CHARGE MOVEMENT IN A FAST TWITCH SKELETAL MUSCLE FROM RAT

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ABSTRACT Voltage-dependent charge movement in the rat omohyoid muscle was investigated using the three microelectrode voltage clamp technique. The charge that moved during a depolarization from the holding potential (−90 mV) to the test potential, V, increased with increasing V, saturating around 0 mV. The charge vs. voltage relationship was well fitted by $Q = Q_{max}/\{1 + \exp[-(V - \overline{V})/k]\}$, with $Q_{max} = 28.5$ nC/μF, $\overline{V} = -34.2$ mV, and $k = 8.7$ mV. Repolarization of the fiber from the test potential back to the holding potential caused an equal but opposite amount of charge to move. The kinetics of ON charge movement could be well described by a model developed for frog muscle by Horowicz and Schneider (1981*b*), which suggests that rat and frog charge movements are similar. This model failed to describe the kinetics of OFF charge movement for steps in potential from 0 mV to test potentials of −10 to −90 mV. OFF-charge movement rose to a peak more slowly and decayed more slowly than predicted by the theory.

INTRODUCTION

A voltage-dependent, nonlinear charge movement has been extensively studied in frog twitch (Chandler, et al., 1976; Adrian and Almers, 1976) and slow muscle fibers (Gilly and Hui, 1980). This charge movement (Kovacs et al., 1979; Horowicz and Schneider, 1981*b*) or a component of it (Huang, 1982; Hui, 1982) may be involved in the coupling of the depolarization of the transverse tubular system (t-system) to the release of calcium from the sarcoplasmic reticulum and subsequent activation of contractile proteins. A detailed model for how charge movement might function during this process has appeared (Horowicz and Schneider, 1981*b*), and in frog muscle has been shown to describe the kinetics of the charge that moves following a depolarizing step (ON charge movement). Recently, Hollingworth and Marshall (1981) have described similar charge movements in fast (extensor digitorum longus [EDL]) and slow twitch (soleus) muscles of the rat. A comparison of charge movement in these two muscles was of interest because Dulhunty (1980) had earlier reported that the strength-duration curve for EDL was shifted ~20 mV in the depolarizing direction compared with soleus. Hollingworth and Marshall found a similar difference between the two muscles in the relationship between steady-state charge distribution and voltage; in EDL the midpoint of this relationship was shifted in the depolarizing direction, and the slope at the midpoint was less steep than in soleus.

The aim of our study was to characterize charge movement in the rat omohyoid for comparison with measurements of charge movement in other muscle preparations. Based on histochemistry, the omohyoid contains only fast twitch fibers (Müntener et al., 1980) and it was therefore of particular interest to determine whether the voltage dependence of charge movement in the omohyoid is similar to that found in EDL by Hollingworth and Marshall (1981). It was also of interest to compare the kinetics of charge movement in a mammalian muscle with those in frog muscle because any significant differences would have important implications for the role of charge movement in excitation-contraction (EC) coupling. As a standard of comparison we used the model of Horowicz and Schneider (1981*b*) because it gives a good account of ON charge movement in the frog. Some of the results presented here have appeared in abstract form (Simon and Beam, 1982).

METHODS

Omohyoid muscles from 400–500-g male Sprague-Dawley rats were voltage clamped using the three microelectrode technique (Adrian et al., 1970). The omohyoid was studied because most of its fibers terminate on a tendon transversing the center of the muscle, allowing for the easy and accurate placement of microelectrodes. The two voltage-measuring electrodes V_1 and V_2 and the current-injecting electrode were inserted at distances of 210, 420, and 490 μm from the tendon. The controlled voltage was the potential at V_1. The voltage electrodes were unshielded because coupling between them and the current electrode was found to be inconsequential. Command pulses were rounded with a time constant of 50–330 μs. Data were sampled with a digital computer at 4 kHz. A more detailed description of the voltage-clamp procedures is given elsewhere (Beam and Donaldson, 1983).

Total charge moved was normalized in terms of linear fiber capacitance, which has the advantage of minimizing errors associated with leaks at the sites of electrode impalement (Schneider and Chandler, 1976).

During the measurement of charge movements, the muscle was continuously perfused with an oxygenated solution that contained: 146 mM TEA Br, 5 mM CsBr, 10 mM $CaAc_2$, 1 mM $MgAc_2$, 400 mM sucrose, 1 μm TTX, and 10 mM HEPES, pH titrated to 7.4 with NaOH. Br and acetate were used as the extracellular anions to reduce the Cl conductance, which is known to be nonlinear and time dependent in mammalian skeletal muscle (Palade and Barchi, 1977). Two pulse protocols were used. In a single sequence of the standard protocol, four control steps 45 mV in amplitude were made from a base potential of -135 mV. These were followed by 31-ms test steps of increasing amplitude from the holding potential (-90 mV). The currents from the control steps were summed, appropriately scaled, and subtracted from each of the test currents. This individual sequence was repeated four times and the test currents at each voltage were averaged. In the second protocol, which was used to measure stepped OFF charge movements, the test pulse consisted of a 31-ms step to 0 mV, followed by a 31-ms step to test potentials of varying amplitude. The control steps and averaging procedure for the stepped OFF protocol were the same as for the standard protocol, except the scaling and subtraction were done in two 31-ms segments appropriate for the test pulses in those intervals. Records of charge movement were corrected as necessary by subtraction of a sloping baseline (Horowicz and Schneider, 1981*a*).

RESULTS

Fig. 1 shows a series of uncorrected charge movements measured in a fiber of a rat omohyoid muscle at 16.6°C for test pulses varying from -50 to $+20$ mV. In a number of respects, these charge movements in the omohyoid resemble charge movements measured previously in frog (Chandler et al., 1976; Adrian and Almers, 1976) and rat (Hollingworth and Marshall, 1981) leg muscles. Thus, increasing the potential during the test step causes the ON transients to become faster, without appreciable effect on the kinetics of the charge movement that follows the termination of the test pulse (OFF transient). Additionally, for a given test pulse Q_{on} and Q_{off}, the area under the ON and OFF transients have about the same value. Finally, the amount of charge moved increases with test-pulse amplitude until saturation occurs at ~0 mV. (In Fig. 1, the charge moved during the ON transients at -10, 0, and $+10$ mV were 30, 29.5, and 29.4 nC/μF, and during the OFF transients were 29.6, 30.3, and 31 nC/μF, respectively.)

For depolarizations more positive than $+10$ mV, the charge movements were frequently contaminated by ionic currents as evidenced by nonlinear baselines and inequality of Q_{on} and Q_{off}. For example, in Fig. 1, the test depolarization to $+20$ mV caused the activation of an outward current and caused Q_{off} to exceed Q_{on} (35 vs. 27 nC/μF). The presence of these time-dependent ionic currents made it difficult to accurately estimate Q_{on} and Q_{off}, but in the few cases where ionic currents were minimal, very little extra charge moved for test steps to $+50$ mV.

Fig. 2 graphs the normalized amount of charge moved, Q, as a function of test potential from eight fibers. These Q vs. V data are fit with a two-state Boltzmann model in

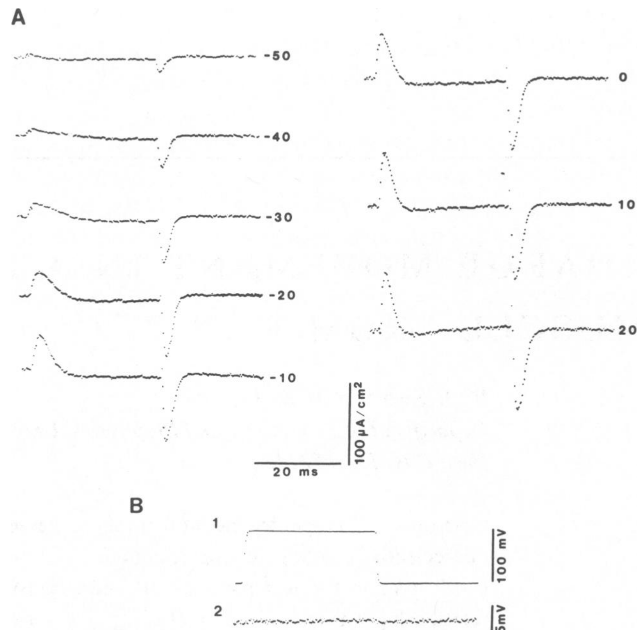

FIGURE 1 (*A*) Uncorrected charge movements for test pulses to the indicated potentials. (*B1*) $V_1(+10)$, the potential recorded by the V_1 electrode for the step to $+10$ mV. (*B2*) the difference between $V_1(+10)$ and the scaled V_1(control). Muscle 101-5. T = 16.6°C.

which potential governs the distribution of charge according to $Q = Q_{max}/\{1 + \exp[-(V - \overline{V})/k]\}$, where Q_{max} is the maximum charge that can be moved, $\overline{V}$ is the potential at which half the charge has moved, and k is a constant related to the steepness of the curve. For the eight fibers illustrated in Fig. 2, Q_{max} was 28.3 ± 3.4 nC/μF (mean ± SD). A linear regression analysis of $\ln(Q_{max}/Q - 1)$ vs. V yielded the values $\overline{V} = -34.2$ mV and $k = 8.7$ mV. The solid line in the figure is a plot of the Boltzmann model with these values. Changing temperature between 7 and 25°C had no obvious effect on Q vs. V.

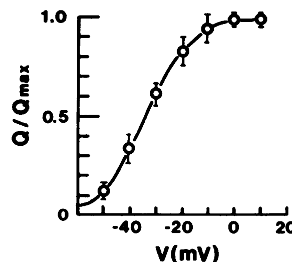

FIGURE 2 Normalized steady-state distribution of charge as a function of potential. Charge movements were measured in eight fibers at ~15°C and corrected as necessary by subtraction of a sloping baseline. At each potential, V, $Q(V)$ was taken to be the average of Q_{on} and Q_{off}, which were obtained by integration of the ON and OFF transients. The $Q(V)$ values for a given fiber were normalized by the largest value of Q (Q_{max}) for that fiber and the $Q(V)$ values were then averaged. The smooth curve represents $Q = Q_{max}/\{1 + \exp[-(V - \overline{V})/k]\}$, where $\overline{V} = -34.2$ mV, $k = 8.7$ mV, and $Q_{max} = 28.3 \pm 3.4$ nC/μF. Error bars denote ± 1 SD.

Horowicz and Schneider (1981*b*) have described a model for charge-movement kinetics in frog. According to this model, depolarization causes a charged particle, described by a variable u obeying first-order kinetics, to move from its rest or standby position to an initiator position. When three such particles occupy the initiator position, a fourth particle with charge R instantaneously moves through the membrane, effecting the release of calcium from the sarcoplasmic reticulum. If u is the probability of a particle being in the initiator position, then the total charge moved is given by

$$Q = Q_{max}(u + Ru^3)/(1 + R), \quad (1)$$

where $R = 2.6$, a value that optimizes the fit of charge movement in frog. The time course of u after a step in potential is given by

$$u = u_f + (u_i - u_f)\exp(-t/\tau_u), \quad (2)$$

where u_i and u_f are the initial and final values of u respectively, and τ_u is a time constant that depends only on potential.

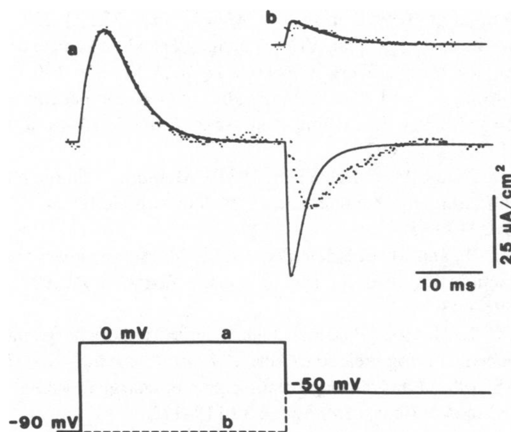

FIGURE 3 Comparison of corrected ON and stepped OFF charge movements with the predictions of Eqs. 1 and 2. The theoretical curves are shown by solid lines. (*a*) Potential was stepped from the holding potential to 0 mV and then to -50 mV. (*b*) Potential was stepped directly from the holding potential to -50 mV. Prior to fitting individual charge transients the steady-state value of u as a function of potential was calculated with Eq. 1. Using these values of u, τ_u was adjusted for a visual best fit of Eqs. 1 and 2 to an individual ON transient. τ_u was 3.3 ms for the ON transient at 0 mV (left-hand portion of trace *a*), and 4.1 ms for the ON transient at -50 mV (trace *b*). This same value of τ_u was used for the theoretical curve, which is compared with the stepped OFF at -50 mV (right-hand portion of trace *a*). Voltage does not actually change instantaneously, as assumed by Eq. 2, but instead rises exponentially with a time constant that was 350 μs for the fiber illustrated here. Therefore, the illustrated solutions for charge movement were computed by integration of the differential equation for u, $du/dt + (\alpha + \beta)\, u = \alpha$, where α and β are the forward and reverse rate constants with voltage dependence given by: $\alpha(V) = \alpha(\overline{V})\exp[\eta(V - \overline{V})/k]$ and $\beta(V) = \alpha(V)\exp - [(V - \overline{V})/k]$ (Horowicz and Schneider [1981*b*]). For this fiber $\overline{V} = -40$ mV, $\alpha(\overline{V}) = 0.1\ ms^{-1}$, $k = 11.5$ mV, and $\eta = 0.3$. The falling phase of the computed currents was the same whether computed with this integration procedure or with Eqs. 1 and 2. The steady-state values of u were 0, 0.35, and 0.99 at -90, -50, and 0 mV. Muscle 99–2. T = 6.6°C.

Fig. 3 compares the predictions of Eqs. 1 and 2 with ON charge transients for steps to 0 (*a*) and -50 mV (*b*). The charge movements were measured at 7 rather than 15°C because the kinetics were slower and hence easier to study. The theory gives a good fit of these ON charge transients, accounting for both the time to peak and the time course of the falling phase. Similarly good fits were obtained for ON transients at other test potentials and in other fibers. These fits were obtained by using values of τ_u, which are similar, both in magnitude (assuming a Q_{10} of 1.4) and in voltage-dependence to the values describing charge movement in frog muscle (Horowicz and Schneider, 1981*b*). The behavior of ON charge movement in the rat omohyoid, therefore, appears to be very similar to that in the frog. As a further test of the model, we examined the ability of Eqs. 1 and 2 to describe stepped OFF transients in which potential was first stepped for 31 ms to 0 mV, and then back to potentials ranging from -90 to -10 mV (e.g., pulse sequence *a* in the lower portion of Fig. 3). This protocol allows a direct comparison of kinetics of ON and stepped OFF transients over the same potential range. Fig. 3 *a* demonstrates that the theory summarized by Eqs. 1 and 2 fails to account for the kinetics of a stepped OFF charge movement at -50 mV. The theoretical curve, which was calculated using the same value of τ_u describing the ON transient at -50 mV, peaks too soon and decays too fast. Because the rate constants that determine the kinetics of u are functions of voltage only, τ_u should have been identical for the ON and stepped OFF. The discrepancy between the model and the stepped OFF at -50 mV was representative; at most potentials examined, the model similarly failed to describe the kinetics of stepped OFF's.

DISCUSSION

The potential dependence of steady-state charge distribution, which we have measured in the rat omohyoid, is different from that reported by Hollingworth and Marshall (1981) for the rat EDL. Based on Boltzmann fits, they found $Q_{max} = 48.9$ nC/μF, $\overline{V} = -23$ mV, and $k = 13$ mV for EDL, whereas we have found the values $Q_{max} = 28.3$ nc/μF, $\overline{V} = -34$ mV, and $k = 8.7$ mV in the omohyoid. Because the calcium concentration used in our bathing medium was higher than that used by Hollingworth and Marshall (10 vs. 2 mM), the discrepancy in the values of $\overline{V}$ and k may actually be larger, since it has been reported in frog muscle that raising calcium shifts $\overline{V}$ in the depolarizing direction and increases k (Shlevin, 1979).

It is not clear why the steady-state distribution of charge measured in the omohyoid is different from that measured in EDL as both muscles are composed of predominantly fast-twitch fibers. It is possible that EC coupling is different in the two muscles, or that differences in experimental procedure account for the disparity. For example,

Hollingworth and Marshall studied the center of the fiber, whereas we studied the end, and in our experiments Br and Ac replaced extracellular Cl. Probably the most important difference between the two studies, however, is that Hollingworth and Marshall obtained values of k and $\bar{V}$ by fitting Boltzmann's relation to charge movements in EDL for test potentials as large as +50 mV, whereas our best fit parameters for the omohyoid are for test potentials of +10 mV and less. The absolute amount of charge (nanocoulombs per microfarad) moved in EDL and omohyoid are rather similar for test pulses up to +10 mV. Thus, the conclusions about similarities or differences between the EDL and omohyoid depend largely on the reliability of charge measurements for test potentials that exceed +10 mV.

The model of Horowicz and Schneider (1981*b*) successfully describes both the strength-duration curve and the kinetics of ON charge movement in frog muscle, but the ability of the model to describe OFF transients in frog has not yet been tested. We have found that the model provides an excellent description of ON charge movement in the omohyoid, but does not describe OFF kinetics there. Specifically, the kinetics of ON and stepped OFF charge movements at a given potential are similar, whereas the model predicts that the kinetics of the stepped OFF should be faster. The inability of the model to describe OFF kinetics in rat suggests that it will be important to determine whether the theory can account for OFF kinetics in the frog.

The slow rising phase of both ON and stepped OFF charge movements at some voltages suggests that the rearrangement of charge within the membrane occurs in two or more steps, as posited by the Horowicz and Schneider model for ON charge movement. However, the measured kinetics of charge movement are complicated by the presumed tubular location of the mobile charge. Such a tubular location would slow the measured kinetics because the change in voltage within the t-system would be expected to lag by a varying amount the change in voltage at the surface of the fiber, and the current produced by the movement of charge within the t-system would be delayed in its appearance at the surface of the fiber. (An estimate of these delays can be obtained from the slow phase of the capacity transient for small voltage steps, which in the omohyoid has a time constant of 0.8–1.2 ms.) We are presently attempting to model the kinetics of measured charge transients by assuming that the mobile charge is located within a distributed tubular system (Adrian and Peachy, 1973) in order to determine whether we need to assume that the mobile charge obeys higher-order kinetics, or if it is sufficient to suppose that the charge obeys first-order kinetics.

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