Short communication

Initial charge distribution and capacity transients in frog skeletal muscle

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Abstract
The effect of prior charge transfers on intramembrane charge movements was investigated in voltage-clamped frog skeletal muscle fibres. 10mV steps between fixed prepulse and test voltages were imposed at varying intervals after a 5mV prepulse step made from a previous conditioning level. The amount of charge transfer prior to the test step was varied by altering the extent to which the test step intercepted slow (qF) currents elicited in the prepulse. The greater the prior charge transfer, the more rapid the transients in the subsequent test step. In contrast, where the prepulse had not reached the threshold of slow currents, or at larger depolarizations where the then faster charging currents were not intercepted, charge movements induced by the test step had a constant size and form. Charging kinetics thus varied with preceding charge transfer, consistent with higher-order cooperative (Huang, 1984), or sequential (Almers, 1978) schemes for the nonlinear charge.

Key words: charge movements, skeletal muscle

Introduction
It has been suggested on several occasions that charge movements reflect configurational changes in potential sensors within cell membranes, which regulate physiological processes. In skeletal muscle, the charge may contain at least three components, of which qa and q6 produce quasi-exponential transients over different voltage ranges. In contrast, the qT component produces complex, steeply voltage-dependent currents responsible for 'humps' and prolonged phases at potentials near contractile threshold (e.g. Adrian 1978). This suggests a sequential system (Horowicz & Schneider, 1979) or the existence of rate constants with cooperative features (Huang, 1984) rather than a single-step first order Hodgkin-Huxley (1952) transition depending uniquely upon membrane potential. In the higher order schemes, charging kinetics should depend not only upon voltage, but also upon charge transfers prior to the test pulse. Horowicz & Schneider (1979) investigated the effect of holding potential, but it is possible that slow charge inactivation could have contributed to the results. The experiments here explored the effect of charge distribution upon subsequent responses to test steps from a constant prepulse voltage to a fixed test voltage for the first time. Thus, the only variable was the charge moved prior to the test step. This was accomplished by using the test pulse to intercept to different degrees slow ('qF') transients elicited by a prepulse. Such slow currents extending over 100 ms occur over a narrow (±10mV) voltage range near contraction threshold. Earlier work has shown that charge is conserved through such pulse procedures and so the currents observed reflect capacitative charge (Adrian & Huang, 1984a).

Methods
A three microelectrode voltage clamp of Rana temporaria sartorius muscles employed conventional glass microelectrodes (resistance 4-10Mohm) inserted 1 = 375mm (voltage control electrode V1), 2 = 750mm (electrode V2) and 875mm (current injection I0 electrode) from the fibre pelvic end. The membrane current i(t) was deduced from the voltage drop V1 - V2. Fuller details of data acquisition and analysis are available elsewhere (see Adrian, 1978; Adrian & Huang, 1984a). Records presented here were obtained from averages of five test and five control sweeps; the latter also yielded linear cable constants used to monitor fibre stability and condition. Experiments were performed at 3-4°C in a solution consisting of Rb2SO4 2.5mM, tetraethylammonium sulphate 80mM, tetraethylammonium chloride 15mM, CaSO4 8mM, tetrodotoxin 2x10^-7M, Tris buffer 3mM, CoSO4 20mM, sucrose 310mM, pH 7.0.

Results
A double pulse procedure was imposed at a fixed time, 500ms, after varying conditioning depolarizations from the -90mV holding voltage. It consisted of a 5mV prepulse, which was followed by the 10mV test pulse applied at varying intervals Δt of 0, 50, 110 or 290ms after the prepulse (Fig. 1A). Linear charge was obtained by a similar procedure except that prepulse and test pulses were performed from the -90mV holding voltage rather than superimposed on a conditioning depolarization. Nonlinear currents were obtained by subtracting the
Figure 1

(A) Pulse procedure incorporating (a) conditioning, (b) 5 mV prepulse and (c) 10 mV test pulses to potential V_T. (B) Prepulse charge intercepted by test step ΔQ at different prepulse-test pulse intervals Δt. (C) - (E) charge movements in response to (b,b') prepulses and (c) test pulses, responses to the latter superimposed, at different Δt, at test voltages of (C) -34 mV, (D) -30 mV & (E) -27 mV respectively. (E) shows some evidence of delayed rectifier current. Prepulse responses at Δt = 50 & 110 ms included. Test pulse responses superimposed. (F) Semilogarithmic plots of charge movements at -30 mV test steps with different Δt (arrowed) on superimposed time scales. Slow currents did not occur in prepulse responses but prolonged 'shoulders' appeared in currents induced by the larger test depolarizations (cf. Horowicz & Schneider, 1979). Charge transfer prior to the test step is therefore similar at the three Δt. Fig. 1C shows that under such conditions test transients at the prepulse-test pulse intervals indicated in Fig. 1B, were superimposable.

Fig. 1D shows records obtained at the -30 mV test voltage at which decay of prepulse charge was intercepted by the test step to different degrees. Slow currents in the prepulse responses are indicated by an arrow. The charge movements elicited by test steps were now not superimposable as the balance of the intercepted charge moved in the test step. In the resulting family of traces, transients associated with slow test step delays enveloped those from longer delays but the traces did not simply scale in magnitude (see below).

In contrast, at still larger test depolarizations (-27 mV in the fibre illustrated), the slow currents now became more rapid, and decayed completely before even the earliest test step, consistent with earlier accounts of q_K charge (Figs. 1B & 1E; Adrian & Huang, 1984a). Consequently,