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EFFECTS OF LOCAL ANAESTHETICS ON THE RELATIONSHIP BETWEEN CHARGE MOVEMENTS AND CONTRACTILE THRESHOLDS IN FROG SKELETAL MUSCLE

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SUMMARY

1. Charge movements to 10 mV steps at different potentials were studied in voltage-clamped frog skeletal muscle fibres in isotonic solutions that minimized ionic currents, under different pharmacological conditions. The earliest onset of detectable mechanical movement ('threshold') was assessed visually under magnification.

2. Charge movements in isotonic solutions were similar to those reported in hypertonic solutions, under identical pulse procedures.

3. In the absence of local anaesthetics, threshold occurred at a mean membrane potential of −55 mV, after the movement of 4·0 nC/μF of non-linear charge and when the membrane capacitance approximated values that corresponded to the onset of the 'Q_γ' charge movement component.

4. Lidocaine shifted the threshold in the hyperpolarizing direction to −62 mV, and reduced the amount of non-linear charge needed to reach threshold to 1·4 nC/μF.

5. The presence of tetracaine shifted threshold in the depolarizing direction to −39 mV, and more than doubled the amount of non-linear charge that had to move to reach threshold to 10·8 nC/μF. Much of this increase was attributed to the Q_γ component of charge movement.

6. It is concluded that more non-linear charge is required to initiate mechanical movement when calcium release by sarcoplasmic reticulum is inhibited by tetracaine, whereas less charge is so required when calcium re-uptake is being inhibited by lidocaine. Assuming earlier interpretations of the strength duration curve, this is consistent with charge movement preceding, rather than being a consequence of calcium release.

INTRODUCTION

It has been suggested that the contractile threshold in skeletal muscle is achieved when a particular amount of non-linear intramembrane charge has moved with depolarization (Schneider & Horowicz, 1979). This paper examines the effect of the local anaesthetics lidocaine and tetracaine on the relationship between charge movements and threshold. Tetracaine was selected because it is thought to inhibit the release of calcium in response to membrane depolarization, without compromising subsequent steps in the activation of muscle (Almers, 1976; Almers & Best, 1976). In contrast, lidocaine is thought to inhibit the re-uptake of calcium by the

sarcoplasmic reticulum, and this would increase its intracellular concentration for any given rate of its steady release. This was on the basis of studies of contractures (Bianchi & Bolton, 1967), of sarcoplasmic reticular preparations (Johnson & Inesi, 1969), and of its effect on the strength–duration curve (Almers, 1977). If charge movement is related to, and is the cause of calcium release, these pharmacological actions would predict that tetracaine would increase the amount of non-linear charge that would have to move in order to attain a particular level of intracellular calcium, whereas lidocaine should decrease it. However, it is at least possible that part of the charge movement may be a consequence of the release of calcium, and that a mechanism of this kind may contribute to the complex nature of the kinetics of charge movements at particular voltages (Adrian & Peres, 1979). One example of a hypothesis of this kind is that the calcium released, possibly by the quasi-exponential part of the charge movement (Chandler, Rakowski & Schneider, 1976), could bind to fixed sites on the inner tubular membrane surface and so alter the transmembrane field and cause an additional, delayed, non-linear transient whose time course may explain the delayed 'hump' (Huang, 1981). However, hypotheses of this second kind would predict that part of the non-linear charge is a consequence of the intracellular calcium level, and that its total amount would therefore assume a fixed magnitude for any given steady-state change in calcium concentration whatever the pharmacological condition. The present experiments attempt to differentiate between these possibilities. They employ the assumption that a fixed, albeit indeterminate, steady-state level of calcium has been attained at the contractile threshold to a long imposed voltage step. Such an interpretation has been used in earlier work in the analysis of the rheobase of strength–duration curves of muscle (Adrian, Chandler & Hodgkin, 1969*a*; Almers, 1978).

METHODS

Frog sartorius (*Rana temporaria*) muscle fibres were mounted in a bath and stretched to around 1·2 times their slack length. A three-electrode voltage clamp was imposed at the fibre pelvic end. The experimental apparatus has been fully described elsewhere (Adrian & Rakowski, 1978) and so only an outline of the methods used is given below. The micro-electrodes were inserted at $l = 500\,\mu$m (V_1), $2l = 1000\,\mu$m (V_2) and $1250\,\mu$m (I_0) from the end of the fibre respectively, and the clamped potential was at V_1. Experiments were done at 4–6 °C in the following isotonic solution: Rb_2SO_4, 5 mM; $(TEA)_2SO_4$, 80 mM; (TEA)Cl, 15 mM; $CaSO_4$, 8 mM; TTX, 2×10^{-7} M, Tris buffer 3 mM. The fibres were therefore able to contract. Pharmacological studes were done in the presence of 7 mM-lidocaine *or* 1 mM-tetracaine, at neutral pH; solution pH was checked both before and after each experiment with a PHM 62 pH meter (Radiometer, Copenhagen) at the temperature at which the experiments were conducted. Capacitative currents were measured in response to 10 mV steps superimposed 300 msec after the clamped potential was prepulsed to a voltage V'_T from a -90 mV holding potential; the effective test voltage V_T about which the step was taken is thus $V'_T + 5$ mV. The particular pulse programme used enabled results obtained in the isotonic solutions to be compared with earlier results in hypertonic solutions (Adrian & Peres, 1979). The currents obtained were displayed as admittances of unit surface membrane area

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

Membrane capacitance was measured as the integral of the transient part of $Y(t)$ at the beginning and end of each voltage step. Cable constants λ, r_i and r_m were calculated from the steady values of $V_1(t)$, $V_2(t)$ and of the injected current I_0 at the end of a 10 mV step from the holding potential. The fibre diameter, d, and specific membrane constants R_m and C_m were then calculated employing

a value of the sarcoplasmic resistivity R_i of 250 Ω cm in isotonic solution at 2 °C and with a Q_{10} of 1·37 (Hodgkin & Nakajima, 1972).

Arrays of $V_1(t)$, $V_2(t) - V_1(t)$ and $I_0(t)$ obtained by analogue-to-digital conversion were obtained by sampling the transients at an interval of 200 μsec per point, through a sampling window of 1080 points, which included on and off parts of the 10 mV step, each of duration 105 msec. Successive sweeps were separated by an interval of 1 sec, and for each voltage examined five or six sweeps were averaged for filing on an RKO5 disk. A series of control sweeps was taken every two to three test sweeps to assess fibre condition and stability. During the course of an experiment, fibres were observed visually for the onset of a just perceptible mechanical movement in order to detect the 'threshold'.

TABLE 1. Fibre cable constants. Diameters were calculated using values for the sarcoplasmic resistivity R_i from Hodgkin & Nakajima (1972). The mean values, with their standard errors, are shown with the results of t testing against the control results obtained in the presence of local anaesthetic, for significant discrepancies.

	Controls (four fibres)
Length constant	4·0 ± 0·35 mm
Diameter	89·3 ± 8·42 μm
Capacitance	6·7 ± 1·53 μF/cm²
	Lidocaine-treated fibres (eight fibres)
Length constant	4·0 ± 0·51 mm ($t = 0·73$; $P > 5\%$)
Diameter	86·9 ± 8·88 μm ($t = 0·16$, $P > 5\%$)
Capacitance	8·0 ± 1·04 μF/cm² ($t = 0·66$, $P > 5\%$)
	Tetracaine-treated fibres (six fibres)
Length constant	4·9 ± 0·36 mm ($t = 1·45$, $P > 5\%$)
Diameter	91·8 ± 8·53 μm ($t = 0·18$, $P > 5\%$)
Capacitance	7·8 ± 0·61 μF/cm² ($t = 0·67$, $P > 5\%$).

RESULTS

Different fibres were studied for each pharmacological condition: it was not practicable to examine the effect of different agents on the same fibre as each experiment was usually terminated by mechanical movement. However, the following measures were taken to ensure that the effects reported are not merely the result of cable effects reflecting the different sets of fibres used. (1) Capacitances were normalized to the control capacitance at a reference voltage of −85 mV, and so are expressed as a ratio of test to control capacitance, C_T/C_C; estimates of charge, Q, were similarly normalized. (2) Length constants, fibre diameters and control capacitances computed as described in the Methods were not significantly different from the controls ($P > 5\%$) in the presence of either lidocaine or tetracaine (see Table 1). The following nomenclature (Adrian & Peres, 1979) will be used below when describing results: Q_β is the monotonically declining phase of the charge movement; Q_γ is the 'hump' which occurs at particular voltages, and Q_α is the component that persists on prolonged depolarization to −20 mV. The effects to be described concern primarily the first two components, since there is only a small contribution by Q_α, whose broad voltage-dependence makes it unlikely to have any direct bearing on contractile activation. Thus: (1) lidocaine and tetracaine did not have a significant effect on the dependence of Q_α on potential. Thus, when hyperpolarized voltages between the potentials of −85 and −145 mV were examined, the membrane capacitance declined from −85 mV, with slopes of 0·15, 0·19 and 0·18 per mV in

controls, lidocaine-treated and tetracaine-treated fibres respectively, and analysis of the variance of these slopes indicated that they were *not* significantly different from each other ($P > 5\%$); it is therefore unlikely that these agents greatly affect the amount of Q_α charge that is known to be present in control steps at -85 mV (see Adrian & Almers, 1976); (2) by inactivating the Q_β and Q_γ components by prolonged depolarization to -20 mV, it was possible to find the contribution of the non-linear charge that could be attributed to Q_α that had occurred over the voltage range examined in experiments below. This was not more than 1–2 nC/μF, which is a small proportion of the differences that were found. Therefore the effects described below concern only the Q_β and Q_γ components of the charge movement.

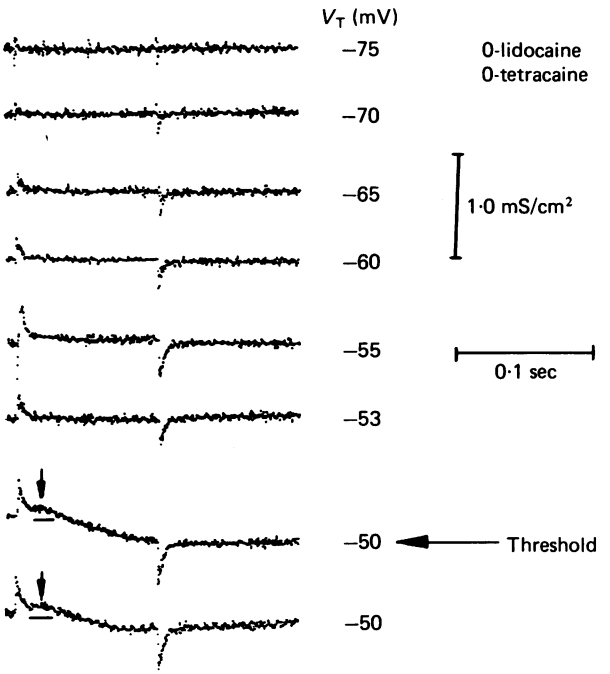

Fig. 1. Charge movements to 10 mV steps in isotonic solutions in the absence of local anaesthetic. Threshold occurs at those capacitances and voltages close to the appearance of Q_γ.

Charge movements versus the onset of mechanical movement

The fibres were subjected to a series of test voltages depolarized to the holding potential of -90 mV, and upon this test voltage was superimposed a 10 mV depolarizing step of duration 105 msec, which was made 300 msec after the beginning of the main pulse. Transients in response to the small step were sampled, and test voltages were increased in small 2 or 3 mV steps as threshold voltages were approached. This made it possible to follow closely changes in charge movements and membrane capacitance, as well as to obtain accurately the voltage at which threshold occurred. At the same time the fibre studied was observed at a $\times 100$ magnification for the onset of just visible mechanical movement. The latter procedure is identical to the one used

earlier by Adrian *et al.* (1969*a*), in which the results obtained were in close agreement with those derived from the use of more refined techniques employing polarized light and cinephotography in determining the onset of contractile activation (Adrian, Costantin & Peachey, 1969*b*; Costantin, 1974). Furthermore, whereas the earlier experiments included pulse lengths of very short duration, the present experiments assessed the onset of mechanical movement from a *constant*, and a *long* pulse length, at which such visual titrations would be at their most precise. Thus this method of

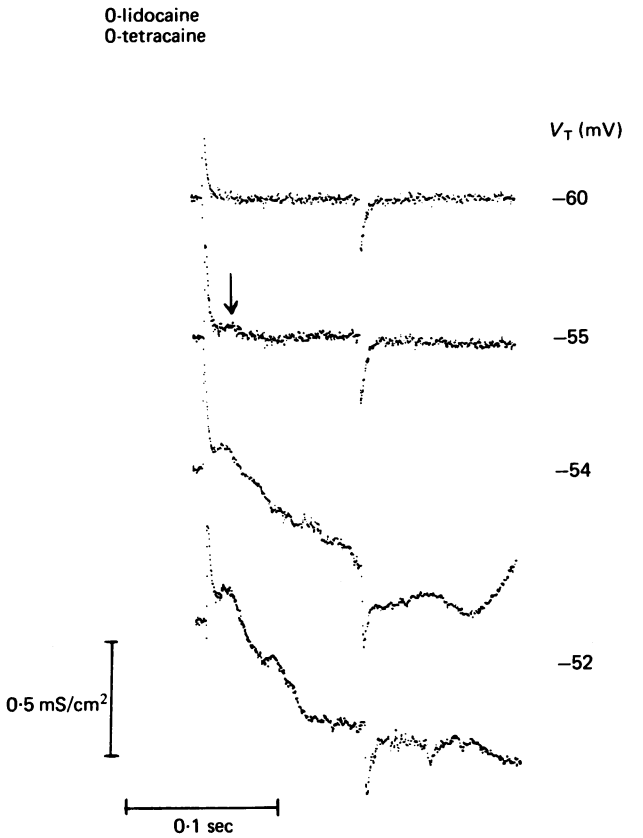

Fig. 2. Muscle fibre partly stretched to 1·4 × slack length to postpone the onset of contractile artifact (which occurs in the traces at −54 and −52 mV) to show a trace containing Q_γ free of such artifacts (at −55 mV).

determination of the threshold gave results that were repeatable to within 1–2 mV. As the length of pulse is long, the 'threshold' so determined by this approach corresponds to the rheobase of the strength–duration relationship of the contractile activation of muscle. At this voltage, the steady-state rate of intracellular release of calcium is at equilibrium with its re-uptake into the sarcoplasmic reticulum, and the resulting intracellular concentration of calcium ions is therefore at that level just sufficient to activate the contractile apparatus (Adrian *et al.* 1969*a*; Almers, 1977, 1978).

Capacitative transients to the 10 mV steps were recorded at the same time, from which control transients obtained from similar steps of about −85 mV were subtracted to compute the charge movements in the *isotonic* solutions used. The transients are measured in units of admittances of unit surface membrane area. In the absence of local anaesthetics, charge movements resembled those reported earlier in *hypertonic* media using the same pulse structure (Adrian & Peres, 1979), over the potentials where study in isotonic solutions was possible. Thus, with depolarization from the control voltage, the quasi-exponential Q_β appears first (Fig. 1), and with stronger depolarization the Q_γ 'hump' appears in the 'on' part of the response,

TABLE 2. Effect of local anaesthetics on the relationship between threshold and charge movements. Mean results are shown with their standard errors. The *t* tests were performed on the effects of each drug against the control results, and are shown in parentheses

	Number of fibres	Threshold voltage (mV)	Capacitance ratio C_T/C_C at threshold	Non-linear charge that had moved at threshold (nC/μF)
Controls	4	$-55 \pm 1{\cdot}7$	$1{\cdot}28 \pm 0{\cdot}10$	$4{\cdot}0 \pm 0{\cdot}71$
Lidocaine, 7 mM	7	$-62{\cdot}3 \pm 0{\cdot}82$ ($t = 4{\cdot}0$, $P < 0{\cdot}1\%$)	$1{\cdot}16 \pm 0{\cdot}04$ ($t = 1{\cdot}1$, $P > 5\%$)	$1{\cdot}4 \pm 0{\cdot}24$ ($t = 3{\cdot}51$, $P < 1\%$)
Tetracaine, 1 mM	6	$-39{\cdot}3 \pm 1{\cdot}43$ ($t = 6{\cdot}3$, $P < 0{\cdot}1\%$)	$1{\cdot}63 \pm 0{\cdot}07$ ($t = 2{\cdot}6$, $P < 5\%$)	$10{\cdot}8 \pm 1{\cdot}04$ ($t = 4{\cdot}4$, $P < 1\%$)

although the 'off' response remains monotonic but larger. The appearance of Q_γ coincided with voltages at which mechanical movement was just detectable, and this produced a movement artifact in the traces which made their interpretation difficult. However, fibres could be stretched to 1·4 times their slack length to postpone the onset of movement, and the Q_γ transients could then be demonstrated before the electrical traces suffered such mechanical artifact (Fig. 2). Membrane capacitances could be obtained from the traces for voltages up to, but excluding threshold, and were normalized to the control capacitance at −90 mV, to give a test/control capacitance ratio C_T/C_C, as defined above. The mean values (±S.E. of the mean) of the threshold voltages, capacitance at threshold, and computed non-linear charge that had to be moved to reach threshold (see below), are summarized in Table 2, which also gives the results of t-testing against the control results to assess for the statistical significance of the observed effect of the local anaesthetics used below. In the absence of local anaesthetics, the mean threshold voltage was −55 mV, and at this voltage, the capacitance was 1·28 times its value at the control voltage. It has been shown in earlier work (Huang, 1981), that the Q_γ component of the charge movement appears when the membrane capacitance is close to such values; thus, Q_γ may appear at voltages close to threshold.

Adding 7 mM-lidocaine to the bathing solution shifted the observed threshold to more hyperpolarized voltages, so the onset of a just perceptible movement occurred at potentials when the charge movement was much smaller. This is shown in Fig. 3. In the presence of lidocaine, mean threshold was at −62·3 mV, and at this voltage the capacitance was only 1·16 times its control value. Fig. 4 shows the contrasting effect in the presence of 1 mM-tetracaine. Even though there was a distinct Q_γ component when the membrane potential was at −40 mV, contraction did not occur

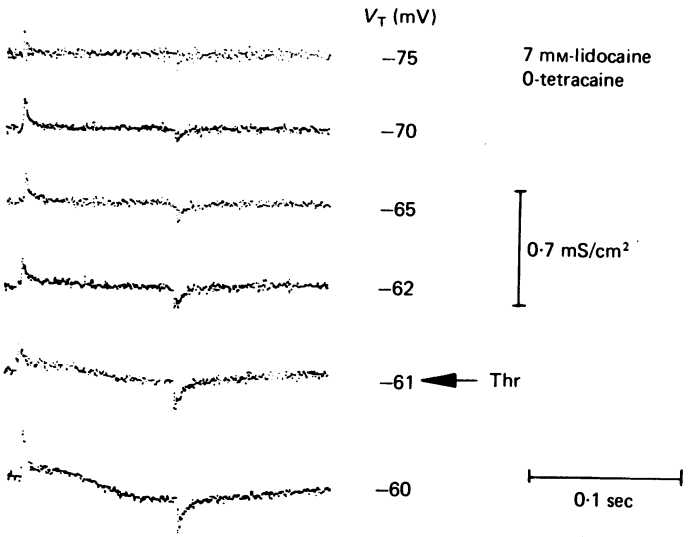

Fig. 3. 'Threshold' occurred at more hyperpolarized voltages in the presence of 7 mM-lidocaine at which charge movement transients were smaller.

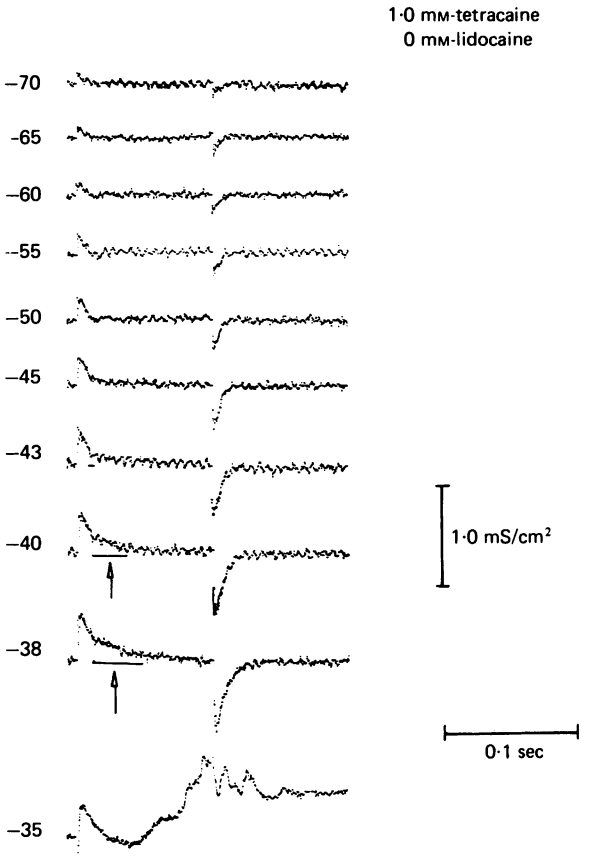

Fig. 4. Charge movements in *isotonic* solutions containing 1 mM-tetracaine. At −43 mV, the capacitance increases sharply in this particular fibre; Q_γ was visible at −40 mV, but threshold did not occur until −35 mV, at which potential a substantial amount of Q_γ was in evidence.

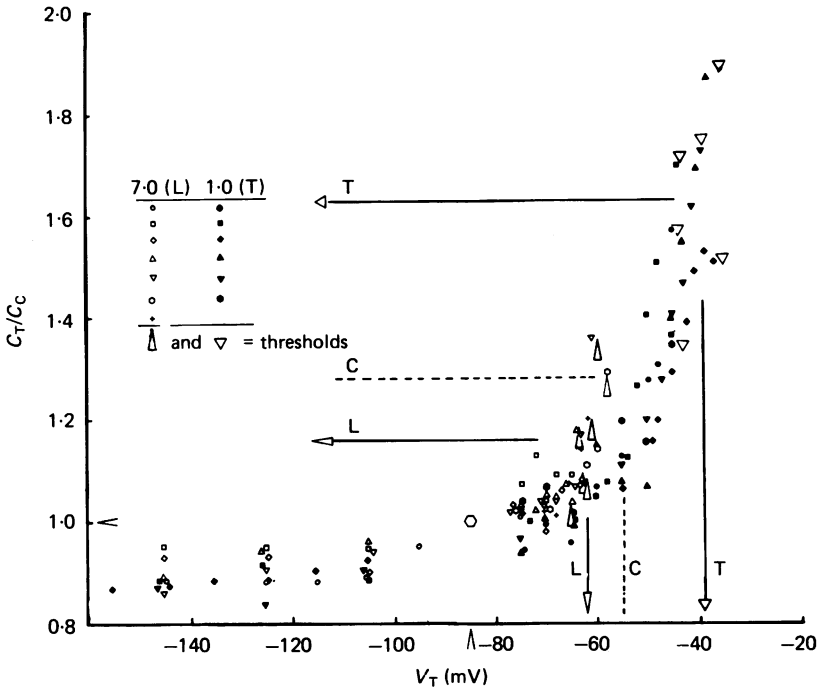

Fig. 5. Pooled fibres: capacitances in lidocaine (open symbols) and in tetracaine (filled symbols), with the threshold points (large triangles) of the fibres studied. Mean threshold is hyperpolarized relative to the control (C) in lidocaine (L), but shifted in the depolarizing direction in tetracaine (T), and that corresponding capacitances at threshold are substantially greater in tetracaine.

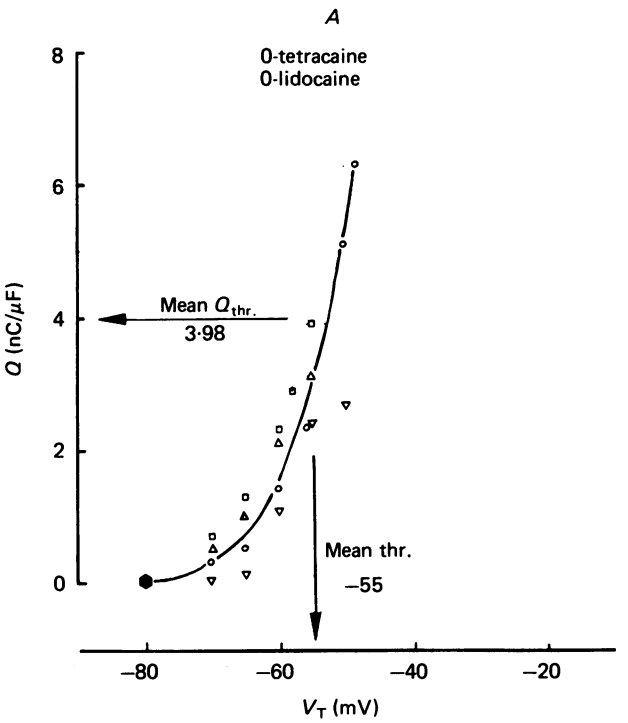

Fig. 6.

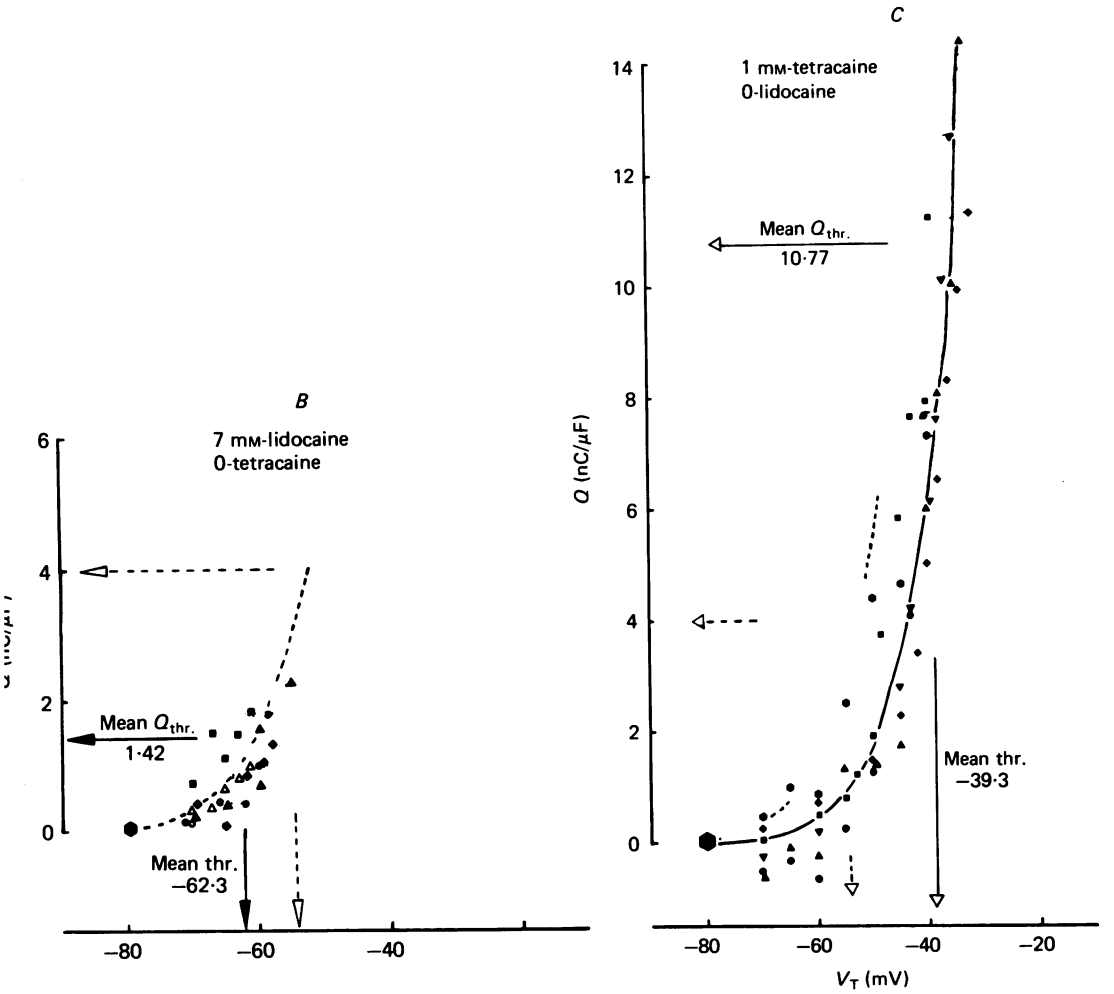

Fig. 6. Derived charge–voltage curves in isotonic solutions in: *A*, controls, *B*, lidocaine and *C*, tetracaine. Lidocaine decreases the non-linear charge needed to attain threshold (thr.); tetracaine increases it. Each symbol represents a different muscle fibre, under the conditions described.

until the membrane was subjected to a 10 mV step at about −35 mV. Thus, for the six fibres studied, the mean threshold was significantly shifted in the *depolarizing* direction to −39·3 mV, and the onset of just perceptible mechanical movement did not occur until the capacitance was at least 1·63 times the control value at −90 mV (Fig. 5). Values of capacitance of this size are consistent with the presence of a substantial amount of Q_γ charge in the non-linear transients (see Huang, 1981; Adrian & Peres, 1979). Thus, the presence of tetracaine significantly increases the amount of Q_γ that has to be present in capacitative transients that occur at voltages where the fibre is sufficiently depolarized just to contract, as compared to the controls where the tetracaine is absent.

Since the capacitance ratio C_T/C_C is an indication of the *slope* of the dependence

of non-linear charge upon voltage, integrating the capacitances with respect to potential would enable the amount of non-linear charge that had to move to reach threshold to be calculated. The results of such a numerical integration for the three cases examined are summarized in Fig. 6*A–C*. When local anaesthetics were absent the mean amount of non-linear charge that had to move to reach rheobase was 4·0 nC/μF. Adding lidocaine halved this amount to 1·4 nC/μF. Adding tetracaine instead of lidocaine more than doubled the required charge to 10·8 nC/μF, and (as indicated above) the values of capacitance that were obtained at the threshold voltage suggested a substantial amount of Q_γ charge as contributing to this increase.

DISCUSSION

The experiments suggest that the charge movement in muscle fibres in isotonic solutions agrees with those obtained in earlier experiments that used a similar procedure of small voltage steps performed in hypertonic solutions (Adrian & Peres, 1979). Although in isotonic solutions it was not possible to study charge movements over a wide range of voltages owing to the onset of mechanical movement, it was possible to study the relationship of charge movements to the onset of contraction. The experiments described simultaneously measured charge movements to small 10 mV steps of long duration superimposed upon different test voltages, and assessed the voltage at which a just perceptible mechanical movement ocurred. This enabled the amount of charge that had moved at the rheobasic voltage to be calculated. One possible interpretation of the effects of adding local anaesthetics on this relationship might assume the following results.

(1) Tetracaine inhibits the release of calcium ions from the sarcoplasmic reticulum, but does not affect the relationship between the concentration of calcium and the resulting steady-state contractile activation (Almers, 1976; Almers & Best, 1976). Lidocaine, however, inhibits the uptake of calcium by the sarcoplasmic reticulum and therefore, for any particular rate of release of calcium, would be expected to increase the steady-state sarcoplasmic calcium concentration (Almers, 1977; Bianchi & Bolton, 1967; Johnson & Inesi, 1969).

(2) The rheobase as measured in the present experiments corresponds to that applied voltage at which the steady-state release of calcium is at a rate that just equals that of its re-uptake by the sarcoplasmic reticulum, and the resulting intracellular concentration of calcium is at a constant level just sufficient to cause detectable contractile activation (see Almers, 1978; Adrian *et al.* 1969*a*). Thus it is a reflexion of a particular steady-state calcium concentration that is the result of the two opposing processes. Hence pharmacological agents that affect either of these processes should alter the rheobase.

The results show that, in order to reach the rheobase, less charge need move in the presence of lidocaine, where the inhibition of calcium uptake that is produced would be expected to enhance the level of calcium for any steady rate of its release. In contrast, more charge has to move to reach the rheobase when calcium release is inhibited by the addition of tetracaine. Both examination of the transients obtained, and the values of the capacitance at the observed rheobase potential, showed that changes in the amount of Q_γ made a substantial contribution to this

effect. Thus, Q_γ was present in substantially greater amounts in transients obtained in tetracaine, as compared to the controls, when the membranes were at potentials about which a superimposed 10 mV step just elicited detectable mechanical movement. If the rheobase corresponds to a fixed, steady-state level of calcium in the conditions tested, this is not consistent with the Q_γ component being a consequence of a particular steady level of calcium having been achieved in the sarcoplasm. Rather, it suggests that the change in intracellular calcium is a consequence rather than a cause of this charge movement.

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