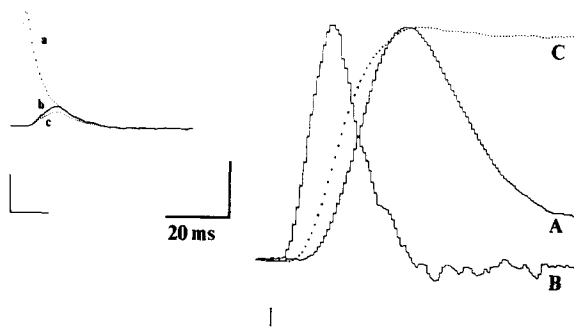


waveform followed that of the charging transient. For example, e-fold factors for tetracaine-sensitive charge and peak $\Delta[\text{Ca}^{2+}]$, determined within 5 mV on either side of the contraction threshold, were estimated at 7.8 ± 4.6 and 4.1 ± 1.9 mV (cf., Maylie et al., 1987), respectively. This voltage dependence was considerably steeper than that for total charge. This suggests that the tetracaine-sensitive charge in cut fibers may be equivalent to the q_γ of intact muscle fibers.

Time Course of Tetracaine-sensitive Charge in Cut Fibers

Finally, closer comparisons of nonlinear charge and $\Delta[\text{Ca}^{2+}]$ signals were made in response to rheobase voltage steps where the kinetics of both would be relatively slow and easy to resolve. Records were the average of 24 optical and electrical traces obtained before the addition of tetracaine, and 36 traces were obtained after the

FIGURE 6. Comparison of (A) the time course of changes in myoplasmic $[\text{Ca}^{2+}]$ with (B) heavily signal-averaged and filtered (see Methods) q_γ transients and (C) corresponding charge transfer. Vertical scale bar represents a charging current of $0.05 \mu\text{A}/\mu\text{F}$ and a total charge of $0.96 \text{ nC}/\mu\text{F}$, or a $\Delta[\text{Ca}^{2+}]$ of $0.22 \mu\text{M}$. Inset (left): comparison of (a) total charge movement in the absence of local anesthetic with (b) maximum tetracaine-sensitive current obtained by scaling (c) transient that was abolished by $200 \mu\text{M}$ tetracaine to the envelope (a). Vertical bar, $0.5 \mu\text{A}/\mu\text{F}$; horizontal bar, 20 ms. Fiber 0320: diameter, $97.5 \mu\text{m}$; sarcomere spacing, $2.36 \mu\text{m}$; temperature, 3.5°C ; antipyrilazo III concentrations, $545 \mu\text{M}$. Linear capacitances at the beginning and end of the procedure were 7.39 and 7.37 nF, respectively. Nonlinear charge in the first and last records in the run taken in the absence of tetracaine was 20.17 and 19.9 nC/ μF , respectively. When tetracaine was added, the first and last values were 17.08 and 17.15 nC/ μF . The changes through each run thus were not $>1.3\%$.



addition of $200 \mu\text{M}$ tetracaine. Such averaging was intercalated every 12 sweeps with sets of 12 control sweeps in response to -30-mV steps from the -100-mV holding potential. The latter checked linear fiber capacitance and its stability through the procedure. This was necessary in view of the long duration of the experiment. Additionally, by averaging sweeps in sets of 12 it was possible to control for any drifts in quantities of nonlinear charge while data were being collected (see the legend of Fig. 6). If transfers of tetracaine-sensitive charge resulted from changes in myoplasmic $[\text{Ca}^{2+}]$, they should lag the antipyrilazo III signal. Fig. 6 compares records of (A) heavily averaged $\Delta[\text{Ca}^{2+}]$ transients with (B) the tetracaine-sensitive portion of the charge movement and (C) its integral. When all traces were normalized to their peak values, $\Delta[\text{Ca}^{2+}]$ signals clearly followed rather than preceded either the nonlinear currents or the charge moved. This confirms an earlier

report (Vergara and Caputo, 1983) and is not consistent with such charge movement being a response to myoplasmic $[Ca^{2+}]$ changes (but see Discussion).

Finally, records of the time course of the different charging currents enabled us to make an estimate of the upper limit of tetracaine-sensitive charge at contractile threshold in addition to the lower limit provided earlier. Fig. 6 (inset) compares (a) total charge movement before adding local anesthetic with (b) maximum possible tetracaine-sensitive charge movement obtained by scaling (c) experimentally obtained transients abolished by 200 μM tetracaine to the envelope represented by a. The scaling factor obtained in six experiments suggested that the maximum tetracaine-sensitive charge would not exceed 1.83 ± 0.39 times the lower limit obtained here.

DISCUSSION

These experiments compared the effects of tetracaine on simultaneously measured charge movements, $\Delta[Ca^{2+}]$ signals, and contractile threshold in cut amphibian skeletal muscle fibers in which antipyrilazo III was introduced into the intracellular space.

Ca^{2+} signals, in particular the early $\Delta[Ca^{2+}]$ peak, were used to indicate changes in Ca^{2+} release. This requires the properties of antipyrilazo III and of 'fast' cytosolic buffering to be not appreciably affected by tetracaine treatment (see Melzer et al., 1986b). The first point has been checked by Vergara and Caputo (1983). We noted here that peak $\Delta[Ca^{2+}]$ associated with barely visible contraction did not alter. This agrees with relationships observed earlier between contraction and either peak $\Delta[Ca^{2+}]$ or the threshold of arsenazo III signals (Kovacs and Szucs, 1983; Miledi et al., 1983). It is consistent with an unchanged distribution between cytosolic-free, troponin-buffered, and other 'fast'-buffered Ca^{2+} at least at a constant end-point of troponin activation. Alternative hypotheses would be considerably more complex and would not be consistent with earlier observations that tetracaine did not alter the pCa-tension relation in skinned fibers (Almers and Best, 1976). It is conceivable that changes in 'slow' Ca^{2+} buffering could influence the optical transients (Melzer et al., 1986a). Decays of $\Delta[Ca^{2+}]$ after the peak were sometimes more prolonged in tetracaine, and this could reflect reductions in Ca^{2+} reuptake (e.g., Fig. 1, also see Bianchi and Bolton, 1967). However, the latter would affect $\Delta[Ca^{2+}]$ in a direction opposite to the net changes observed here and allowing for such phenomena would merely reinforce our present conclusions.

In any event, $\Delta[Ca^{2+}]$ measurements offer a more direct indication of Ca^{2+} release than earlier measurements of tension generation (Almers and Best, 1976). Furthermore, even low tetracaine concentrations (25–40 μM) caused large (44–80%) reductions of $\Delta[Ca^{2+}]$ signals with little effect on charge movements. It would be difficult to disregard such changes in Ca^{2+} release when accounting for such large effects. These findings, therefore, suggest that low tetracaine concentrations could reduce Ca^{2+} release by the sarcoplasmic reticulum, and consequently suppress contraction without altering nonlinear charge. This explains earlier findings by Almers (1976; see Introduction) and the inhibition of caffeine contractures by tetracaine (Luttgau and Oetliker, 1968).

Tetracaine-sensitive charge has been associated with slow transients at threshold

voltages in intact fibers (Adrian and Huang, 1984*a, b*). However, the latter currents are frequently less prominent or indistinguishable in cut fibers (Adrian and Peres, 1979; Melzer et al., 1986*b*). High tetracaine concentrations that approached those used on intact fibers, were toxic to cut fibers (see also Lamb, 1986). Nevertheless, our lower limit for 'threshold' tetracaine-sensitive charge of 2.8 nC/ μ F did not alter appreciably between tetracaine concentrations of 100–200 μ M. The voltage dependence of its time course and relative amounts suggested an equivalence to the ' q_γ ' described in intact fibers. It is therefore possible that discrepancies between cut and intact preparations might reflect quantitative kinetic differences in particular components rather than the presence or absence of entire charge species.

The findings under the higher tetracaine concentrations are consistent with observations in hypertonic solutions where tetracaine abolished a discrete, steeply voltage-dependent (q_γ) charge (Huang, 1981, 1982). We were unable to maintain viable cut (as opposed to intact) fibers in hypertonic solutions. Nevertheless, the finding that tetracaine acts both at Ca^{2+} release and charge transfer sites to degrees that depend differently on concentration, partly reconciles the earlier apparently conflicting findings.

Tetracaine has been used when studying charge movements in mammalian muscle, which were thought not to alter with such treatment (e.g., Dulhunty and Gage, 1983; Dulhunty et al., 1987). However, such results would be difficult to interpret if tetracaine not only abolished Ca^{2+} release but also reduced part of the charge movement. Indeed, reported quantities of maximum charge in mammalian fibers varied by as much as a factor of two depending on the choice of external solution and whether tetracaine was present (see Hollingworth and Marshall, 1981; Hollingworth et al., 1987). A significant number of agents used on charge movements could have more than one site of action. Besides the local anesthetics studied here, the dihydropyridines D600 and nifedipine affect surface or transverse tubular Ca^{2+} channels in addition to excitation-contraction coupling (Hui et al., 1984; Rios and Brum, 1987). Nevertheless, these findings do not preclude the use of tetracaine or other agents that may have more than one site of action in empirical separations of charge movement components, provided that the concentrations used entirely abolish the component being isolated (e.g., Huang, 1981; Hui, 1983).

Finally, the results exclude one possible hypothesis for the origin of the nonlinear charge. It has frequently been suggested in the literature that some of the charge movement, particularly the delayed (q_γ) transients, is the consequence of changes in intracellular [Ca^{2+}] in excitation-contraction coupling (see Horowicz and Schneider, 1981; Huang, 1981; Hui, 1983; Caille et al., 1985; Lamb, 1986; Melzer et al., 1986*b*; Dulhunty et al., 1987). For example, an increase in myoplasmic [Ca^{2+}] could cause surface (Gouy-Chapman) charge neutralization on the cytoplasmic face of transverse tubular membrane through Ca^{2+} binding (Aveyard and Haydon, 1973). This would shift transmembrane field in a direction that would cause additional and delayed charge movement following the time course of free $\Delta[Ca^{2+}]$.

However, there have been relatively few attempts to test this kind of model. Yet, such hypotheses conveniently explain the entire tetracaine-sensitive charge, including delayed transients otherwise difficult to model, simply as secondary to Ca^{2+}

release. They also explain tetracaine actions on both Ca^{2+} release and nonlinear charge through a single action at sarcoplasmic reticular Ca^{2+} release. This would also clarify the effect of tetracaine on caffeine contractures (Luttgau and Oetliker, 1968). However, in our experiments, the $\Delta[\text{Ca}^{2+}]$ signal followed rather than preceded charge transfer. Interpretation of such results must allow for the optical measurements representing an average $\Delta[\text{Ca}^{2+}]$ along the sarcomere, rather than at the site of Ca^{2+} release. However, the additional steady state findings contrasted an 80% reduction in peak $\Delta[\text{Ca}^{2+}]$ with the absence of major effects on charge movements. This was observed even when the lower limit for tetracaine-sensitive charge at contraction threshold was $\sim 2.8 \text{ nC}/\mu\text{F}$, as was determined at high drug concentrations. This greater sensitivity of $\Delta[\text{Ca}^{2+}]$ signals than charge movements to tetracaine, combined with the kinetic results, are clearly contrary to a hypothesis in which intracellular $\Delta[\text{Ca}^{2+}]$ causes charge movement.

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