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THE EFFECTS OF TETRACAINE ON CHARGE MOVEMENT IN FAST TWITCH RAT SKELETAL MUSCLE FIBRES

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SUMMARY

1. The effects of tetracaine, a local anaesthetic that inhibits muscle contraction, on membrane potential and intramembrane charge movements were investigated in fast twitch rat muscle fibres (extensor digitorum longus).

2. The resting membrane potentials of surface fibres from muscles bathed in isotonic Ringer solution containing 2 mM-tetracaine were well maintained, but higher concentrations of tetracaine caused a time-dependent fall of potential. Muscle fibres bathed in hypertonic solutions containing 2 mM-tetracaine were rapidly depolarized. In both isotonic and hypertonic solutions, the depolarizing effect of tetracaine could not be reversed.

3. Charge movement measurements were made using the middle-of-the-fibre voltage clamp technique. The voltage dependence of charge movements measured in cold isotonic solutions was well fitted by a Boltzmann distribution ($Q(V) = Q_{max}/(1+\exp(-(V-\bar{V})/k))$ where $Q_{max} = 37{\cdot}3 \pm 2{\cdot}8$ nC μF^{-1}, $\bar{V} = -17{\cdot}9 \pm 1{\cdot}2$ mV and $k = 12{\cdot}6 \pm 0{\cdot}8$ mV ($n = 6$, 2 °C; means ± S.E. of means). Similar values were obtained when 2 mM-tetracaine was added to the isotonic bathing fluid ($Q_{max} = 40{\cdot}6 \pm 2{\cdot}3$ nC μF^{-1}, $V = -14{\cdot}1 \pm 1{\cdot}3$ mV, $k = 15{\cdot}3 \pm 0{\cdot}8$ mV; $n = 8$, 2 °C).

4. Charge movements measured around mechanical threshold in muscle fibres bathed in hypertonic solutions were reduced when 2 mM-tetracaine was added to the bathing fluid. The tetracaine-sensitive component of charge was well fitted with an unconstrained Boltzmann distribution which gave: $Q_{max} = 7{\cdot}5$ nC μF^{-1}, $\bar{V} = -46{\cdot}5$ mV, $k = 5{\cdot}5$ mV. The e-fold rise of the foot of the curve was 9·3 mV.

INTRODUCTION

One action of the local anaesthetic tetracaine is to inhibit the contraction of amphibian and mammalian skeletal muscle fibres (Lüttgau & Ötliker, 1968; Almers & Best, 1976; Dulhunty & Gage, 1983; Lamb, 1986; Csernoch, Huang, Szucs & Kovacs, 1988), most probably by inhibiting the release of calcium ions from the sarcoplasmic reticulum (SR) (Almers & Best, 1976; Csernoch *et al.* 1988). Calcium release from the SR is controlled by the transverse-tubular (T-tubular) membrane potential and the majority of the intramembrane charge movement (Schneider & Chandler, 1973; see Huang, 1989 for a review) which can be measured in skeletal

muscle fibres is thought to arise from the T-tubular voltage sensors of the calcium release process. Hollingworth & Marshall (1981) and Dulhunty & Gage (1983) studied charge movements in intact fast and slow twitch rat muscle fibres and, while their results are in broad agreement, Dulhunty & Gage (1983) measured only about one-half of the charge reported by Hollingworth & Marshall (1981). One possible explanation for this difference is that the 2 mM-tetracaine used by Dulhunty & Gage (1983) to prevent fibre movement had blocked a component of mammalian charge.

Some support for this comes from experiments on intact frog muscle fibres where, although at first tetracaine was considered not to affect charge movement (Almers & Best, 1976), recent studies have revealed a tetracaine-sensitive charge component (Huang, 1982, 1986; Hui, 1983). This tetracaine sensitive component is thought to be Q_γ, the component of frog charge that was first identified by Adrian & Peres (1979) from the complex kinetics of charge movement close to mechanical threshold. The importance of Q_γ in frog muscle is that it has a steep voltage dependence near mechanical threshold (k_γ 3–4 mV; Hui, 1983; Hui & Chandler, 1988). This steepness is similar to that of calcium-related optical signals in frog muscle (e-fold for 2–4 mV; Baylor, Chandler & Marshall, 1979, 1983; Miledi, Nakajima, Parker & Takahashi, 1981; Maylie, Irving, Sizto & Chandler, 1987) and Q_γ may thus be the component of charge directly linked to SR calcium channel opening.

Charge movements in intact mammalian fibres do not have the complex kinetics shown by frog fibres (Hollingworth & Marshall, 1981), suggesting that a Q_γ component of charge may not be present. If Q_γ is indeed absent in mammalian muscle, then tetracaine might not have an effect on mammalian charge movement. We have investigated, therefore, the effects of tetracaine on mammalian fast twitch charge movement. In isotonic solution tetracaine (2 mM) has no effect on charge movement, but in hypertonic solution it reduces the charge. The voltage dependence of the tetracaine-sensitive component in hypertonic solution is centred between -40 and -45 mV but is less steep than that reported for Q_γ in frog muscle. The conditions under which charge is blocked by tetracaine also cause a fall in resting membrane potential.

METHODS

Experiments were carried out on isolated extensor digitorium longus (EDL) muscles dissected from male and female Wistar rats that were at least 12 weeks old (weight range 210–240 g). Animals were killed by cervical dislocation and muscles were kept moist during dissection with oxygenated Ringer solution that contained (mM): NaCl, 150·8; KCl, 4·0; $CaCl_2$, 2·0; $MgCl_2$, 1·0; glucose, 11·0; HEPES buffer, 2·0. The pH was set to 7·4. Once in the experimental chamber, the muscles were stretched to 10–15% of their resting length to reduce muscle contractions and facilitate microelectrode penetration.

Tetracaine was added to the bathing solution just before use and the pH readjusted to 7·4. When changing solutions, the chamber was washed through at least three times with the new solution. Charge movement solutions were designed to eliminate ionic currents and contained (mM): tetraethylammonium chloride, 150·8; RbCl, 4·0; $CaCl_2$, 2·0; $MgCl_2$, 1·0; glucose, 11·0; HEPES buffer, 2·0. The pH was set at 7·4 and 7 μM-tetrodotoxin was added to block any residual sodium currents. Solutions were made hypertonic by the addition of 350 mM-sucrose. All charge movement experiments were carried out at 2 °C.

Electrophysiological methods

The use of the middle of the fibre voltage clamp technique (Adrian & Marshall, 1977) to measure charge movements has been described previously in some detail (Hollingworth & Marshall, 1981; Hollingworth, Marshall & Robson, 1984; Baylor, Hollingworth & Marshall, 1989).

The membrane current at time t, $i_m(t)$, is calculated from

$$i_m(t) = (1/l) \times [I_o(t)/2 - \Delta V(t)/(r_i l)], \quad (1)$$

where I_o is the current delivered to a fibre from an electrode placed as close as possible to the centre of the fibre and $\Delta V(t)$ is the voltage difference between two voltage-recording electrodes V_1 and V_2. The electrode V_1 is placed at a measured distance, $l/2$, from the I_o electrode and V_2 is inserted at a distance l ($\leqslant 500\ \mu$m) from the V_1 electrode. The longitudinal resistance, per unit length of fibre, is r_i.

Values of space constant, λ, r_i and r_m ($1/g_m$), the membrane resistance per unit length of fibre, were calculated, according to the theory of Adrian & Marshall (1977), from the response to +10 mV control depolarizations from the holding potential (−90 mV). The membrane capacity, C_{eff}, is obtained from the integral of the transient part of the membrane current (Adrian & Almers, 1976):

$$C_{eff} = 1/\Delta V_1(\infty) \int_o^\infty (\Delta i_m(t) - g_m \Delta V_1(t))\, dt, \quad (2),$$

where V_1 (∞) is the final value of V_1 (t), the clamped voltage at the V_1 microelectrode. The symbol Δ denotes changes with respect to the values at the holding potential. Fibre diameter was calculated from r_i assuming a value for the specific resistance of the sarcoplasm, R_i (Adrian & Marshall, 1977).

At least two control depolarizations preceded each depolarization to a test potential, and the state of the fibre, as judged from the calculated linear cable properties, was thus monitored throughout the course of an experiment. The linear component of the membrane current response to a test depolarization was estimated and corrected for by appropriately scaling and subtracting the membrane current response to the control depolarizations (Hollingworth & Marshall, 1981). The resulting non-linear membrane currents were baseline corrected for residual non-linearities in membrane conductance by fitting the decaying charge transient with a single exponential plus constant offset and sloping baseline and subtracting the offset and baseline components.

RESULTS

Passive properties

The effect of 2 mM-tetracaine on linear fibre constants in muscles bathed in isotonic and hypertonic charge movement solution is shown in Table 1. Most constants were unaffected, but there is a suggestion that 2 mM-tetracaine may have reduced the muscle fibre membrane resistance. In muscle fibres bathed in hypertonic solution containing 2 mM-tetracaine there appeared to be a fall in the resting membrane potential (RMP). The effects of tetracaine on the RMP were therefore further investigated.

Figure 1*A* shows that in isotonic Ringer solution (20 °C) 2 mM-tetracaine had little effect on the RMP which was well maintained for at least 100 min, but that higher concentrations of tetracaine in isotonic solution caused a time-dependent fall in RMP. In contrast, in hypertonic Ringer solution at 20 °C and in hypertonic charge movement solution at 2 °C (Fig. 1*B*), 2 mM-tetracaine depolarized the RMP and 4 mM-tetracaine caused a more rapid depolarization than 4 mM-tetracaine in isotonic solutions.

TABLE 1. Mean values of constants in charge movement solution

Solution	Temperature (°C)	n	Membrane potential (mV)	λ (mm)	R_i (Ω cm)	R_m (kΩ cm^2)	Diameter (μm)	Effective capacitance (μF cm^{-2})
Isotonic	1·3±0·9	13	−76·2±1·03	1·21±0·08	305·3±2·4	3·49±0·41	51·9±0·13	4·69±0·31
Isotonic + 2 mM-tetracaine	2·2±0·2	8	−77·0±1·7	1·03±0·03	296·2±2·1	2·67±0·20	49·5±1·78	6·48±0·54
Hypertonic	1·5±0·1	32	−82·7±1·1	0·76±0·02	406·0±1·3	1·76±0·07	55·2±1·40	4·43±0·23
Hypertonic + 2 mM-tetracaine	1·5±0·2	15	−68·7±1.0	0·72±0·02	405·8±2·6	1·44±0·08	60·6±1·80	4·80±0·21

Means±S.E. of means.

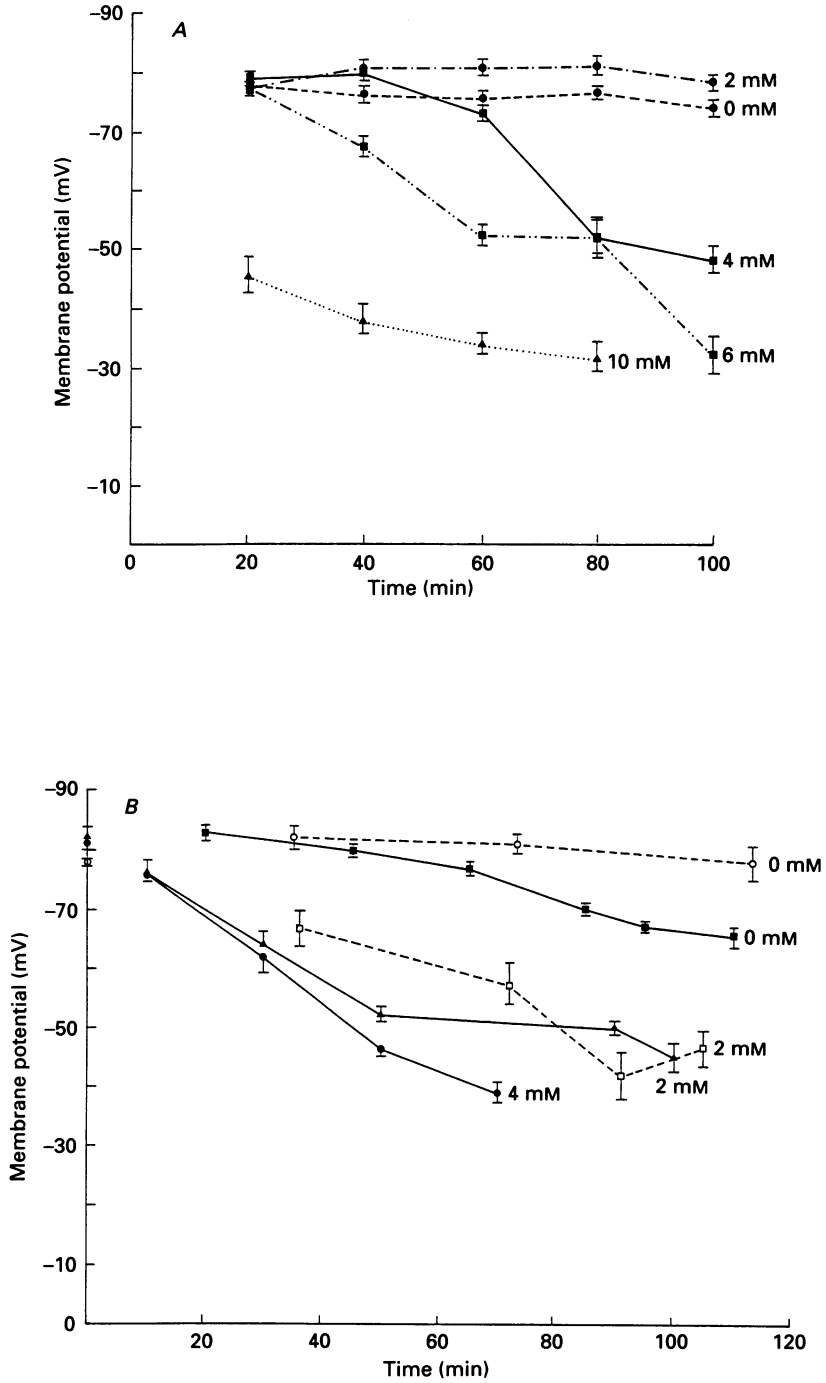

Fig. 1. The effect of tetracaine on the resting membrane potential of EDL muscles. *A*, isotonic Ringer solution, 20 °C. *B*, hypertonic solutions: open symbols, charge movement solution, 2 °C; filled symbols, Ringer solution, 20 °C. The different concentrations of tetracaine are shown alongside each curve. Means ± S.E. of means of ten or more observations.

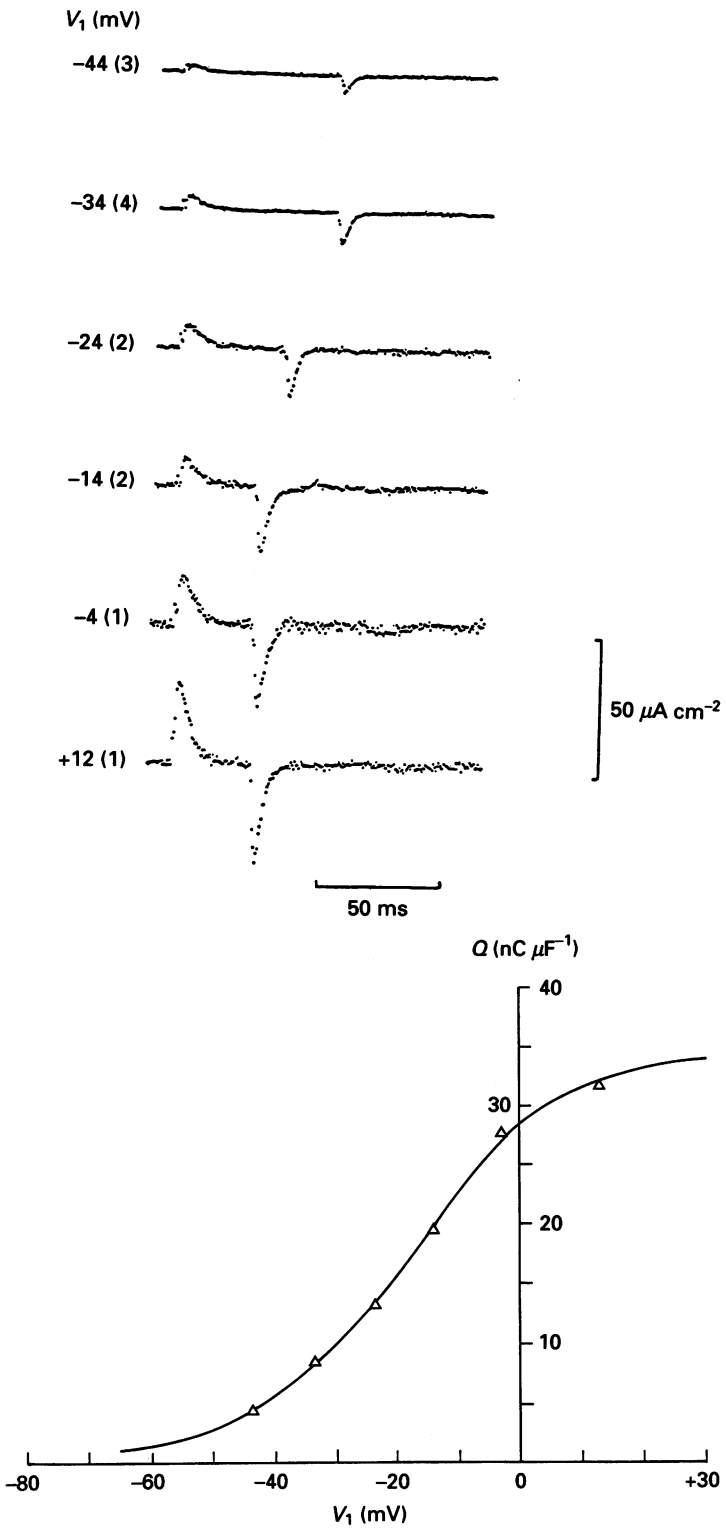

Fig. 2. For legend see facing page.

Results very similar to those in Fig. 1 were also obtained in frog sartorius muscles (*Rana temporaria*). Frog fibres were not depolarized by 2 mM-tetracaine in isotonic solution. This result is probably consistent with those of Lüttgau & Ötliker (1968) who found that 1·0 and 1·5 mM-tetracaine caused a small (5–10 mV) hyperpolarization of frog muscle fibres bathed in isotonic solutions (high potassium (40 mM); constant $[K^+]$ $[Cl^-]$ product). Frog fibres were, however, depolarized by higher concentrations of tetracaine in isotonic solution and by 2 mM-tetracaine in hypertonic solution.

In both isotonic and hypertonic solutions the tetracaine-induced fall in RMP could not be reversed when tetracaine was removed from the bathing solution. Possible changes in membrane conductance underlying the tetracaine-induced depolarization were not further investigated.

Charge movements

Hollingworth, Marshall & Robson (1987) showed that 2 mM-tetracaine in cold, isotonic solutions had no effect on the amounts of charge moved around mechanical threshold, but they did not explore more depolarized levels of membrane potential since in isotonic solutions such experiments are difficult to perform. Although contractions are greatly reduced when mammalian muscle fibres are cooled to 2 °C (Stephenson & Williams, 1980; Hollingworth & Marshall, 1981; Hollingworth *et al.* 1984), fibres can still move and damage themselves on the inserted microelectrodes when voltage clamp pulses are taken beyond mechanical threshold. However, by using brief voltage clamp pulses, in a number of fibres it was possible to obtain charge movements over a broad range of membrane potentials.

Figure 2*A* shows charge movements measured in a fibre bathed in cold, isotonic solution without tetracaine and Fig. 2*B* shows the amount of charge move plotted as a function of membrane potential ($Q(V)$). The smooth curve in Fig. 2*B* is an unconstrained least-squares best fit to the data assuming a Boltzmann distribution for charge between two locations ($Q(V) = Q_{max}/(1+\exp(-(V-\bar{V})/k))$; Schneider & Chandler, 1973). The mean value for the saturating amount of charge (Q_{max}) estimated from $Q(V)$ fits in this and five other individually analysed fibres at 2 °C was $37{\cdot}3 \pm 2{\cdot}8$ nC μF^{-1}. The mean value of the membrane potential at which charge was equally located between two locations ($\bar{V}$) was $-17{\cdot}9 \pm 1{\cdot}2$ mV, and the mean value for k, a steepness factor, was $12{\cdot}6 \pm 0{\cdot}8$ mV (means ± S.E. of means). Eight muscle fibres bathed in isotonic solutions at 2 °C containing 2 mM-tetracaine gave

Fig. 2. Charge movements obtained from a fibre bathed in isotonic charge movement solution at 2 °C. *A*, records of charge movement obtained with voltage clamp depolarizations to the membrane potential indicated beside each trace. Briefer voltage clamp pulses were used at more depolarized potentials. The number of averages in each sweep is shown in parentheses. *B*, the averages of the 'on' and 'off' amounts of charge from the fibre shown in Fig. 2*A* plotted as a function of membrane potential during the voltage clamp depolarization. There was good equality between 'on' and 'off' charge with a maximum difference of 5 nC μF^{-1} at −4 mV. The smooth line is the best fit of an unconstrained Boltzmann distribution which gave $Q_{max} = 35{\cdot}4$ nC μF^{-1}, $\bar{V} = -17{\cdot}2$ mV, $k = 12{\cdot}8$ mV.

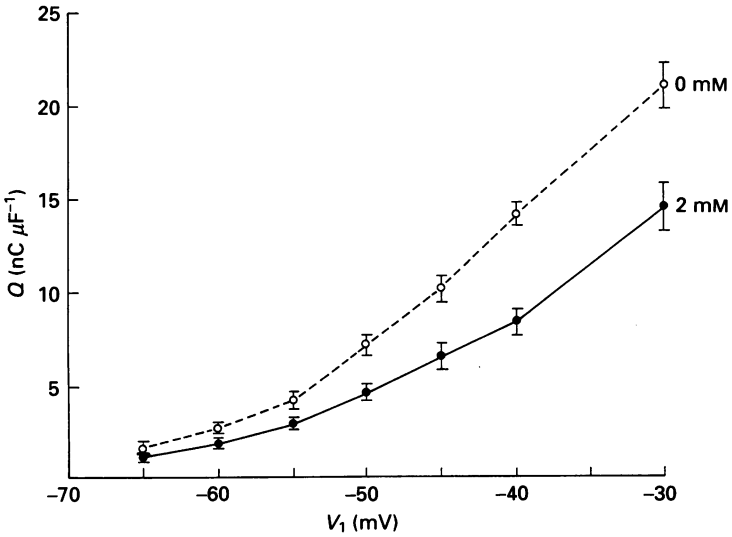

Fig. 3. The effect of 2 mM-tetracaine on the amount of charge moved in muscles bathed in hypertonic charge movement solutions at 2 °C. Means ± S.E. of means of four to sixteen observations. Concentrations of tetracaine shown alongside each curve.

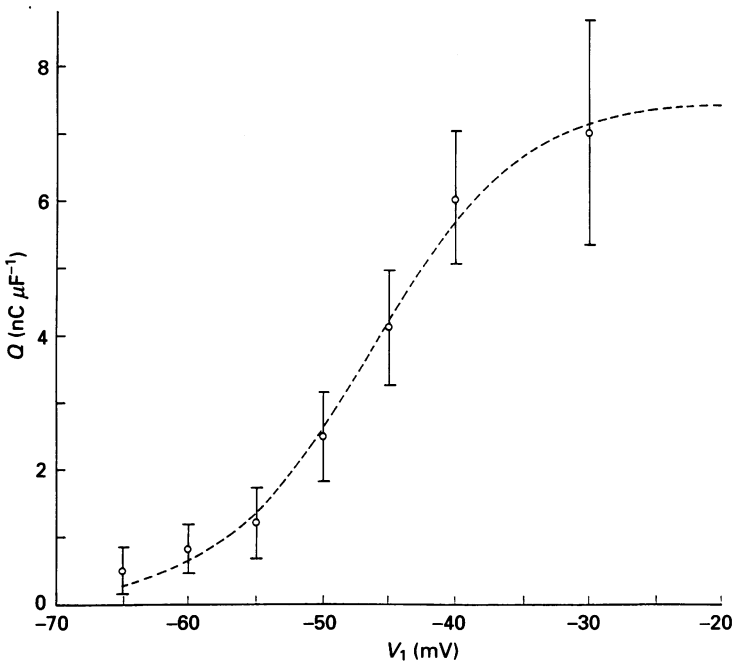

Fig. 4. Voltage dependence of the tetracaine-sensitive charge component. ○, the differences between the mean values shown in Fig. 3. Error bars give ± S.E. The dashed curve is an unconstrained least-squares fit of a Boltzmann distribution (Q_{max} = 7·5 nC μF^{-1}, $\bar{V} = -46{\cdot}5$ mV, k = 5·5 mV).

similar values ($Q_{max} = 40{\cdot}6 \pm 2{\cdot}3$ nC μF^{-1}, $\bar{V} = -14{\cdot}1 \pm 1{\cdot}3$ mV and $k = 15{\cdot}3 \pm 0{\cdot}8$ mV) to those obtained from fibres in the absence of tetracaine. Thus 2 mM-tetracaine does not appear to effect the amount or voltage distribution of charge in isotonic solutions.

Since hypertonicity potentiated the effect of tetracaine on muscle RMP, the action of tetracaine on charge moved in hypertonic solutions was investigated. Figure 3 shows the amount of charge moved around mechanical threshold in muscles bathed in hypertonic solutions containing zero and 2 mM-tetracaine. It is clear from this figure that 2 mM-tetracaine reduced the amount of charge moved around threshold. The differences between the two curves of Fig. 3 is shown in Fig. 4, where the smooth curve drawn through the points is an unconstrained least-squares fit of a Boltzmann distribution. The fit gave: $Q_{max} = 7{\cdot}5$ nC μF^{-1}, $\bar{V} = -46{\cdot}5$ mV and k $-5{\cdot}5$ mV. The e-fold rise of the foot of the curve was 9·3 mV. If k was constrained to 9·3 mV, the fitted value of Q_{max} was 9·5 nC μF^{-1} and V was $-41{\cdot}5$ mV. Thus tetracaine appears to remove a component of at least 8–10 nC μF^{-1} of charge. The experiments do not distinguish between a complete block of this charge or a tetracaine-induced shift in the voltage dependence of the charge component to more positive membrane potentials than those explored in Fig. 3.

The charge movement measurements were carried out under conditions (2 °C; hypertonic solution) in which fibre contraction is largely inhibited and a detailed study of the effects of tetracaine on contraction was not carried out. However, in both isotonic and hypertonic solutions, residual movement, as judged from the ease with which depolarizing pulses beyond mechanical threshold could be applied, was greatly reduced by tetracaine. Tetracaine was undoubtedly acting to further inhibit contraction in these fibres.

DISCUSSION

We report here measurements of full Q–V curves that have been made for the first time in intact mammalian muscles bathed in isotonic solutions in the absence of tetracaine. One clear finding in isotonic solutions is that the amount, and voltage distribution, of charge movement is unaffected by 2 mM-tetracaine. This confirms and extends our earlier report (Hollingworth *et al.* 1987) that the foot of the Q–V curve in fast twitch fibres was unaffected by tetracaine in isotonic solution. In addition we report that when mammalian fibres are bathed in hypertonic solution a component of charge becomes sensitive to tetracaine.

The absence of an effect of 2 mM-tetracaine in isotonic solution indicates that the 2-fold higher Q_{max} values measured by Hollingworth & Marshall (1981) compared to those of Dulhunty & Gage (1983) cannot be explained by Dulhunty and Gage's use of tetracaine to prevent muscle contraction. One possible explanation for the difference, suggested by the results of Hollingworth *et al.* (1987), is that charge movements may be reduced in amplitude by bromide, which was the principle anion in the solutions used by Dulhunty & Gage (1983). Another possibility is that the latter were measuring charge movement at the end of a mammalian muscle fibre where charge might be reduced, when compared to the middle of a fibre. This is not, however, the case in frog muscle (Hollingworth & Marshall, 1981).

Intact mammalian fibres in hypertonic solution (350 mM-sucrose added) have 7–10 nC μF^{-1} of charge that is blocked by 2 mM-tetracaine (Figs 4 and 5). In cut rabbit fibres about 15% of maximum charge is blocked in moderately hypertonic solutions (80 mM-sucrose added) by low concentrations of tetracaine (50–200 μM). Increasing the tetracaine concentration to 1 or 2 mM causes a 'catastrophic leak' (Lamb, 1986). The voltage dependence of the tetracaine component in cut rabbit fibres appears to be more positive when compared to the results reported here on intact fibres. Low concentrations of tetracaine (100–200 μM) also block a component of charge in cut frog fibres bathed in isotonic solution, and 2–4 mM-tetracaine is rapidly toxic (Csernoch *et al.* 1988). Thus cut fibres, when compared to intact fibres, are differently sensitive to tetracaine.

Our results on the influence of tonicity on the action of tetracaine probably explain the different accounts of the action of tetracaine on intact frog muscle fibres. Experiments in which tetracaine was thought to have little effect on frog charge movement were carried out in isotonic solution (Almers & Best, 1976; Huang, 1981*b*) whereas those in which tetracaine was shown to block charge were carried out in hypertonic solution (Huang, 1981*a*, 1982, 1986; Hui, 1983). We found the concentration- and time-dependent effect of tetracaine on resting membrane potential in frog muscle was very similar to its effect on mammalian muscle (Fig. 2). Thus it is likely, as in mammalian muscle, that the block of frog charge by tetracaine in hypertonic solution occurs under conditions in which there is a fall in resting membrane potential. For example, Huang (1986) reported mean membrane potentials of −61·3 mV from control fibres (350 mM-sucrose, 5 mM-rubidium) but of −50·0 mV when tetracaine was added to the hypertonic bathing solution.

Since the contraction of frog and rat skeletal muscle fibres is blocked by tetracaine in isotonic solution (Almers & Best, 1976; Dulhunty & Gage, 1983; see Results), the absence of an effect of tetracaine on charge movement in isotonic solution suggests that the primary effect of tetracaine in inhibiting contraction is at another step in the excitation–contraction coupling process. In support of this, Csernoch *et al.* (1988) showed that myoplasmic calcium signals recorded from cut frog fibres were reduced by tetracaine at concentrations (25–40 μM) which had no effect on charge movements.

In frog muscle where charge movements are complex tetracaine appears to eliminate the secondary rising phases or 'humps' characteristic of Q_γ (Huang, 1982, 1986; Hui, 1983). In contrast to frog muscle, the decay of mammalian charge movements in hypertonic solution is well fitted by a simple exponential (Hollingworth & Marshall, 1981) and the tetracaine-sensitive charge cannot be readily associated with a distinct kinetic component. Although the voltage dependence of the tetracaine-sensitive component, centred between −40 to −45 mV (Fig. 4), is in the region expected for an involvement in contractile activation, the tetracaine component has a less steep dependence on membrane potential (e-fold for 9 mV) than has been reported for Q_γ in frog muscle (3–4 mV: Hui, 1983; Hui & Chandler, 1988). A more detailed understanding of the involvement of mammalian charge movement in excitation–contraction coupling must, therefore, await studies of SR calcium release in mammalian muscle since the voltage dependence of this process is as yet unknown.

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