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DIFFERENTIAL EFFECTS OF TETRACAINE ON DELAYED POTASSIUM CHANNELS AND DISPLACEMENT CURRENTS IN FROG SKELETAL MUSCLE

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SUMMARY

1. Delayed K^+-currents and displacement currents were studied with a voltage-clamp technique.

2. In normal fibres, the conductance of the delayed channel grows e-fold per 3 millivolts at sufficiently negative potentials and reaches a limiting value of 2–10 m-mho/cm^2 (mean 5·8 m-mho/cm^2) at positive potentials. Adding tetracaine (2 mM) reduces the limiting conductance, shifts the voltage-dependence of the delayed channel to +25 mV more positive potentials and slows the kinetics fourfold.

3. By contrast, the displacement currents are virtually unaltered by 2 mM tetracaine. Their voltage-dependence is shifted by less than 5 mV and their kinetics are unaffected.

4. Tetraethylammonium ions (TEA) are known to slow the kinetics of delayed K^+-channels fivefold but fail, like tetracaine, to change the kinetics of the displacement currents.

5. Both tetracaine and TEA have thus large effects on the 'gating' of the delayed channel, yet little or none on the displacement currents. This suggests that the displacement currents in skeletal muscle are for the most part unrelated to the opening and closing of delayed channels. It is estimated that 'gating' the delayed channel in muscle may require no more than 1 or 2% of the observed charge displacement.

INTRODUCTION

Recent studies of displacement currents across the cell membrane of skeletal muscle (Schneider & Chandler, 1973; Chandler, Rakowski & Schneider, 1976*a*; Almers, 1975; Adrian & Almers, 1976*b*) and of the potential dependence of muscle membrane capacity (Adrian & Almers, 1976*a*; Schneider & Chandler, 1976) have shown that the membrane

contains polar molecules, which are mobile enough and carry sufficient charge or dipole moment to respond strongly to membrane potential changes of physiological magnitude. These molecules could therefore serve as 'gating molecules' regulating potential-dependent events in muscle fibres, and the displacement currents accompanying their movement under a changing electric field could be 'gating currents' representing a first step in the regulatory process.

Though it has been suggested that the displacement currents are involved in regulating muscle contraction (Schneider & Chandler, 1973; Chandler *et al.* 1976*b*), there are theoretical (Hodgkin & Huxley, 1952*b*) and experimental (Armstrong & Bezanilla, 1974) reasons to expect that Na and K channels would also contribute to the displacement currents. Na channel gating probably accounts for less than 10 % of the maximal charge carried by displacement currents (Almers & Levinson, 1975), but the question as to whether or not K channels are major contributors (Chandler *et al.* 1976*b*) seems more difficult to answer.

The present experiments show how the local anaesthetic tetracaine modifies the 'gating' of the delayed K channel, yet fails to influence displacement currents in a corresponding manner. Tetraethylammonium ions, known to slow the kinetics of the delayed channel (Stanfield, 1970) also show little effect on the displacement currents. Both findings suggest that the molecules giving rise to the displacement currents in skeletal muscle are for the most part not those from which delayed channels derive their voltage dependence. Preliminary reports of this work have been included elsewhere (Almers, Adrian & Levinson, 1975; Almers & Best, 1976*a*).

METHODS

Experiments were carried out at 2–5° C with the three micro-electrode voltage-clamp technique (Adrian, Chandler & Hodgkin, 1970). The three electrodes were inserted into the pelvic end of a sartorius muscle fibre from the frog *Rana temporaria*. Placed at distances l, $2l$, and $(2l+l')$ from the end, they served to record the voltages V_1, V_2, and inject current I_0 respectively. Unless indicated otherwise, V_1 was controlled by electronic feed-back. Membrane current density I_m at distance l from the fibre end was measured by

$$I_m = \frac{a}{3l^2R_i}\Delta V, \tag{1}$$

where $\Delta V = (V_2 - V_1)$, the myoplasmic resistivity R_i was assumed 299–270 Ω cm depending on the exact temperature, and the fibre radius a was determined from measurements of the longitudinal myoplasmic resistance r_i (Ω/cm fibre length) by $a = \sqrt{(R_i/\pi r_i)}$. r_i was measured from steady-state displacement of V_1, ΔV, and I_0 as described by Adrian *et al.* (1970). When r_i was not measured, $a = 40$ μm was assumed or the membrane current was referred to the membrane capacity. This and other experimental details are described in Almers & Best (1976*b*).

In some experiments, membrane currents during and after step displacements in

membrane potential were automatically corrected for capacitive and leakage admittances by on-line use of a digital computer (see Almers & Best, 1976*b*, for details). After correcting for capacity and leakage, records of membrane current were usually 'smoothed' in order to reduce high-frequency noise. This was done by replacing a data point y_n obtained at time n by $0{\cdot}25\ (y_{n-1}+2y_n+y_{(n+1)}$; $y_{(n-1)}$, y_n, and $y_{(n+1)}$ are successive data points spaced at 0·4 msec intervals. Exceptions are the first and last data points y_0 and y_m in a series, which were replaced by $0{\cdot}25\ (3y_0+y_1)$ and $0{\cdot}25\ (y_{(m-1)}+3y_m)$, respectively. Similarly, if a step change in voltage occurred between data points $n = k$ and $n = (k+1)$, y_k was replaced by $0{\cdot}25\ (y_{(k-1)}+3y_k)$ and $y_{(k+1)}$ by $0{\cdot}25\ (3y_{(k+1)}+y_{(k+2)})$. Such special treatment of initial and final data

TABLE 1. Composition of solutions

Reference	Rb^+	K^+	Na^+	TEA^+	SO_4^{2-}	Sucrose
A	2·5	—	187·5	—	95	—
B	10	—	180	—	95	—
C	10	—	—	180	95	—
D	—	10	180	—	95	—
E	—	10	70	—	40	463

Concentrations in mM. All solutions contained 5 mM-$CaSO_4$, 0·4 μM tetrodotoxin and 1·5 mM-Na phosphate buffer at pH 7·0.

points in a given interval preserved the time integral of membrane current over the interval as well as the discontinuities expected at beginning and end of voltage square pulses. Each corrected current record was subjected twice to the above procedure. This did not significantly distort the recorded transients as could be shown by performing the operation twice on a digitally generated exponential decline of rate constant $1/\tau = 300$/sec sampled once every 0·4 msec. The declining exponential giving the best least-squared fit to the result had a rate constant of $1/\tau = 297{\cdot}87$/sec differing from the original value by less than 1%.

Bathing media were designed to minimize ionic transmembrane currents other than through the delayed potassium channel; their compositions are given in Table 1. They all contained 1·5 mM-Na phosphate buffer at pH 7·0 and 0·4 μM tetrodotoxin. Tetracaine-HCl (Sigma) was added either in crystalline form or from a 40 mM stock solution of neutral pH. The pH of experimental media containing tetracaine was checked before use and, if necessary, adjusted to pH 6·9–7·2.

RESULTS

Tetracaine and delayed currents

Shift of voltage dependence

The left column of Fig. 1 shows membrane currents during depolarizations from −94 mV to the potential given next to each trace. The muscle was stretched to 1·3 times its *in situ* length in order to minimize myofilament overlap and V_2 (the potential at distance $2l$ from the fibre end) was controlled by electronic feed-back. Final values of V_1 (potential at distance l from the end) are given next to each trace. At potentials between −41 and −34 mV, it was possible to record delayed currents relatively undisturbed by fibre movement. However, depolarization beyond this range, e.g. to $V_1 = -32{\cdot}8$ mV, resulted in a strong contraction causing

the wiggle in that trace and disqualifying it from further analysis. Voltage control was then switched to the electrode closest to the end (V_1) and the fibre was briefly depolarized to + 18 mV. The ensuing contraction occurred with sufficient latency to allow relatively undisturbed recording of the large delayed current which resulted. At + 18 mV, the delayed channel should be almost completely activated, so the last trace will give a measure of $\bar{g}_K$, the maximal conductance through the delayed channel.

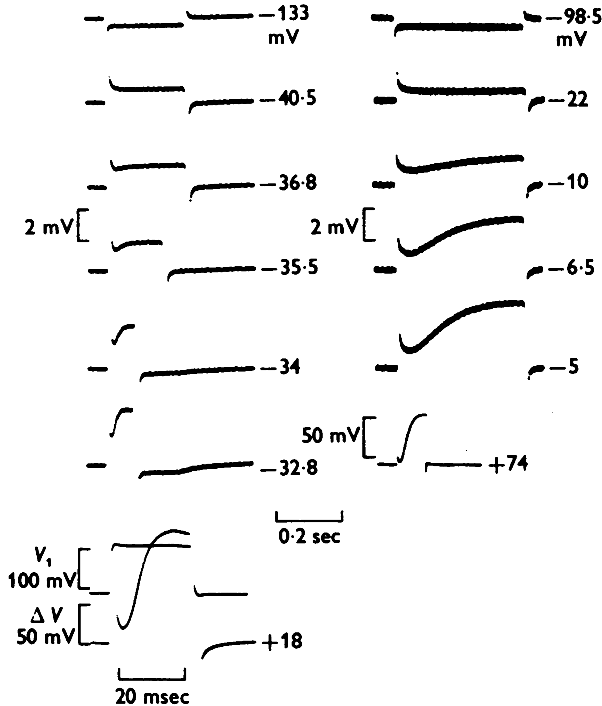

Fig. 1. Left: membrane currents in Na_2SO_4 solution (solution A) during pulses from the holding potential of − 94 mV to the potentials given next to each trace. Feed-back from V_2 in all except the bottom traces taken on a fast time base. The irregularities after the pulse to − 32·8 and − 34 mV are movement artifacts. Fibre 47-2-5, fibre radius 47·9 μm. Right: membrane currents after addition of 2 mM tetracaine. Feedback from V_1 throughout; holding potential − 60 mV, fibre 47-2-7, fibre radius 50·0 μm. Electrode spacing l = 375 μm; 1 mV corresponds to 4·21 $\mu A/cm^2$ on the left and 4·59 $\mu A/cm^2$ on the right. Temperature 5° C.

The right column in Fig. 1 was obtained from the same muscle after adding 2 mM tetracaine. V_1 was controlled throughout, and the holding potential was set to − 60 mV. The combination of tetracaine and a low holding potential inactivated the excitation–contraction coupling mechanism sufficiently to allow prolonged depolarization up to 0 mV without fibre movement. At beginning (not shown) and end of the series, a large

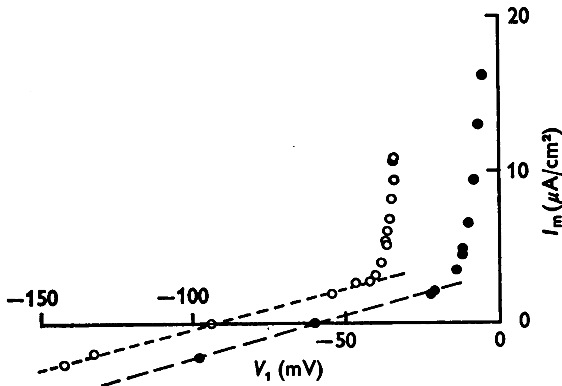

Fig. 2. Current–voltage curves in the absence (open circles) and presence (filled circles) of 2 mM tetracaine. Currents were measured at the ends of pulses and relative to the holding current. Dashed lines are estimates of membrane leakage conductances from fitting a straight line to measurements at potentials negative to −46·5 mV on the left and −22 mV on the right. Zero-current potentials upon initial electrode impalement were −74 and −52 mV, respectively. Same experiment as Fig. 1.

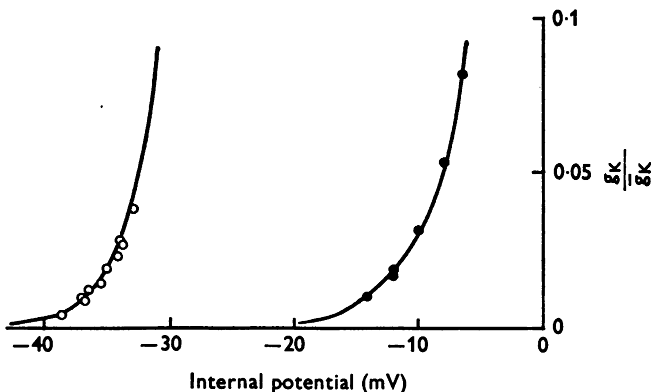

Fig. 3. Final conductances g_K through the delayed channel as a function of membrane potential V_1 in the absence (○) and presence (●) of 2 mM tetracaine. Values are given relative to the maximal conductance $\bar{g}_K$ measured with a pulse to +18 (○) or +74 mV (●). Zero-current potential for the delayed channel was assumed −79·7 mV (○) or obtained by measurement as −78·0 mV (●). Correction for leakage as indicated in Fig. 2. Same experiment as Figs. 1 and 2.

pulse to +74 mV was given for measurement of the maximal conductance through the delayed channel (see later). In between, the conductance of the delayed channel was explored with submaximal depolarizations.

In Fig. 2, the final current during the pulse (relative to a small and steady holding current) is plotted against the membrane potential. The current–voltage curve in both fibres is linear until a sharp increase in

conductance occurs due to the activation of delayed channels. In Fig. 2, this happened at about −15 mV in the presence and at −40 mV in the absence of tetracaine.

TABLE 2. Effect of tetracaine on delayed channels

Fibre no.	$[Rb^+]_o$ (mM)	$\bar{g}_K$ (m-mho/cm²)	k (mV)	$V_{2\%}$ (mV)	V_{rev} (mV)
		A, control			
47-2-2	2·5	10·08	2·99[1]	−39·6	−79·7[2]
47-2-3	2·5	5·09	2·94	−34·3	
47-2-4	2·5	4·43	2·66	−35·5	
47-2-5	2·5	5·91	3·30	−37·7	
47-2-6	2·5	2·28	2·85	−34·8	
Mean ± S.E. of mean		5·56	2·99	−36·4	
		1·28	0·19	1·0	
		B, 2 mM tetracaine			
47-2-7	2·5	1·92	3·59	−11·6	−78
47-2-8	2·5	3·87	4·49	−10·4	−83
47-2-9	2·5	2·31	2·86	−14·9	−78
47-2-10	10·0	2·23	4·98	−11·2	−60
47-2-11	10·0	1·22[3]	5·40	−11·8	−66·5
47-2-12	10·0	1·70[3]	3·46	−16·9	−62
Mean ± S.E. of mean		2·21	4·13	−12·8	
		0·40	0·44	1·1	

Parameters of delayed channel in the absence and presence of 2 mM tetracaine. $\bar{g}_K$ maximal conductance during depolarizing pulses beyond +15 mV (control) and +73 mV (2 mM tetracaine). k is defined by $g_K/\bar{g}_K = \exp[(V-\bar{V}/k]$ where $\bar{V}$ and k are adjusted to give the best least-squares fit of the equation to data such as in Fig. 3. $V_{2\%} = \bar{V} + k \ln 0{\cdot}02$. Electrode spacing $l = 375\,\mu m$ throughout. Solutions A or B. (1) assumed from average; (2) assumed from the mean of measurements in the presence of 2 mM tetracaine and 2·5 mM-Rb^+; (3) measured towards end of experiment after holding fibre at −100 mV for 3 min or longer. All measurements were made on the same muscle and with the same set of micro-electrodes.

The voltage dependence of conductance in normal fibres seemed surprisingly steep, and in order to test whether this could be an erroneous result of voltage non-uniformity or of applying the approximate eqn. (1), I calculated the potential along a model fibre using standard numerical methods. Simulating the experiment of Figs. 1 and 2 it was assumed that the membrane behaved as a linear leak conductance in parallel with an element whose conductance increases exponentially with potential, growing e-fold in 3 mV and equalling the leak conductance at 58 mV depolarization. With linear properties and electrode spacings as in the experiment, the calculated values of V_2 and V_2 gave, when analysed as above, a value of $k = 2{\cdot}98$ in good agreement with the assumed $k = 3$ mV.

In the tetracaine-poisoned fibre, the reversal potential of the delayed channel was found to be −78 mV; in the control fibre it was assumed to be −79·7 mV (see later). After subtracting leakage, the final conductance

due to the delayed channel can be determined for each of the pulses in Fig. 1. In Fig. 3, their values relative to $\bar{g}_K$ are plotted against the membrane potential. The continuous curves are least-squares fits of rising exponentials; they describe the data well.

Table 2 summarizes a number of similar experiments on the same muscle. At sufficiently negative potentials, conductance through the delayed channel grows e-fold in 2·99 mV in the control fibres; at more positive potentials the voltage dependence becomes less steep (see also legend of Table 2). The mean value for $\bar{g}_K$ was 5·56 mm-ho/cm² in these fibres if referred to the surface of a circular cylinder of radius a; the radius was obtained as described in Methods from an electrical measurement of the cross-sectional area. The over-all mean of nine fibres with S.E. of mean was 5·7 ± 0·8 m-mho/cm². In the presence of tetracaine, the maximum conductance appears reduced more than twofold; the effect is not due to the low holding potential since seven other fibres from the same animal were held at −100 mV instead of −60 mV and gave essentially the same value for $\bar{g}_K$ (2·13 ± 0·23 mm/cm² measured at 70–80 mV). The voltage dependence of conductance (e-fold in 4·13 mV) appears less steep in tetracaine. Most importantly of all, however, the potential at which conductance of the delayed channel has risen to 2 % of the maximum, is −36 mV in the control and −13 mV in tetracaine. The drug therefore shifts the voltage dependence of the delayed channel to 23–24 mV more positive potentials.

The value of $\bar{g}_K$ for the normal K channel was obtained here in an isotonic solution and is smaller than values obtained in 2·5 times hypertonic solutions by Adrian *et al.* (1970; 8·5–20 m-mho/cm²), Stanfield (1975; 17·8 m-mho/cm²) and myself (10·3 m-mho/cm², see legend of Fig. 6). The difference is probably due to the increased potassium activity inside osmotically shrunken fibres. It could be explained if currents, through the delayed channel obeyed the 'independence principle' (Hodgkin & Huxley, 1952*a*).

Reversal potential of the delayed channel in 2 mM tetracaine

The reversal potential was measured with double-step experiments as described by Adrian *et al.* (1970). The first step went to between 50 and 70 mV and opened a large fraction of the delayed channels. The second step returned the membrane potential to negative values, causing 'tails' of current which declined with time as the delayed channels closed. The 'tails' were corrected for capacitive transients and used to construct a segment of the 'instantaneous' current–voltage curve pertaining immediately after the first step. From it, the potential where the 'tail' reversed direction could be obtained. In three fibres in 2·5 mM-Rb^+-containing sulphate saline (fibres 47-2-7 to 47-2-9, solution A) the reversal potential was −79·7 ± 1·7 mV, three other fibres (47-2-10 to 47-2-12) in

10 mM-Rb^+-saline (solution B) gave a mean value of $62 \cdot 8 \pm 2 \cdot 0$ mV (mean ± S.E. of mean). This change in reversal potential is consistent with a permeability ratio P_{Rb}/P_{Na} of 40 and smaller than expected from results on frog myelinated nerve (Hille, 1973; $P_{Rb}/P_{Na} > 100$). The difference could be explained, for instance, if there were delayed channels in the transverse tubules causing K^+-accumulation. The reversal potentials given above were assumed to apply also to delayed channels in the absence of the drug, where kinetics are faster and reversal potentials harder to measure.

Inactivation in the presence of 2 mM tetracaine

As in normal fibres, delayed channels inactivated under maintained depolarization. The inactivation behaviour was not explored in detail, but the following incidental observations may be of interest. One fibre with a resting potential of −40 mV when initially impaled was held at −30 mV; a peak conductance of 0·07 mmho/cm² was elicited by depolarization to +71 mV. 34 min thereafter, the holding potential was set to −100 mV; after 3 min at −100 mV the conductance during a pulse to 71 mV had increased to 0·33 m-mho/cm². In this fibre, delayed channels were therefore about 78% inactivated at −30 mV. Little or no inactivation takes place at −60 mV, since freshly impaled fibres gave virtually the same values for $\bar{g}_K$ after being held for several minutes at −100 or −60 mV. These results would suggest that the steady-state relationship between inactivation and membrane potential is about the same as reported for normal fibres by Adrian *et al.* (1970). Recovery from inactivation seemed exceedingly slow at −60 mV. Although care was taken to keep depolarizing pulses short or small, inactivation often accumulated so that after 30 min experimentation, the maximum available conductance was often less than half of its initial value. Rest periods of up to 4 min brought little recovery, but 3–4 min rest at −100 mV often did. Values of $\bar{g}_K$ entered in Table 2 are always the highest value recorded from a given fibre, usually at the beginning of an experiment. Values for calculating $g_K/\bar{g}_K$ in experiments as in Fig. 3 were the mean of two values obtained immediately before and after runs such as those in Fig. 1 (right).

Kinetics of currents in the presence of tetracaine

The right column of Fig. 4 shows delayed currents in the presence of 2 mM tetracaine, the left column in the absence of the drug. The left traces were recorded in a hypertonic sulphate solution of the same ionic strength as Ringer fluid (solution E). As is seen in Fig. 4, delayed channels in the presence of the drug not only require abnormally positive potentials for activation, but also open more slowly. Though the potential along the end of the fibre was not very uniform in some traces of Fig. 4, this non-uniformity is unlikely to explain the slowing of kinetics, since at a given potential, final values of ΔV are not very different in the two columns. For a quantitative study of the effect, currents were corrected for leakage and capacity and analysed assuming the conductance to be proportional to the fourth power of a variable n obeying first-order kinetics (Hodgkin & Huxley, 1952*b*):

$$I(t) = I(\infty)n^4 = I(\infty)\,[1-\exp(-t/\tau_n)]^4 \qquad (2)$$

$I(\infty)$ is the final current, t the time variable and τ_n the time constant with which n varies at a given potential. τ_n was obtained by analysing the time course of currents; the steady-state values of n, n_∞, were obtained by

$$n_\infty = \left(\frac{I(\infty)}{\bar{g}_K(V - V_{rev})}\right)^{\frac{1}{4}} \quad (3)$$

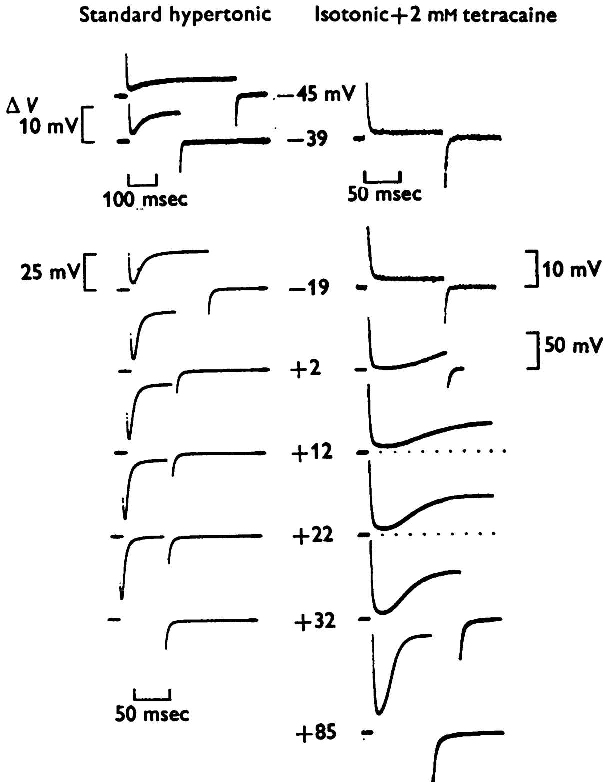

Fig. 4. Right, membrane currents in the presence of 2 mM tetracaine (solution B, electrode spacing l = 375 μm, fibre 39-2-2), left, currents in the absence of the drug (solution E, l = 125 μm, fibre 3/5-1-5). ΔV = 1 mV corresponds to 17·5 $\mu A/cm^2$ of cylinder surface on the left and to 3·39$\mu A/cm^2$ on the right assuming fibre radii of 30 and 40 μm, respectively. Pulses went from the holding potential of −100 mV to the approximate values indicated between traces; the correct values were 0−2 mV more negative (left) or positive (right).

where $\bar{g}_K$ and V_{rev} are maximal conductance and reversal potential of the delayed channel. V_{rev} ranged from −60 to −65 mV in the group of fibres in hypertonic solution, and was assumed to be −62·8 mV in solution B. $\bar{g}_K$ was measured with depolarizations beyond +20 mV in the absence of the drug and beyond +55 mV in its presence.

Eqn. (2) is known to describe delayed currents in normal fibres adequately

(Adrian *et al.* 1970; Stanfield, 1970). Except at extreme potentials, eqn. (2) also fits in the presence of the drug if one appropriately shifts the origin of the data points (heavy black lines in Fig. 5), in other words, if one allows that a depolarization from -100 mV affects the variable n only after a delay. The traces in Fig. 4 could have been fitted without a delay

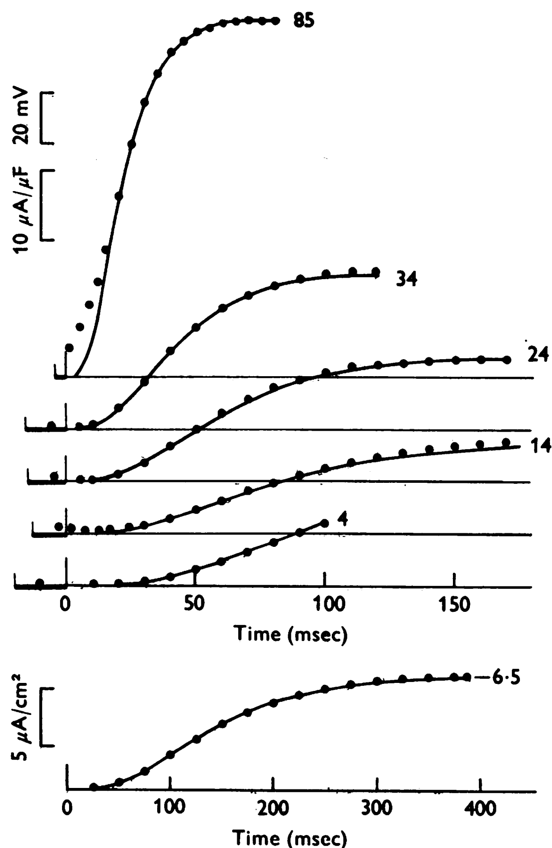

Fig. 5. Kinetic analysis of delayed currents in the presence of 2 mM tetracaine. Continuous lines are drawn by eqn. (2); filled circles are measured membrane currents from fibre 39-2-2 (top, same as Fig. 4) and fibre 47-2-7 (bottom, same as Fig. 1) corrected for capacity and leakage. In order to account for the delay with which K channels open in 2 mM tetracaine, the time origin for the data points was shifted where necessary to the left and by the amounts indicated by the heavily drawn horizontal bars. For experimental details see legends of Figs. 1 and 4.

had one chosen slower time constants (e.g. τ_n = 32 instead of 22 msec at 34 mV and τ_n = 39 instead of 31 msec at 24 mV) but the fit would have been substantially worse. Delays of this kind are reminiscent of those observed by Cole & Moore (1960) in squid giant axons after conditioning

the membrane at strongly negative potentials. Their physical significance is unclear in the present experiments, and they have been introduced mainly as a data-fitting convenience. Allowing delays was usually unnecessary when currents were recorded during depolarizations not beyond 0 mV from a holding potential of −60 mV (see bottom trace in Fig. 5).

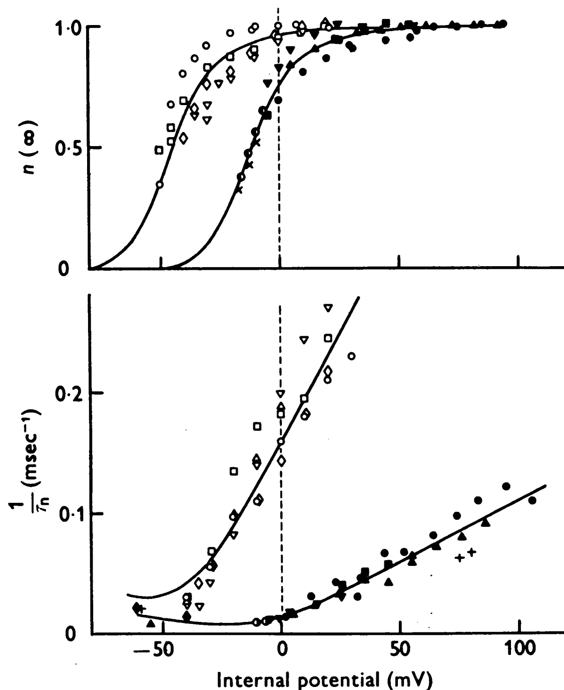

Fig. 6. Parameters n_{∞} and τ_n of the delayed channel from experiments like that in Fig. 4 in hypertonic solution (open symbols, solution E) and isotonic sulphate media containing 2 mM tetracaine (filled and half-filled symbols, solution B). Results from Fig. 4 given by open circles and filled circles (○ fibre 3/5-1-5; ● fibre 39-2-2); other open symbols from fibres 3/2-1-1, 3/2-1-3, 3/5-1-2; filled symbols from fibres 37-1-1, 37-1-2, 37-1-7, 39-2-1, 39-3-4, 47-2-9, 47-2-12. In the lower portion, ◑ indicates means from five to eight fibres each; standard deviation was less than the size of symbol. Lines were drawn according to eqns. (4–7) as described in the text with $\bar{V}$ = −40 mV, $\bar{\alpha}_n$ = 3·77/sec, $\bar{\beta}_n$ = 15·9/sec in the absence and $\bar{V}$ = 7 mV, $\bar{\alpha}_n$ = 1·02/sec, $\bar{\beta}_n$ = 4·29/sec in the presence of 2 mM tetracaine. Filled symbols negative to −25 mV were obtained from the decline of conductance after activation with a depolarizing pulse (not shown in Fig. 4); no delays were necessary when fitting the decline of conductance. Assuming a radius of a = 30 μm and internal resistivity of R_i = 365 Ω cm in the drug-free hypertonic medium, $\bar{g}_K$ = 10·3 ± 1·0 m-mho/cm² (n = 5) was obtained in this series of experiments; the fibres in the isotonic solution B + 2 mM tetracaine gave an average $\bar{g}_K$ = 2·8 ± 0·2 m-mho/cm² (n = 4) with a = 40 μm and R_i = 270 Ω cm. Figures are given ± S.E. of mean and include measurements from a holding potential of −100 mV only.

In Fig. 6, the analysis outlined by eqns. (2), (3) has been carried out on a number of fibres in the absence (open symbols) or presence (filled or half-filled symbols) of 2 mM tetracaine. The continuous curves were drawn according to

$$n(\infty) = \frac{\alpha_n}{\alpha_n+\beta_n}, \tag{4}$$

$$1/\tau_n = \alpha_n+\beta_n, \tag{5}$$

$$\alpha_n = \frac{\bar{\alpha}_n(V-\bar{V}_n)}{1-\exp[-(V-\bar{V}_n)/7]}, \tag{6}$$

$$\beta_n = \bar{\beta}_n \exp[-(V-\bar{V}_n)/40] \tag{7}$$

(Adrian *et al.* 1970). Data in the absence of the drug were fitted with the values used by Adrian *et al.* (1970) except that their values for $\bar{\alpha}_n$ and $\bar{\beta}_n$ were multiplied by 0·857. In order to fit data in the presence of 2 mM tetracaine, it was necessary to introduce delays as in Fig. 5, $\bar{\alpha}_n$ and $\bar{\beta}_n$ each had to be reduced 3·7-fold and $\bar{V}_n$ had to be shifted by 33 mV in the positive direction. The shift of + 33 mV in Fig. 6 is larger than the 23–24 mV from Table 2, probably because relative to the fibres in tetracaine, the control fibres in Figs. 4 and 6 were exposed to a lower external and higher internal ionic strength.

The effect of 2 mM tetracaine on delayed channels can be summarized by saying that the drug shifts the voltage dependence to 23–24 mV more positive potentials, delays the opening of the channels and slows the kinetics about 3·7-fold.

Displacement currents in tetracaine

In 2 mM tetracaine, displacement currents are of similar size and carry similar charge as those seen in drug-free hypertonic media (Almers & Best, 1976*b*). However, hypertonicity *per se* might change the electrical properties of muscle membrane. For studying drug effects quantitatively, it seemed therefore preferable to work in isotonic media throughout, even though fibre movement restricts the potential range over which small currents can be recorded.

Fig. 7 shows membrane currents in an isotonic sulphate medium (solution B). The two top traces on the left show single sweeps across the oscilloscope screen of voltage (uppermost) during a pulse from − 100 to − 38 mV ('test pulse') and current (below) during a small 'control pulse' from − 107 to − 93 mV. A signal-averaged version of the current during the 'control pulse' was used to correct currents accompanying pulses to other potentials for the leakage and capacitive admittances existing around − 100 mV. All other traces on the Figure show membrane currents

corrected in this way; they accompanied 'test pulses' from −100 mV to the potential indicated and show transient displacement currents which are outward during and inward after the pulse.

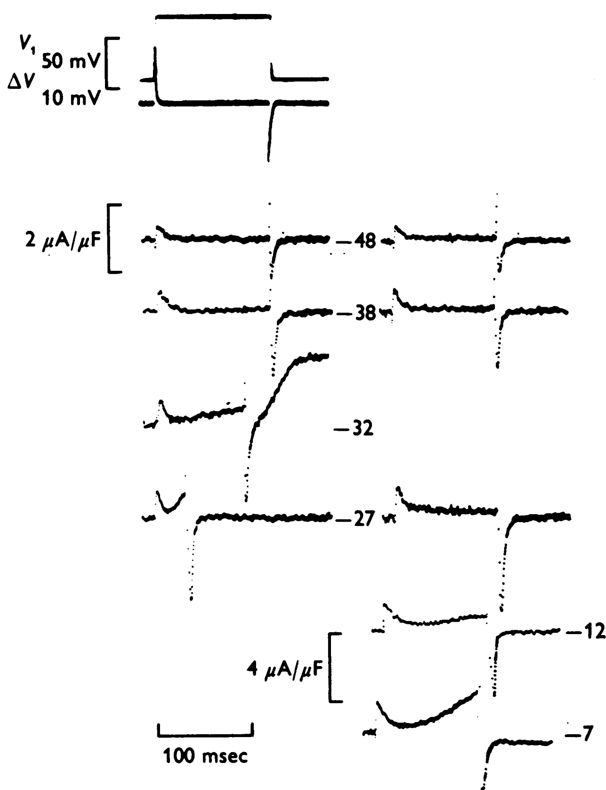

Fig. 7. Displacement currents in the absence (left) and the presence (right) of 2 mM tetracaine during test pulses to the potentials indicated in mV. Upper left shows voltage during a test pulse from −100 to −38 mV and current during the control pulse from −107 to −93 mV. Assuming a fibre radius of 40 μm and r_i = 5·57 MΩ/cm, one obtains C_m = 8·11 μF/cm² on the left (fibre 39-3-3) and C_m = 6·83 μF/cm² on the right (fibre 39-3-4). Calibration bars only approximate; they were chosen so that equal charges referred to C_m correspond to equal areas. For instance, the time calibration corresponds to 104·5 msec on the left and 95·5 msec on the right. Holding potential −100 mV, electrode spacing l = 375 μm. Both fibres in solution B and from the same muscle.

Currents on the left were recorded without tetracaine. Displacement transients, though relatively small, are clearly visible. The pulse to −32 mV caused contraction; the large upward deflexion after the pulse is a movement artifact. With shorter pulses, depolarization to −27 mV without substantial movement was possible, but the displacement current

transient became partially obscured by outward current through the delayed channel. About 30 min after applying 2 mM tetracaine (right column) fibre movement no longer interfered, and delayed channels did

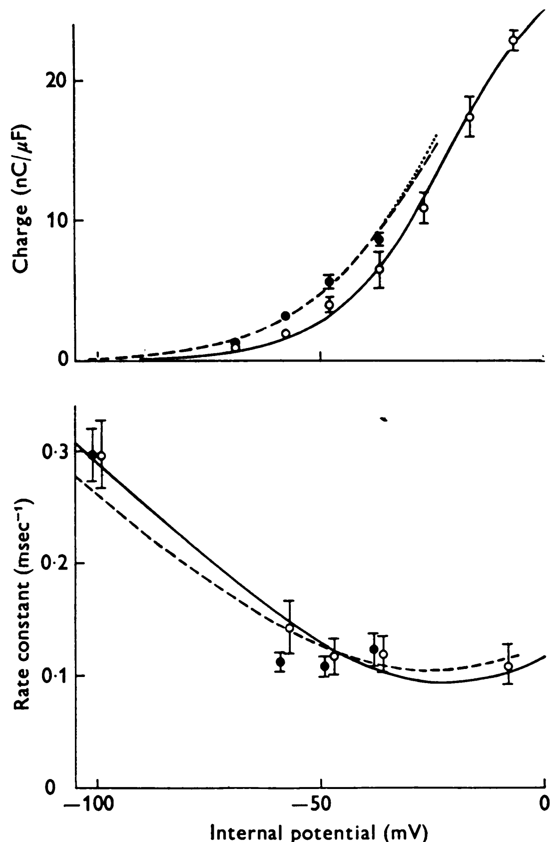

Fig. 8. Charge displacement (top) and decay rate of displacement currents (bottom) as functions of membrane potential V_1. Open symbols in the presence, filled symbols in the absence of 2 mM tetracaine. Error bars give S.E. of mean. Curves are drawn by eqns. (8) and (9) with the mean parameters from Table 3. Top: $Q_{max} = 28{\cdot}6$ nC/μF, $\bar{V} = -25{\cdot}7$ mV, $k = 14{\cdot}9$ mV (dashed curve) or $Q_{max} = 37{\cdot}2$ nC/μF, $\bar{V} = 19{\cdot}4$ mV, $k = 15{\cdot}7$ mV (dotted curve), all in the absence of tetracaine; in the presence of the drug, $Q_{max} = 28{\cdot}6$ nC/μF, $\bar{V} = -22{\cdot}0$ mV and $k = 12{\cdot}3$ mV (continuous curve). Bottom: $\bar{V} = 25{\cdot}7$ mV, $k = 14{\cdot}9$, $\bar{\alpha} = 51{\cdot}9$/sec in the absence (dashed) and $\bar{V} = 22{\cdot}0$ mV, $k = 12{\cdot}3$ mV, $\bar{\alpha} = 45{\cdot}1$/sec in the presence of the drug (continuous curve).

not open unless the membrane was depolarized to beyond -12 mV. Yet the displacment currents do not seem conspicuously affected.

This and similar experiments are analysed in Table 3 and Fig. 8. Horizontal (sometimes sloping) base lines were fitted by eye to the final

sections of traces or to sections preceding voltage changes and the displacement currents measured relative to these base lines. Traces showing delayed currents were fitted with eqn. (2) over the latter half of the pulse, assuming leak asymmetries consistent with other traces recorded immediately before. The delayed current component was then extrapolated by eqn. (2) over the first half of the trace and the remaining transient taken as displacement current. 'Off-charge' was obtained from a subsequent pulse to the same potential, shortened so the delayed conductance increase could not occur to any great extent. Fig. 8 plots the charge displacement (top) and the rate constants with which displacement currents declined (bottom) against the potential during the test pulse. Open symbols were obtained with 2 mM tetracaine and closed symbols without. Rates of decline were obtained by fitting single exponentials to currents flowing between 4–5 and 20–25 msec after a potential change. Single exponentials described the observations fairly well except for data recorded in the voltage range −30 to −10 mV (see later).

In Table 3, each fibre is analysed individually. The maximal charge displacement, Q_{max}, is assumed to be 28·6 nC/μF as found in tetracaine treated fibres in the presence of tetraethylammonium ions (Almers & Best, 1976*b*). Similar values are obtained in fibres immobilized by tetracaine-free hypertonic solutions (Chandler *et al.* 1976*a*; Adrian & Almers, 1976*b*). The entries k and $\bar{V}$ in Table 2 represent steepness and transition potential of the relationship between charge displacement and potential; they are defined by

$$Q = Q_{max}/\{1+\exp[-(V-\bar{V})/k]\}. \quad (8)$$

$\bar{V}$ and k were determined by least-squares fits of eqn. (8) to the charge displacement data as described previously. $\bar{\alpha}$ is defined by

$$\frac{1}{\tau_Q} = \frac{\bar{\alpha}(V-\bar{V})}{k} \coth[(V-\bar{V})/2k] \quad (9)$$

(see Chandler *et al.* 1976*a* and Almers & Best, 1976*b*) where τ_Q is the time constant of the exponential decay fit to displacement currents.

Fig. 8 shows that tetracaine has no effect on the kinetics of displacement currents. The difference in the mean values for $\bar{\alpha}$ in Table 2 is statistically insignificant and model-dependent; it would vanish if k in normal fibres were as small as in the presence of tetracaine. The value for k in tetracaine agrees well with that found previously in a solution containing tetracaine and TEA (Almers & Best, 1976*b*). Tetracaine shifts the charge–voltage relation by about +4 mV, but the shift is of marginal statistical significance ($P < 0·05$, t test).

Q_{max} was not determined independently here, but given that the drug does not change it, the exact value of Q_{max} makes little difference to the conclusion that any

shift produced by tetracaine is small. If Q_{max} is left as a free parameter in fitting eqn. (8) to the data in tetracaine, the mean results are: $Q_{max} = 37 \cdot 2 \pm 2 \cdot 6$ nC/μF; $k = 14 \cdot 8 \pm 0 \cdot 6$ mV and $\bar{V} = -14 \cdot 0 \pm 0 \cdot 9$ mV (mean ± S.E. of means). Re-analysing the data in normal fibres with $Q_{max} = 37 \cdot 2$ nC/μF yields $\bar{V} = -19 \cdot 4 \pm 1 \cdot 4$ mV and $k = 15 \cdot 7 \pm 1 \cdot 0$ mV (dotted curve in Fig. 8, top). k is now larger, but the magnitude of the shift remains about the same.

TABLE 3. Effect of tetracaine on displacement currents

Fibre no.	C_m (μF/cm^2)	k (mV)	$\bar{V}$ (mV)	$\bar{\alpha}$ (sec^{-1})
		Control		
39-3-1	9·23	15·5	−25·7	57·6
39-3-2	6·29	16·6	−26·7	46·9
39-3-3	8·11	13·9	−26·6	53·5
40-1-2	8·15	12·0	−27·2	55·4
40-1-3	7·42	17·4	−20·6	49·8
40-1-4	7·49	14·2	−27·2	46·2
Mean	7·78	14·9	−25·7	51·6
± S.E. of mean	0·40	0·8	1·0	1·9
		2 mM tetracaine		
37-1-6	7·20	11·3	−21·3	58·1
39-2-1	9·04	11·2	−21·3	39·4
39-2-2	7·40	13·3	−18·6	38·1
39-3-4	6·83	11·2	−27·4	52·8
40-2-1	7·48	14·4	−21·5	40·3
Mean	7·59	12·3	−22·0	45·1
± S.E. of mean	0·38	0·7	1·5	4·0
		2 mM tetracaine + 180 mM TEA$^+$		
Mean	6·66	12·9	−26·8	45·7
± S.E. of mean	0·63	1·2	3·3	3·8

Membrane capacity (C_m) at −100 mV and parameters of displacement currents in absence and presence of 2 mM tetracaine. For calculating C_m, $r_i = 5 \cdot 57$ MΩ/cm and $a = 40$ μm were assumed. The parameters k and $\bar{V}$ were adjusted to give the best least-squares fit to eqn. (8); $\bar{\alpha}$ is the mean of determinations from individual transients by eqn. (9), rates of off-transients at −100 mV were evaluated only for test pulses to potentials negative to −35 mV. $Q_{max} = 28 \cdot 6$ nC/μF was assumed throughout as found by Almers & Best, 1976; the last row is taken from that paper. Membrane conductances were $5 \cdot 66 \pm 2 \cdot 87$ μmho/μF in the absence and $12 \cdot 94 \pm 2 \cdot 81$ μmho/μF in the presence of tetracaine. Solution B throughout except for the last row which was obtained in the presence of 180 mM-TEA$^+$ (solution C).

Effect of tetraethylammonium ions

Since tetraethylammonium ions (TEA$^+$) block delayed channels and slow their kinetics (Stanfield, 1970), the effect of TEA$^+$ on displacement currents was investigated. Fig. 9 plots charge displacement (top) and

rates at which displacement currents declined (bottom) against membrane potential. Currents were recorded in the presence of 2 mM tetracaine in an experimental medium containing either 180 mM-Na (solution B) or 180 mM-TEA^+ (solution C). Measurements in the presence (open circles)

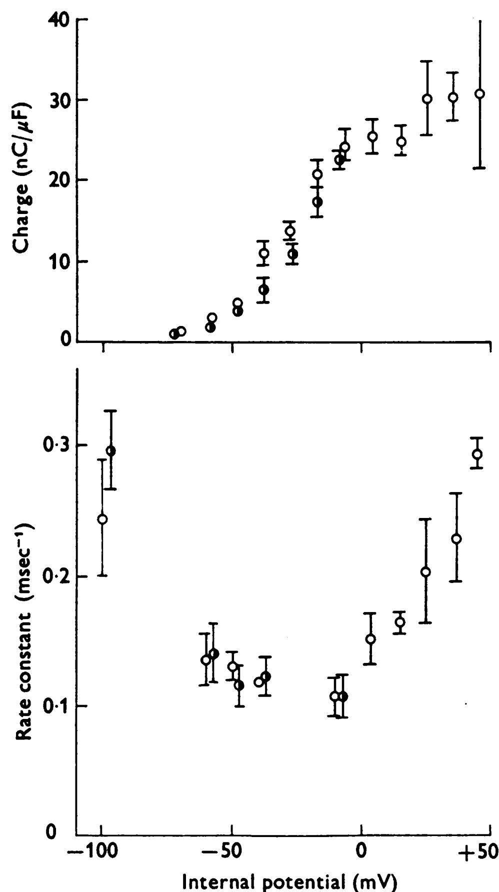

Fig. 9. Lack of effect of 180 mM tetraethylammonium ions (TEA^+). Charge displacement (top) and decay rates of displacement transients (bottom) in the presence (○, solution C) and absence (◑, solution B) of TEA^+. Error bars give S.E. of mean; 2 mM tetracaine was present throughout. Electrode spacing l = 375 μm, holding potential −100 mV.

and absence (half-filled dots) of TEA^+ are means from two groups of four or five fibres, respectively. Parameters from least-squares fits of eqns. (8) and (9) to the data in TEA^+ were given previously (Almers & Best, 1976*b*) and are included in Table 3. TEA^+ at the high concentration used here may reverse the tetracaine-induced 5 mV shift of the charge–voltage relation, but has no other effect on displacement currents.

Time course of displacement currents

Tubular delays. If a substantial portion of the observed charge flowed across the transverse tubular membranes, then the delay with which voltage signals propagate into the tubules could distort the time course of displacement currents. In particular, tubular delays could mask a drug-induced slowing of kinetics if the charging of tubular membranes were rate-limiting. However, this appears highly unlikely in view of impedance

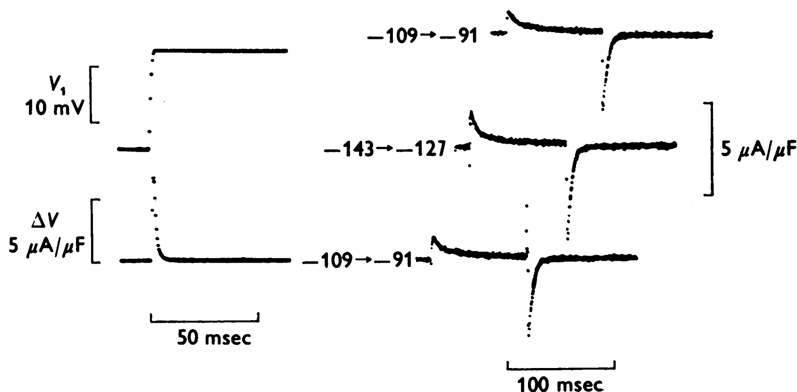

Fig. 10. Effect of control pulse on time course of displacement transients. Left, current and voltage during a control pulse from −109 to −91 mV. Right, displacement transients during and after test pulses from −100 to −28 mV; the potential range spanned by the control pulse is given next to each trace. Digital smoothing was not employed in this experiment. Solution C, fibre 40-3-1; l = 375 μm; 2 mM tetracaine was present. Charge displacements were: top, Q_{on} = 15·3 nC/μF, Q_{off} = 16·8 nC/μF; middle, Q_{on} = 20·4 nC/μF, Q_{off} = 18·7 nC/μF; bottom, Q_{on} = 16·7 nC/μF, Q_{off} = 17·8 nC/μF, all referred to C_m = 7·63/cm² at −100 mV.

analyses of muscle membrane. In the model of Adrian, Chandler & Hodgkin (1969) with the parameters proposed by Hodgkin & Nakajima (1972) or Valdiosera, Clausen & Eisenberg (1974), one would expect, for instance, the peripheral three-quarters of the tubule membrane area in an 80 μm diameter fibre to follow a step potential change across the sarcolemma within about 1 msec to more than 68 % completion. This is less than the shortest decay time constants observed here (about 3 msec) and much less than decay time constants at −50 to −30 mV which were of the order of 10 msec. Furthermore, the capacitive charging transient during small 'control pulses' (see Figs. 7 or 10) always declined more quickly than displacement currents. Therefore, drug-induced slowing of displacement currents should, if present, have been recorded faithfully despite tubular delays.

Non-exponential time course. Exponentially declining displacement currents would be expected only in the simplest situations as, for example, in the transition of a single species of polar molecules between two states. Evidence in hypertonic media (Almers, 1975; Chandler *et al.* 1976*a*) argues against this simple model. The present analysis was performed in terms of single exponentials and eqns. (8), (9) simply to allow quantitative exploration of drug effects.

TABLE 4. Two components of displacement current at −27 to −28 mV

Fibre no.	A (μA/μF)	τ_a (msec)	B (μA/μF)	τ_b (msec)	Q_b/Q	CP (mV)
37-1-6	0·848	4·23	0·266	18·5	0·532	−110, −97
39-2-1	0·864	11·0	0·043	73·0	0·291	−109, −92
39-2-2	1·664	0·83	0·206	28·4	0·617	−109, −92
39-3-4	1·363	3·90	0·394	23·3	0·660	−109, −95
40-2-1	0·793	9·50	0·058	36·6	0·192	−109, −95
40-3-1*	0·686	4·94	0·603	23·3	0·952	−109, −91
	1·838	4·31	0·564	25·7	0·742	−145, −127
	1·044	4·15	0·564	27·9	0·883	−109, −91

* Fibre was in solution C; all others in solution B.

CP, potential range spanned by the control pulse; Q_b, charged carried by the slower component of displacement current, Q total charge. All other symbols as defined by eqn. (10) and in the text.

At most potentials single exponentials fit my observations to within errors expected from tubular delays, delays introduced deliberately into voltage-clamp steps, time-dependence of residual ionic currents and noise. However, between −30 and −10 mV they often do not fit; examples are the trace labelled '−27 mV' in Fig. 7 and the traces on the right of Fig. 10. Currents at these potentials often suggest the presence of a rapid and slow component as shown by the semilogarithmic plots in Fig. 11. The continuous curves in that Figure are least-squares fits of the equation

$$I(t) = A \exp(-t/\tau_a) + B \exp(-t/\tau_b) \qquad (10)$$

to currents sampled from 4 msec onwards after the potential change; A, B, τ_a and τ_6 are adjustable parameters. Eqn. (10) appears to fit fairly well.

Table 4 summarizes results from other fibres. In four of the six, presence of a slow component seems probable; the charge carried by that component (Q_b) was more than half the total (Q) in these fibres. Time constants of both components seem well in excess of the time delays expected from propagation down the tubules. Hence displacement currents at −27 or −28 mV in tetracaine are not exponential.

The experiment of Figs. 10 and 11 suggests that the fast component can be made more visible by taking 'control pulses' at potentials more

negative than the range −109 to −91 mV usually employed. The finding would indicate that the polar molecules responsible for the rapid component re-orient to some extent during 'control pulses' from −109 to −91 mV and therefore are not in a state of dielectric saturation at these potentials. The experiment was tried only once, but similar results were

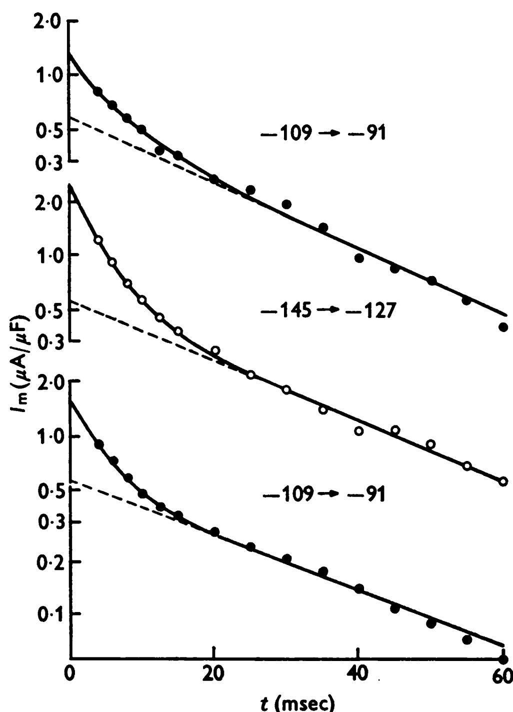

Fig. 11. Semilogarithmic plots of the on-transients in Fig. 10. Open and filled circles are experimental, continuous curves are least-squares fits of eqn. (10) to the data; dashed lines give the second term in eqn. (10). For parameters see Table 4.

obtained in the absence of tetracaine in hypertonic solutions (R. H. Adrian & W. Almers, unpublished; Adrian & Almers, 1976*b*) and are consistent with the voltage-dependence of capacity at very negative potentials (Adrian & Almers, 1976*a*).

DISCUSSION

Tetracaine effects on delayed channels

Effects of local anaesthetics on the early ('Na') channel in excitable membranes have been studied extensively (see, for example, Narahashi, 1971, or Ritchie, 1975, for reviews) but there is little published information

about anaesthetic action on delayed channels (Taylor, 1959; Blaustein & Goldman, 1966). This may be due to the fact that at least in vertebrate nerves, local anaesthetics at pharmacological concentrations have little effect on delayed channels (Hille, 1966; Århem & Frankenhaeuser, 1964). Striking effects are seen, however, at 100–1000 times higher concentration, and the present report describes them in some detail.

Since the drug partitions strongly into the lipid phase (Skou, 1954), its concentration in the present experiments was probably high enough for the number of drug molecules in the membrane to approach that of lipid molecules. For instance, McLaughlin's (1975) conductance measurements on black lipid films suggest that at 2 mM aqueous concentration, a lipid bilayer containing 25 % charged lipids will adsorb tetracaine molecules at a density of one per 100 $Å^2$, or one for every one or two lipid molecules. At such high intramembrane drug concentrations, large effects on delayed channels are not surprising even if there is no 'specific' interaction between drug and channel molecules of the kind observed with tetrodotoxin, saxitoxin and quaternary lidocaine derivatives (Strichartz, 1973; Hille, Courtney & Dum, 1975). For instance, in order to explain the observed 2·5-fold reduction of the maximal conductance through the delayed channel by Gouy–Chapman theory, only one charged tetracaine molecule per 800 $Å^2$ near the inner channel mouth would be enough to change the membrane surface potential there by 22 mV in the presence of 0·15 uni-univalent electrolyte and reduce the local K concentration 2·5-fold. Even if tetracaine molecules adsorbed to the membrane lipid had to act at some distance in order to reduce the local K^+-concentration at the mouths of delayed channels, there may well have been enough drug molecules present in my experiments to accomplish a 2·5-fold depression. The reduction in maximal conductance could therefore be due to a uniform reduction of 'single channel' conductances by surface charge effects rather than to complete blockage of some individual channels.

Shifts in voltage dependence can also result from surface charge effects, but in the present case, the tetracaine-induced positive surface potential would have to be larger on the outer than on the inner membrane surface despite the large external divalent anion concentration. Alternatively, the shift observed here may result because the altered intramembrane environment energetically biases the channel configuration in favour of the closed state. The slowing of rate constants may also be a consequence of an altered channel environment; for example, the Ca^{2+}-pump of the sarcoplasmic reticulum needs a lipid environment to split ATP (Martonosi, 1971). In conclusion, the present data do not require one to postulate a stoichiometric interaction between tetracaine and delayed K channels.

Pharmacological dissociation of delayed and displacement currents

Despite the large expected tetracaine concentration in the membrane, the dielectric membrane properties remain virtually unchanged. As in artificial lipid bilayers (McLaughlin, 1975), no effect of tetracaine on membrane capacity is apparent (Table 2). The data are not accurate enough to rule out a 3–5 % expansion of membrane area said to occur in erythrocytes at high anaesthetic concentrations (Seeman, 1972), but a capacity increase by much more than 10% should have been noticed, and the absence of such an effect makes it unlikely that intramembrane movements of tetracaine molecules have contributed significantly to the observed displacement currents. Although 2 mM tetracaine strongly shifts and slows the voltage dependence of delayed channels, it has no effect on displacement currents other than a 4 mV positive shift. These currents must flow across a region of the membrane which is relatively unaffected by the drug. Tetraethylammonium ions (180 mM), though known to slow the kinetics of delayed channels fivefold (Stanfield, 1970) also have no effect on displacement currents. Therefore, there are at least two agents with large effects on the 'gating' of delayed channels but no corresponding effects on displacement currents. The simplest interpretation of this fact is that displacement currents in skeletal muscle are for the most part not related to the regulation of delayed channels. The same conclusion has been reached by Chandler *et al.* (1976*b*) on the basis of comparing the effects of glycerol treatment on displacement- and K-currents.

Most previous studies of displacement currents have been carried out in hypertonic solutions in order to minimize fibre movement. Under these conditions, the efficiency with which electrical signals spread into the transverse tubular system is thought to be reduced (Valdiosera *et al.* 1974). Since tetracaine inhibits contraction but has so little effect on displacement currents, the drug may be useful as an alternative to hypertonic solutions in further studies of these currents.

Contribution of delayed channels to charge displacement

While a comparison of tetracaine effects give no straightforward estimate for the contribution of delayed channels to displacement currents, a rough idea of this contribution can be obtained as follows. Assuming the conductance of a single open delayed channel to be the same in frog skeletal muscle as in frog myelinated nerve (4 p-mho per channel, Begenisich & Stevens, 1975) the maximal conductance $\bar{g}_K = 5{\cdot}56$ m-mho/cm^2 obtained here indicates that there are about $1{\cdot}4 \times 10^9$ delayed channels per cm^2 of cylinder surface of a cylindrical muscle fibre, roughly twenty-times less than the probable number of Na channels (Almers & Levinson, 1975). If the

charge displacement necessary to open a single K channel were known, the maximal contribution of delayed channels could be estimated. By applying Boltzmann's principle to a relatively simple model of a voltage-dependent ionic channel, Hodgkin & Huxley (1952*b*) have shown that at sufficiently negative potentials, the fraction of open channels should grow exponentially with potential. For our case,

$$g_K/\bar{g}_K = \text{constant}.\exp(zFV/RT), \quad V \rightarrow -\infty, \tag{11}$$

where F is the Faraday, R and T have their usual significance and z is the number of (positive) elementary charges that one needs to transfer through the entire voltage drop across the membrane to open a single K channel. Eqn. (11) also applies in a variety of more general cases (W. Almers, unpublished). To the extent that the potential range explored in experiments as in Fig. 1 and Table 2 was negative enough, the value of $k = 3$ obtained there would suggest that z is equal to about eight electronic charges. Lower values for k might have been obtained had the experimental conditions allowed resolution of smaller delayed currents at more negative potentials and $z = 8$ is therefore a lower limit. For instance, if a model similar to Hodgkin & Huxley's (1952*b*) applied and four charged particles had to move independently across a single energy barrier and combine on one side of the membrane to open a K channel, then the precise value of z in eqn. (11) would have had to be 10·28 (2·57 for each of the four particles) instead of 8 in order to make the model consistent with the data in Table 2. To the extent that the approximate value of $z \geqslant 8$ applies (perhaps to within a factor of two) one would expect the charge displacement accompanying the activation of all K channels to be of the order of $10\text{–}20 \times 10^9$ electronic charges per square centimeter of cylinder surface or, for an average capacity of 7 $\mu F/cm^2$ at a fibre radius $a = 40\ \mu m$, to be 0·25–0·5 $nC/\mu F$. This figure is 1 or 2% of the observed charge and would leave little hope for resolving 'gating currents' for the delayed channel with present methods.

Many thanks are due to Dr P. R. Stanfield who collaborated in some early experiments on delayed K currents and to Dr B. Hille for his critical reading of the manuscript. Some of the experiments reported here were done while the author was on leave of absence at the Physiological Laboratory, Cambridge, and it is a pleasure to thank Dr R. H. Adrian for his kind hospitality. Supported by USPHS grant no. AM17803.

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