PHARMACOLOGICAL DISSECTION OF CHARGE MOVEMENT IN FROG SKELETAL MUSCLE FIBERS

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ABSTRACT When charge movement is measured from muscle fibers bathed in a moderately hypertonic solution, a secondary hump appears in the decay phase of the signal during the "on" of the test pulse. The hump can be suppressed by the application of dantrolene sodium or tetracaine. The amount of charge associated with the hump is ~ 20–25% of the total charge. All the observed properties of the hump charge are consistent with the possibility that it is more closely associated with calcium release from the sarcoplasmic reticulum, and thus more relevant to excitation-contraction coupling, than the rest of the charge.

Voltage-dependent charge movement first observed by Schneider and Chandler (1) in frog skeletal muscle has been considered as the most promising candidate for coupling transverse (T)-tubule excitation to the sarcoplasmic reticulum (SR), the first step in excitation-contraction (E-C) coupling. It is hypothesized that the majority of the moveable charge is involved in E-C coupling. This hypothesis is supported indirectly by the parallelisms between charge movements and contractility (2–4) as well as by the agreement between the density of charge groups and the density of feet in the triads (5). In certain experimental conditions, a secondary hump appears in the on part of charge movement (6–9). It was suggested that the hump might play a role in gating potassium channels (7), but that idea was discarded (8). This work was aimed at isolating the hump by pharmacological and mathematical means and attempting to elucidate its physiological function.

Charge movements were measured from sartorius muscle fibers of *Rana temporaria* with the three-microelectrode voltage-clamp technique. Linear capacitive and ionic currents and nonlinear sodium and potassium currents were subtracted or suppressed as usual (10). It was also necessary to abolish movement artifact by blocking fiber contraction without affecting charge movement. Solutions made hypertonic with sucrose are known to block contraction effectively; if the tonicity is kept below 2.5 times normal (equivalent to 350 mM sucrose) blocking is still effective, whereas calcium release from the SR during a single twitch does not seem to be impaired (11), although calcium release during tetanus appears to be progressively reduced by increasing tonicity (12). This could imply that during a brief depolarization the steps leading to calcium release might not be affected.

Current traces measured from a fiber bathed in 2.5× hypertonic Ringer's solution show marked humps during the decay phase of the on-part (Q_{on}) and resemble those obtained by earlier investigators (6–9). At small depolarizations, the hump is much slower and smaller than the bulk of Q_{on}. As the depolarization is increased, the hump gets larger and faster, until it finally fuses with the main Q_{on}. Thus, the two components appear to have different kinetics. To be consistent with existing convention (8), the main component and the hump will be referred to as Q_β and Q_γ, respectively.

Dantrolene sodium, which suppresses SR calcium release (13), has been widely used clinically as a muscle relaxant. This drug was used to dissect Q_γ from Q_β. Complete runs from the fiber were obtained in the control solution (2.5× hypertonic) and a test solution containing a saturated amount of dantrolene sodium (about 10–15 μg/ml). Traces before and after perfusion are shown in Fig. 1. It is obvious that Q_γ is abolished by dantrolene sodium. The voltage distribution of charge before and after perfusion can be fitted very well by the two-state model proposed by Schneider and Chandler (1). Q_{max} in the presence of dantrolene sodium is a few units (nC/μF) less than that in control solution. The difference could very well be the amount of charge carried by Q_γ. Unfortunately, the drug also slows down the kinetics of Q_β (but probably does not affect its magnitude) so that the waveform of Q_γ cannot be obtained. Besides, it is difficult to rule out whether a residual portion of Q_γ is slowed down and embedded in the decay phase of Q_β.

Tetracaine was found to interfere with SR calcium release and increase the minimum stimulus duration for threshold contraction more than 15-fold (14). Experiments were repeated with tetracaine substituted for dantrolene sodium and results are shown in Fig. 2. It can be seen that tetracaine blocks Q_γ and leaves the magnitude and kinetics of Q_β unaffected (similar results were observed by Huang

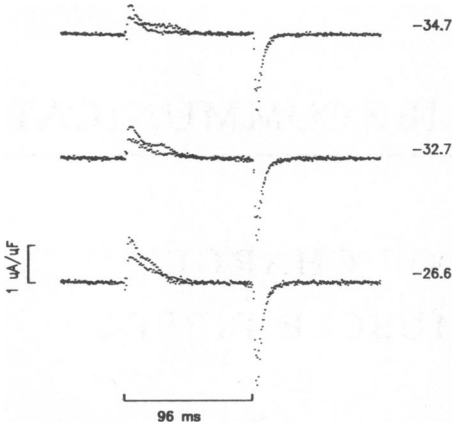


FIGURE 1 Effect of dantrolene sodium on charge movement. Traces are obtained after subtraction of linear current components arising from control and test pulses of identical amplitude. A small residual ionic component, presumably K^+, that appeared to rise linearly with time during the test pulse has been removed by subtracting a sloping baseline from the charge traces (see reference 5 for detail). Each pair of traces obtained by applying the same depolarizing pulse (indicated by numbers on the right) to the fiber in control and test solutions is superimposed for direct comparison. Fiber identification 07181. Electrode spacing, ℓ = 200μm; ℓ' = 40μm; holding potential, −80 mV; temperature, 6°C. Control solution contains 115 mM TEA Cl, 5 mM RbCl, 11.8 mM $CaCl_2$, 1 mM PIPES, 350 mM sucrose, and 10 μg/ml TTX. Test solution has, in addition, 11.3μg/ml dantrolene sodium. Test solution was made fresh just before the experiment by stirring an excess amount of the drug in the solution for at least 15 min, followed by filtering. Traces in test solution (lower one in each pair) were recorded 17–47 min after the start of perfusion with dantrolene sodium. All traces are averages of four sweeps. The ratio of Q_{on}:Q_{off} has an average value of 1.031 ± 0.020 in control solution and 0.942 ± 0.023 in dantrolene solution.

[15]). The earlier finding that tetracaine does not affect charge movement (16) is therefore only true for Q_β. Direct subtractions of pairs of traces before and after perfusion hinted that Q_γ in the control solution has roughly the shape of a symmetrical bell.

To analyze Q_γ more quantatively, mathematical dissection was performed on Q_{on} segments of charge traces obtained in the control solution. As Q_γ merges with Q_β in Q_{off} for all potentials, dissection can only be done on Q_{on}. To the lowest order of approximation, the decay phase of Q_β was assumed to follow a single exponential and Q_γ the form of a symmetrical bell. Least-squares fitting of a sum of an exponential decay and the time derivative of a logistic curve (see legend of Fig. 3) allows dissection of Q_{on} into two charge components as shown in Fig. 3*A*. The choice of the expression for fitting Q_γ appears somewhat arbitrary, but replacement of that expression by other bell-shaped forms gave essentially similar results. It can be seen that Q_γ increases in magnitude and becomes faster in kinetics with increasing depolarizations. The area of Q_γ is plotted against membrane potential in Fig. 3*B* as open circles. The points can be fitted very well by the two-state model. The best-fit values of Q_{max} and k for this experiment are 4.8 nC/μF and 4.6 mV, respectively. Values of Q_γ and Q_{total} normalized with respect to their own Q_{max} are plotted in Fig. 3*C* which shows that the activation curve of Q_γ rises at least twice as steeply as that of Q_{total}.

To assess the reliability of the mathematical dissection procedure, values of Q_γ so obtained were compared in Fig. 4 with those from pharmacological dissection with dantrolene sodium. The two sets of values agree fairly well except for the point marked with an asterisk. This indicates that the values of Q_γ obtained from the mathematical procedure are consistent with the amounts of charge abolished by dantrolene sodium. However, we cannot rule out the possibility that the two sets of data are off by exactly the same amount. The phenomenological model proposed by Horowicz and Schneider (17) fits Q_β very well, but the segment of Q_{on} in which Q_γ appears has to be excluded from the fitting. The mathematical procedure used here fits the expression to the whole decay phase of Q_{on}, although we have to commit ourselves to a specific form of Q_γ.

The average values of Q_{max} and k for Q_γ over six experiments are 5.1 nC/μF and 3.6 mV, respectively. This is intriguing because the calcium signal measured with arsenazo III also increases e-fold for every 3–4 mV (18,19). Such parallelism between Q_γ and calcium signal and similar values of Q_{max} and k for Q_γ were also observed in cut fibers[1]. Besides, all the agents that block Q_γ interfere with calcium release from the SR. It is tempting to speculate that Q_γ may be closely related to calcium release, but whether Q_γ triggers calcium release or arises as a result of the release has to be resolved by further experiments. If the latter is true, part of Q_β may still play the hypothesized role in T:SR coupling. In the other extreme, Q_γ may be solely responsible for triggering calcium release. It is natural to ask, then, what would be the role of Q_β and why is the kinetics of Q_γ so peculiar. A possible answer to these questions is related to the formation of pillars (20) between the T-tubular and SR membranes when the fiber is in the

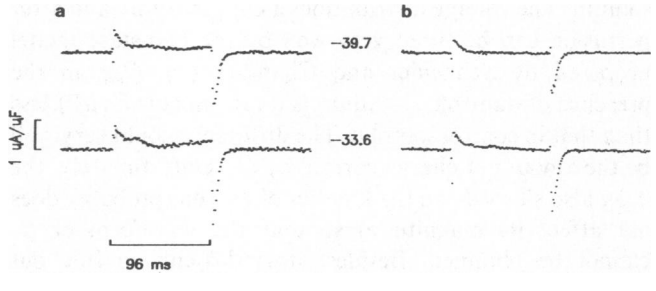
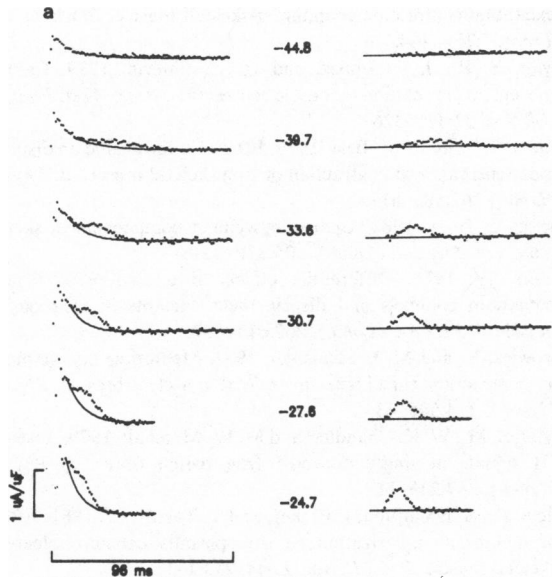
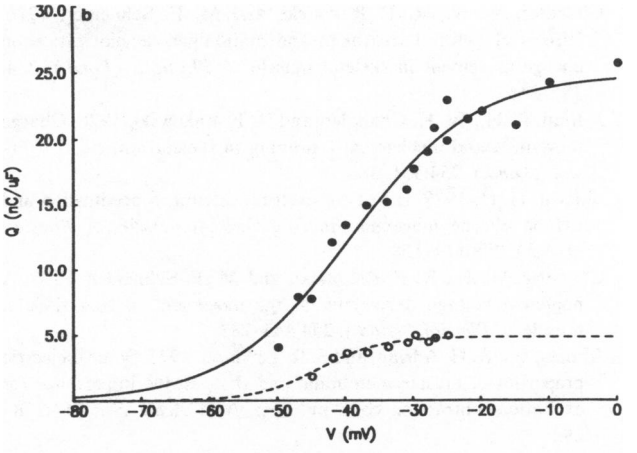


FIGURE 2 Effect of tetracaine on charge movement. Control solution is the same as in the experiment of Fig. 1. Test solution has, in addition, 4 mM tetracaine. Fiber identification 08181. Electrode spacing: ℓ = 200μm; ℓ' = 40μm; temperature, 6°C; holding potential, −80 mV. (*a*) Traces obtained by applying depolarizing pulses (indicated by numbers in the middle) to the fiber in the control solution. The nonlinear ionic currents are purposely displayed. (*b*)Traces in test solution recorded 19–20 min after the application of tetracaine. All traces are averages of four sweeps. The ratio of Q_{on}:Q_{off} has an average value of 1.032 ± 0.031 in control solution and 0.943 ± 0.059 in tetracaine solution.

[1]Vergara, J., and C. Caputo, personal communication.





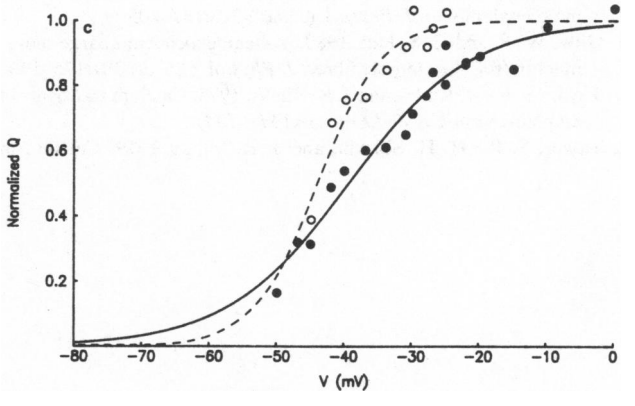


FIGURE 3 Mathematical dissection of Q_{on} in control solution. Fiber 08181. (*a*) On segments of charge movements are displayed in the left column. The expression $C_1\exp(-t/\tau_1) + C_2 \cdot d/dt\,\{1 + \exp[-(t-t_p)/\tau_2]\}^{-1}$ was fitted to the raw data by least squares. The first term corresponds to Q_β and the second to Q_γ. The smooth exponential decay curves are drawn with the values of τ_1 obtained from the fitting. Subtraction of the smooth curves from the experimental curves yields the Q_γ shown in the right column. (*b*) On areas under the Q_γ curves in *a* are plotted against membrane potential as open circles and Q_{total} ($-Q_\beta + Q_\gamma$) as filled circles. The smooth curves were fitted according to Eq. 9 of reference 5. The best-fit parameters are, for Q_{total}, $\bar{V} = -39.2$ mV, $k = 9.1$ mV, $Q_{max} = 24.7$ nC/μF; for Q_γ, $\bar{V} = -43.9$ mV, $k = 4.6$ mV, $Q_{max} = 4.8$ nC/μF. (*c*) Both sets of data are replotted after being normalized w.r.t. their Q_{max}.

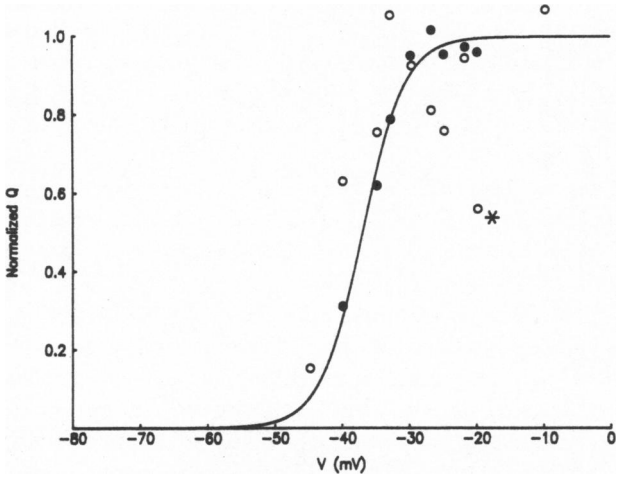


FIGURE 4 Comparison of mathematical dissection and pharmacological dissection of Q_{on}. Same experiment as Fig. 1. The on segments of charge movement traces in the control solution are dissected with the mathematical procedure described in the text and the legend of Fig. 3. The on areas under the Q_γ curve are plotted against membrane potential as filled circles. The smooth curve was fitted to the filled circles according to Eq. 9 of reference 5. The best-fit parameters are $\bar{V} = -36.9$ mV, $k = 3.2$ mV, $Q_{max} = 4.7$ nC/μF. The amounts of charge suppressed by dantrolene sodium at various potentials are shown as open circles. These values were obtained by pair-wise subtractions of the amounts of on charge at each potential before and after the application of dantrolene sodium.

active state. Part of Q_β may be responsible for the formation of the pillars. After the pillars have been formed, a true coupling signal, Q_γ, can then flow across the T-SR junction. This could explain why Q_γ peaks after Q_β begins to decay. Based on the Q_{max} value of 5.1 nC/μF and an effective valence of roughly 7 for the charge groups associated with Q_γ, a rough estimation shows that there might be about 50 groups of Q_γ/μm^2 of tubular membrane, about 1/10 of the density of Q_β-groups. The number of connected pillars is probably a small fraction of the number of feet (21) so that the density of charge groups associated with Q_γ could still agree with the density of pillars.

Acknowledgements are due to Drs. R.H. Adrian, S.M. Baylor, W.K. Chandler, C. Huang, S. Nakajima and F. Sigworth for discussions and comments on the manuscript. This work was supported by research grants from National Institutes of Health, AHA and MDA. Preliminary experiments were supported by a grant from NIH to Dr. Chandler.

Received for publication 15 October 1981 and in revised form 18 March 1982.

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