Charge movement and SR calcium release in frog skeletal muscle can be related by a Hodgkin-Huxley model with four gating particles

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ABSTRACT Charge movement currents (I_Q) and calcium transients ($\Delta[Ca^{2+}]$) were measured simultaneously in frog skeletal muscle fibers, voltage clamped in a double vaseline gap chamber, using Antipyrylazo III as the calcium indicator. The rate of release of calcium from the SR (R_{rel}) was calculated from the calcium transients using the removal model of Melzer, W., E. Rios, and M. F. Schneider (1987. *Biophys. J.* 51:849–863.). I_Q and $\Delta[Ca^{2+}]$ were calculated for 100 ms depolarizing test pulses to membrane potentials from −30 to +20 mV. To eliminate an inactivating component of R_{rel}, each test pulse was preceded by a large, fixed prepulse to +20 mV. The resulting R_{rel} records, which represent the noninactivating component of R_{rel}, were compared with $\int I_Q dt$ (Q), the total charge that moves. The voltage dependence of the steady state R_{rel} was steeper then that of Q and shifted to the right. During depolarization, the R_{rel} waveform was similar to that of Q but was delayed by several ms, while, during repolarization, R_{rel} preceded Q. All of these results could be explained with a Hodgkin-Huxley type model for *E–C* coupling in which four voltage sensors in the *t*-tubule membrane which give rise to I_Q must all be in their activating positions for the calcium release channel in the SR membrane to open. This model is consistent with the structural architecture of the triadic junction in which four dihydropyridine receptors (the voltage sensors for *E–C* coupling) in the *t*-tubule membrane are closely associated with each ryanodine receptor (the calcium release channel) in the SR membrane [Block, B. A., T. Imagawa, K. P. Campbell, and C. Franzini-Armstrong. 1988. *J. Cell. Biol.* 107:2587–2600.]). Some aspects of this work have appeared in abstract form (Simon, B. J., and D. Hill. 1991. *Biophys. J.* 59:64*a*. ([Abstr.]).

INTRODUCTION

Contraction of skeletal muscle is initiated by depolarization of the transverse tubule (*t*-tubule) system which causes opening of calcium release channels in the neighboring sarcoplasmic reticulum (SR). The precise mechanism which underlies *t*-tubule control of SR calcium release is unknown but much research has focused on the relationship between SR calcium release and charge movement (I_Q), the nonlinear current associated with voltage-dependent rearrangement of charged molecules in the *t*-tubule membrane thought to act as voltage sensors for *E–C* coupling (for reviews see Rios and Pizarro, 1991; Kovacs et al., 1979; Melzer et al., 1986; Rakowski et al., 1985; Vergara and Delay, 1986). One obstacle to comparing the activation time course of charge movement ($Q = \int I_Q dt$) and the rate of SR calcium release (R_{rel}) is that during maintained depolarizing voltage steps R_{rel} reaches an early peak and then partially inactivates (Baylor et al., 1983; Melzer et al., 1984). Thus, the overall time course of opening of SR calcium release channels is a convolution of an activation process, presumably related to charge movement, and an inactivation process, believed to be calcium-dependent and unrelated to charge movement (Schneider and Simon, 1988; Simon et al., 1991).

To compare the activation time courses of Q and R_{rel}, it is therefore necessary to either correct R_{rel} for inactivation or to eliminate inactivation. One way to generate R_{rel} records which have no inactivating component is to apply before each test pulse a large depolarizing prepulse (Simon and Schneider, 1988; Simon et al., 1991; Csernoch et al., 1991). Elevation of $[Ca^{2+}]$ during the prepulse suppresses the inactivating component of R_{rel} during the test pulse, presumably via a $[Ca^{2+}]$-dependent inactivation mechanism. The remaining R_{rel} waveform rises monotonically and represents the activation time course of the noninactivating component of R_{rel}. This technique was previously used (Simon and Schneider, 1988) to compare the activation time course of R_{rel} and Q for suprathreshold voltage steps. In those experiments, suprathreshold R_{rel} was found to have a waveform similar to that of Q but delayed by several ms, but no attempt was made to construct a model which could relate the time courses of Q and R_{rel}.

In the experiments described here, we show that both the voltage dependence of the steady-state amplitude and the activation and deactivation time courses of the noninactivating component of R_{rel} can be predicted from Q by a Hodgkin-Huxley type model in which four

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voltage-sensing molecules in the *t*-tubule membrane act in concert to gate the SR calcium release channel. This model is supported by morphological data on the microscopic structure of the triadic gap which indicates that four voltage sensors (dihydropyridine receptors) are associated with each calcium release channel (ryanodine receptor) (Block et al., 1988). Alternatively, each DHP receptor may have an intrinsic Q^4 gating mechanism (Tanabe et al., 1987, 1991) which controls one subconductance level of the ryanodine receptor.

METHODS

Experiments were carried out on single skeletal muscle fibers dissected from frog (*Rana pipiens*) ileofibularis muscles and mounted in a double vaseline gap chamber as described previously (Kovacs et al., 1983). The fibers were stretched to sarcomere lengths of 3.8–4.1 μm to eliminate movement and voltage clamped to a holding potential of −100 mV. Calcium transients ($\Delta[Ca^{2+}]$) were calculated from absorbance changes at 700 nm of the calcium indicator antipyrylazo III (APIII) which diffused into the fiber from the cut ends. The internal solution, applied to the cut ends of the fiber, contained (in mM): Cs^+ glutamate, 90; $MgCl_2$, 5.5; ATP 5 (Na^+ salt); Na^+Tris-maleate buffer, 4; Cs^+Tris-maleate buffer, 12; creatine phosphate (Na^+ salt), 5; APIII, 1; glucose, 5.5; EGTA, 0.1. The external solution, which bathed the intact portion of the fiber in the middle pool, contained (in mM): TEA_2SO_4, 75; Cs_2SO_4, 5; Na^+Tris-maleate buffer, 5; $CaSO_4$, 7.5; TTX, 10^{-7} g/ml. All solutions were adjusted to pH 7.0 at room temperature. Experiments were carried out at 7–10°C.

The rate of release of calcium from the SR (R_{rel}) was calculated according to the general approach developed by Melzer et al. (1987). Briefly, the calcium removal capabilities of each fiber were characterized by fitting a calcium removal model to the decay of $\Delta[Ca^{2+}]$ after several pulses of varying amplitudes and durations. The rate constants and concentrations of the various calcium binding proteins and the properties of the SR calcium pump determined this way were then used in the removal model to calculate R_{rel} from the $\Delta[Ca^{2+}]$ records. R_{rel} records were corrected for a slow decline due to depletion of calcium from the SR (Schneider et al., 1987).

The removal model used for the present calculations included the calcium binding properties of troponin C and parvalbumin and the SR calcium pump. The troponin C sites were assumed to be present at a concentration of 250 μM with on and off rate constants of 1.3×10^8 M^{-1} s^{-1} and 1,000 s^{-1}, respectively. The on rate constants for binding of calcium and magnesium to parvalbumin were assumed to be 1.6×10^8 M^{-1} s^{-1} and 4×10^4 M^{-1} s^{-1}, respectively (Klein et al., 1991). The SR calcium pump was assumed to be present at a concentration of 100 μM and to be in instantaneous equilibrium with Ca^{2+} with a dissociation constant of 1.5 μM. The concentration of parvalbumin, the off rate constants of calcium and magnesium binding to parvalbumin and the maximum pump rate were determined by least squares fitting of the removal model to the decay of $[Ca^{2+}]$ transients after several pulses.

Charge movement currents (I_Q) were calculated from total current recorded simultaneously with APIII absorbance changes by adding scaled control currents to each test current (Melzer et al., 1986). Sequences of test pulses were bracketed by averages of eight control pulses recorded at a gain of 3.16 times that of the gain for the test pulses to improve the signal to noise ratio of the controls. The control pulses consisted of 20 mV hyperpolarizing steps from the holding potential of −100 mV. The scaled control current consisted of a temporally weighted average of the two bracketing control currents so as to minimize any drift in the electrical properties of the fiber which may have occurred during the sequence of test pulses. Constant and slowly changing ionic currents were removed from the I_Q records by subtracting a sloping baseline fit to the last 50 ms of a 100 ms record. In the case of 40 ms pulses, the last 20 ms were used for the fit for determining the sloping baseline. For the 20 ms interval between the prepulse and test pulse no sloping baseline correction was used but the value of the current at the last point was subtracted from the record so as to force the current to be zero at the end of the 20 ms.

Optical signals and membrane currents were digitized using an INDEC BASIC-FASTLAB system controlled by a COMPAQ 386/20 computer and stored on disk for later analysis. Non-linear curve fitting was carried out using NFIT (Island Products, Galveston, TX) or MLAB (Civilized Software, Bethesda, MD).

RESULTS

Inactivation of R_{rel} by a prepulse

Fig. 1 shows $\Delta[Ca^{2+}]$ records and the corresponding R_{rel} records calculated from them for test pulses to varying potentials each preceded by a fixed prepulse to +20 mV. For a maintained step in potential R_{rel} rises to an early peak and then partially inactivates due to a $[Ca^{2+}]$-dependent mechanism. This inactivation can be seen in the R_{rel} waveform during the prepulse. If a test pulse follows the prepulse after a relatively brief delay (20 ms) the inactivating component of R_{rel} will not have time to recover and the resulting R_{rel} waveform will exhibit only

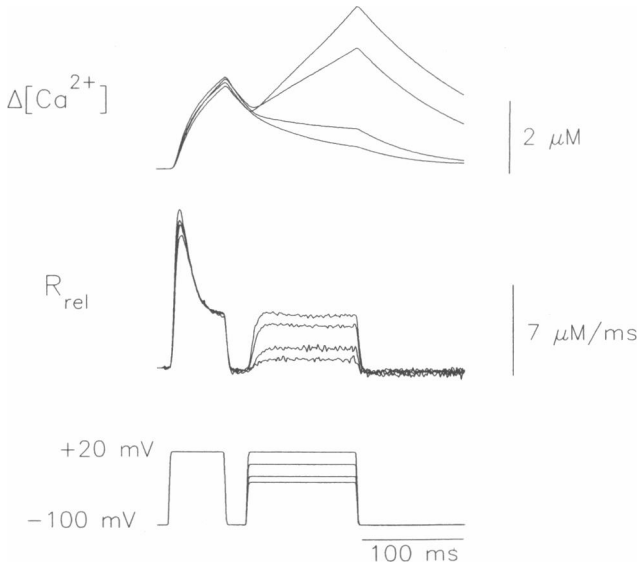

FIGURE 1 Inactivation of the peak component of R_{rel} by a prepulse. (*Top*) $\Delta[Ca^{2+}]$ records for 100 ms test pulses to −30, −20, 0, and +20 mV following a fixed 40 ms prepulse to +20 mV. (*Middle*) R_{rel} records calculated from the $\Delta[Ca^{2+}]$ records and corrected for depletion as described in Methods. Note the inactivating component of release seen in the prepulse R_{rel} is completely absent in the test pulse R_{rel} waveforms. Fiber 183; 7°C.

an activating component. The R_{rel} waveforms for such test pulses to four different membrane potentials are shown here. The test pulse R_{rel} waveforms rise monotonically, suggesting that inactivation is completely absent, and thus represent the pure activation time course of the opening of those calcium release channels in the SR which do not inactivate.

Although a monotonic rise of the test pulse R_{rel} does not guarantee that an inactivating component of release is not also present, double pulse recovery experiments suggest that this is unlikely. Recovery from inactivation of the peak R_{rel} after a large prepulse exhibits a delay of ~100 ms followed by an exponential recovery with a time constant of ~100 ms (Schneider et al., 1987). Thus, in the 20 ms between the pre and test pulses recovery should have been negligible (Simon et al., 1991).

Fig. 2 shows charge movement records obtained simultaneously with the $\Delta[Ca^{2+}]$ shown in Fig. 1. The top trace shows a single charge movement current record, I_Q, for the test pulse to −20 mV. The middle series of records are the $\int I_Q(t)\,dt\,(Q)$ calculated from I_Q records for test pulses to −30, −20, 0, and +20 mV. If I_Q represents the current resulting from movement of charged groups on the voltage sensor molecules for *E–C* coupling, and if these voltage sensors can make transitions between only two states (a resting state and an activated state), then Q represents the number of voltage sensors which become activated as a function of time. Thus, this prepulse technique allows for a direct comparison of the activation time course of the voltage sensors in the *t*-tubule membrane and the opening of the calcium channels in the SR, at least of those channels which do not inactivate.

Comparison of the voltage dependence of steady-state Q and R_{rel}

Fig. 3 is a plot of the voltage dependence of the steady-state amplitude of Q (*circles*) and R_{rel} (*squares*) calculated as the average of the last 50 ms of Q or R_{rel}, respectively, from the records shown in Figs. 1 and 2 and for additional test pulses to −40 and −60 mV. As shown previously (Melzer et al., 1986; Rakowski et al., 1985), the voltage dependence of either the peak or steady level of R_{rel} is shifted to the right compared to the voltage dependence of Q and is steeper.

If one assumes that all of Q is involved in *E–C* coupling then an explanation for the different voltage dependencies of Q and R_{rel} is that charge movement is coupled to calcium release via a sequential, multi-state mechanism in which the voltage sensors undergo two or more voltage-dependent transitions before the opening of the release channel. Such a three-state model with two voltage-dependent transitions was shown to give

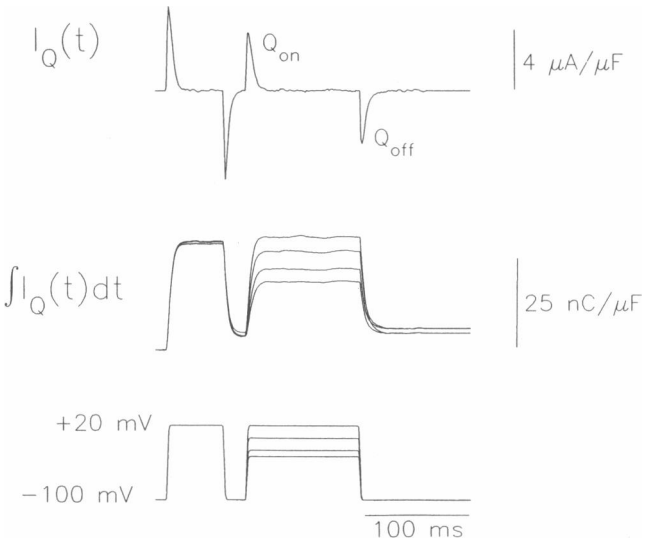

FIGURE 2 Charge movement records calculated from currents recorded simultaneously with $\Delta[Ca^{2+}]$ for the experiment described in Fig. 1. (*Top*) A single charge movement record (I_Q) for the pulse protocol shown at the bottom for the test pulse to −20 mV. (*Middle*) The integrals of I_Q for charge movement records with test pulses to −30, −20, 0, and +20 mV.

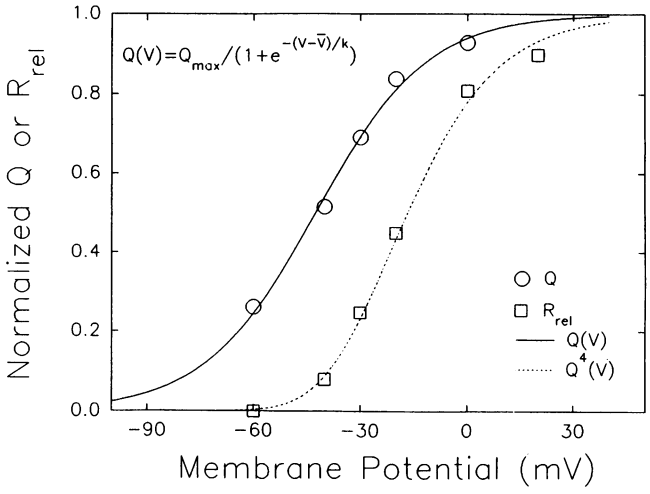

FIGURE 3 Normalized steady-state voltage dependence of total charge moved (Q) or R_{rel} for test pulses inactivated by a prepulse. (*Circles*) Voltage dependence of Q during the test step from the data in Fig. 2 and from steps to −60 and −40 mV. (*Squares*) Voltage dependence of the noninactivating component of R_{rel} during the test step. The solid and dashed lines represent the simultaneous fit of the equation of a Boltzmann two-state model (Eq. 1) to Q and the same equation raised to the fourth power fit to R_{rel}, respectively. $Q_{max} = 25.9\,nC/\mu F$; $R_{max} = 4.45\,\mu M/ms$; $k = 15.5$ mV; $\bar{V} = -42.6$ mV.

satisfactory fits to Q and R_{rel} under the same experimental conditions as those used here (Melzer et al., 1986). The authors also showed that the Q and R_{rel} data could be fit just as well assuming a Hodgkin-Huxley type model with ~3 gating particles. In a Hodgkin-Huxley model (which is a subset of a general n-state sequential model) one assumes that n identical, independent gating particles, which can each make transitions between a resting and an activated state, must all be in their activated states for the release channel to open (Hodgkin and Huxley, 1952). In this model, the probability of the release channel being open is equal to the n^{th} power of the probability of a single gating particle being activated.

The data in Fig. 3 could be well described by a Hodgkin-Huxley model with four gating particles. The solid and dashed lines in Fig. 3 represent the simultaneous fits of the equation for a Boltzmann two-state model [$Q(V)$] to charge movement, and $Q^4(V)$ to R_{rel}, respectively. The Boltzmann two-state model is given by:

$$Q(V) = \frac{Q_{max}}{1 + e^{-V-\overline{V}/k}}, \quad (1)$$

where Q_{max} is the maximum charge moved, k is the steepness factor and $\overline{V}$ is the voltage at which $Q = Q_{max}/2$. The Boltzmann two-state model assumes that all of Q is due to a single species of charge and that this charge can make voltage-dependent transitions between only two states. The values obtained for Q_{max}, R_{max}, $\overline{V}$ and k in this fiber (see Fig. 3, legend) were similar to those reported previously in cut fiber experiments from frog (Melzer et al., 1986). In similar fits in six experiments where n was allowed to vary the average values of these parameters were (mean ± SD): n = 3.76 ± .67; Q_{max} = 25.9 ± 2.16 $nC/\mu F$; R_{max} = 4.81 ± 1.77 μM/ms; $\overline{V}$ = −36.3 ± 3.64 mV, and k = 16.9 ± 2.83 mV.

Comparison of the kinetics of the activation of Q and R_{rel}

A more stringent test of the model than fits of steady-state R_{rel} and Q is a prediction of the kinetics of R_{rel} from Q during activation of release when both I_Q and R_{rel} are changing. In Fig. 4 we compare the time courses of Q and R_{rel} for the first 50 ms following the depolarizing step in potential during the test pulse. For each set of records Q and R_{rel} were scaled and superimposed. The two solid lines in Fig. 4 represent Q and Q^4 (as indicated) and the open circles the R_{rel} data. All records were scaled to the same amplitude so that the fits for the smaller, noisier records could be seen more clearly. At each membrane potential R_{rel} was well fit by the fourth power of charge movement as predicted by a Hodgkin-Huxley model with four gating particles. Similar fits were obtained in six other fibers.

Although each set of records were scaled individually, equally good fits would have been obtained using the same scaling factor for all records. This follows from the goodness of fit of Q^4 to the steady-state R_{rel} as can be seen in Fig. 3.

Comparison of the kinetics of deactivation and R_{rel}

For depolarizing steps in potential, a four-state Hodgkin-Huxley model predicts that R_{rel} should be delayed compared with Q. This is because all four gating particles must be activated before the release channel will open. Thus, movement of the first three gating particles will give rise to subthreshold charge movement before any calcium release. However, during repolarization the model predicts that R_{rel} should precede Q. When the first gating particle moves from the activated to the

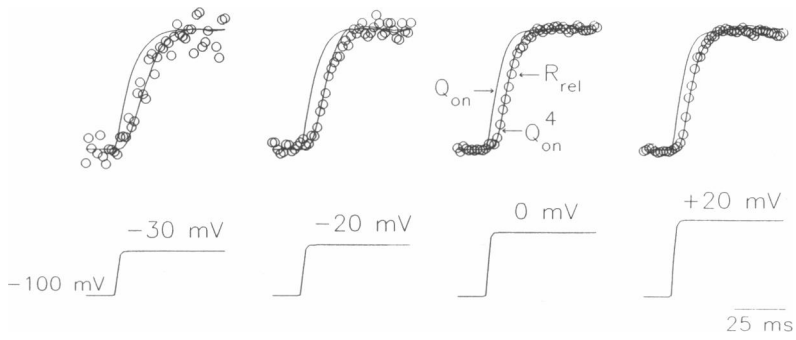

FIGURE 4 A comparison of the activation time course of $Q(t)$ and $R_{rel}(t)$ during the depolarizing step in potential. The first 50 ms during the test step in potential of $R_{rel}(f)$ and $Q(t)$ from the data in Figs. 1 and 2 were compared. For each set of records $R_{rel}(f)$ and $Q(t)$ were scaled so that the last 25 ms coincided. The solid line passing through the R_{rel} record is $Q^4(t)$. Note that during the on step $Q(t)$ precedes R_{rel}. Same fiber and conditions as in Figs. 1–3.

resting position, the calcium release channel will close, but Q will not fall to zero until all the remaining gating particles return to rest. This is precisely what is observed during the off step in potential. Fig. 5 shows a comparison of Q (*solid lines,* as indicated) and R_{rel} (*open circles*) from the data in Figs. 1 and 2 during 10 ms preceding and 50 ms after repolarization. Q and R_{rel} have been scaled so that the average values of Q and R_{rel} coincide during the 10 ms before repolarization. The decline of R_{rel} is more rapid than that of Q as predicted by a Hodgkin-Huxley model. The solid line through the R_{rel} points is Q^4 and provides an excellent fit to the time course of R_{rel}. Similar fits were obtained in six other fibers.

In some experiments, the fiber was repolarized to potentials intermediate between the test pulse potential and the resting potential (−100 mV). At these potentials the turn-off of release was slower than for steps to −100 mV but Q^4 still provided as good a fit to the decline of the R_{rel} records as those shown in Fig. 5.

Thus, a Hodgkin-Huxley model with $n = 4$ successfully predicts the time course of the non-inactivating component of R_{rel} from Q under a variety of pulse protocols during both activation and deactivation of calcium release.

DISCUSSION

In this study we have compared the steady-state voltage dependence and the time course of the noninactivating component of R_{rel} with those of Q during activation and deactivation of SR calcium release. For this comparison, the inactivating component of release was eliminated by applying a fixed prepulse before each test pulse. Both the kinetics and steady-state amplitude of R_{rel} closely followed the fourth power of Q suggesting that R_{rel} and Q can be described by a Hodgkin-Huxley type model with four gating particles.

This model differs from a classic Hodgkin-Huxley model in that in the present case the gating currents and conductance changes occur in different proteins expressed in different membranes. The voltage sensors which give rise to I_Q are in the t-tubule membrane whereas the calcium release channels are in the SR membrane. The coupling between these proteins may involve a direct mechanical link (Block et al., 1988; Caswell et al., 1990) but in our model we only assume that whatever the coupling mechanism, delays between the movement of the fourth gating particle and the opening of the release channel must be small.

The results of this study are in basic agreement with those of previous studies comparing Q and R_{rel}. Simon and Schneider (1988) compared the kinetics of activation of Q and R_{rel} for pulses preceded by a just subthreshold prepulse. Their scaled records of Q and R_{rel} are similar to those shown here in Fig. 4 and are consistent with simulations of a Hodgkin-Huxley model which includes the effects of the prepulse. Melzer et al. (1986) compared the steady-state voltage dependence of Q and peak R_{rel}. They found a good fit could be obtained with either a three-state sequential model or a Hodgkin-Huxley model with three gating particles. The smaller number of gating particles obtained in the fits to their data (3 vs 4) may be due to the fact that they used the peak R_{rel} as a measure of the degree of activation in steady-state rather than the final inactivated level used here. The peak R_{rel} represents a complicated product of activation and inactivation. Because the kinetics of these processes change differently with membrane potential,

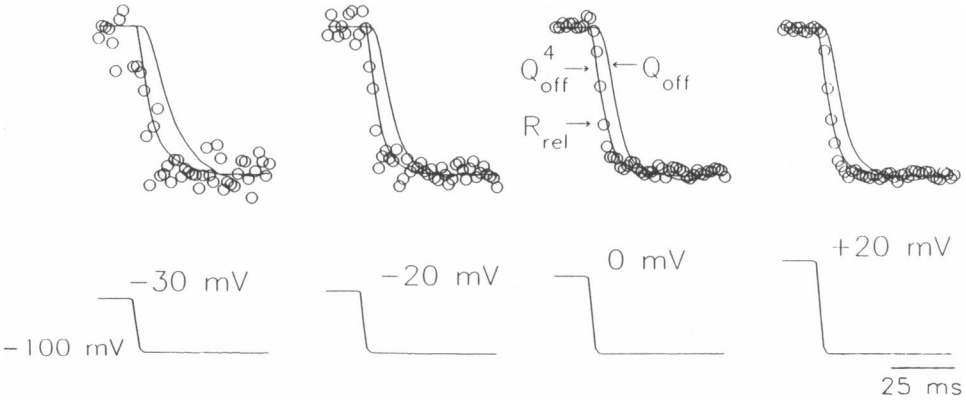

FIGURE 5 A comparison of the deactivation time course of $Q(t)$ and $R_{rel}(t)$ during the repolarizing step in potential. For each test potential the $R_{rel}(t)$ and $Q(t)$ records were scaled so that the first 10 points preceding the off step coincided. The solid line passing through the R_{rel} record is $Q^4(t)$. Note that during deactivation of the test depolarization, $R_{rel}(t)$ precedes $Q(t)$. Same fiber and conditions as in Figs. 1–4.

the actual degree of activation occurring at the peak will vary with the test pulse potential. Melzer et al. (1986) also compared Q and peak R_{rel} for short test pulses following a just subthreshold prepulse and found a linear relationship between Q and R_{rel}. A Hodgkin-Huxley model predicts a somewhat nonlinear relationship between suprathreshold Q and R_{rel} under these experimental conditions. We cannot account for this discrepancy with the predictions of our model except to suggest, as did the authors, that the peak R_{rel} may not be a faithful measure of the maximum activation, especially during the short test pulses used in the experiment.

Removal of Q_γ by the prepulse

In some charge movement experiments with cut and intact fibers, a "hump" component of charge movement, Q_γ, is observed (Huang, 1982; Hui, 1983; for review see Huang, 1989; Horowicz and Schneider, 1981) in addition to the Q_β component. It has been suggested that Q_γ is a consequence of SR calcium release, arising from a positive feed-back mechanism in which calcium released from the SR binds to the t-tubule membrane, shifting the effective voltage across it by altering the surface potential, which causes further movement of charge (Csernoch et al., 1991; Garcia et al., 1991; Szucs et al., 1991; Pizarro et al., 1991). This hypothesis is supported by the observation that the Q_γ component of charge in a test pulse can be suppressed by preceding the test pulse with a conditioning pulse and that suppression of Q_γ restores equality of Q_{on} and Q_{off}, the total charge moved after depolarization and repolarization, respectively (Csernoch et al., 1991). In our experiments and in similar experiments on cut fibers performed in other laboratories (Melzer et al., 1986), a Q_γ component is not observed in the I_Q records and small conditioning prepulses have little effect on I_Q elicited by test pulses which follow them (Simon et al., 1991). However, in our experiments with large prepulses, a small inequality of Q_{on} and Q_{off} is removed by the prepulse suggesting the presence of a small component of Q_γ during the prepulse not apparent in the I_Q records. The $\int I_Q(t)\,dt$ records in Fig. 2 show that $Q_{off} < Q_{on}$ for the prepulse but that $Q_{off} = Q_{on}$ for the test pulse. This was consistently observed in all experiments and may reflect the presence of a Q_γ component of charge movement but may also be due to inappropriate baseline subtraction or to contaminating ionic currents, a likely possibility given the large amplitude of the prepulse (Simon et al., 1991). In any case, the prepulse should have minimized any Q_γ in the test pulse simplifying models relating Q and R_{rel}.

Based on the fits of the sum of two Boltzmann distribution functions to Q vs V data, Hui and Chandler (1990) have proposed that Q_γ is not a consequence of calcium release but is a distinct component of charge movement with voltage dependence similar to that of R_{rel}. They found that the valences of Q_γ and Q_β differed by a factor of 4–7 and suggested that the Q_β component might reflect the rearrangement of a single protein molecule whereas Q_γ reflects the rearrangement of a group of four protein molecules. This interpretation would be consistent with our Q^4 model if the opening of the release channel had associated with it the movement of all four gating particles as a unit. Indeed, such a model was first proposed by Horowicz and Schneider (1981) to account for the kinetics of Q_γ. However, this model would require the additional assumption that a prepulse somehow prevents this cooperative movement because it suppresses Q_γ (Csernoch et al., 1991).

Effects of t-tubule delays on the kinetics of Q and R_{rel}

It is not possible to control precisely the membrane potential of skeletal muscle sarcolemma due to the distributed nature of the t-tubule system (Simon and Beam, 1985; Heiny and Jong, 1990). Consequently the time course of charging of the t-system is slower during the test pulse than the control pulse due to the extra nonlinear charge which moves during the test pulse. This introduces a delay in I_Q but does not affect R_{rel}. Thus, in principle, if the model were correct, Q^4 would be delayed by a few ms compared with R_{rel}. However, several factors suggest that the measured $\Delta[Ca^{2+}]$, from which R_{rel} is derived, is also several ms slower than the actual $\Delta[Ca^{2+}]$. First, the change in absorbance of AP III has a delay of $\sim$1 ms compared to the change in $[Ca^{2+}]$ (Baylor et al., 1985). Second, modeling of calcium gradients which exist in the restricted space between the t-tubule and SR membranes during release indicate that the calculated R_{rel} (neglecting these gradients) is also $\sim$1 ms delayed from the actual R_{rel} (Pizarro et al., 1991). It is possible, therefore, that these two small effects cancel each other accounting for the remarkably good fits obtained with the model.

Applicability of the model to the inactivating component of R_{rel}

The model presented here predicts the time course of the non-inactivating component of R_{rel} from the time course of Q but may not apply to the inactivating component of R_{rel}. That is, the activation time course of the inactivating component of R_{rel} may be different from that of the noninactivating component used here to compare Q and R_{rel}, despite that fact that I_Q is little affected by a prepulse (Simon et al., 1991).

An alternative way to determine the activation time

course of R_{rel} is to correct the R_{rel} waveform for inactivation rather that to eliminate it. To do this requires an appropriate model for inactivation. Schenider and Simon (1988) described a calcium-dependent inactivation model in which inactivation occurs in parallel with activation and for large pulses has an exponential time course. Using these assumptions they corrected R_{rel} records for inactivation. The corrected R_{rel} records, when scaled, were shown to be identical to those obtained if the same test pulses were preceded by a prepulse to suppress the inactivating component of R_{rel}. We also found that the corrected R_{rel} waveforms were similar to the preinactivated waveforms. We chose, however, to use the prepulse technique to compare the noninactivating component of R_{rel} with Q because it required less mathematical manipulation and also minimized any possible confounding effects of calcium release on Q (see above).

Thus, both components of R_{rel} appear to have the same activation time course and the Q^4 model described here should equally well apply to the time course of activation of the inactivating component of R_{rel}. Furthermore, deactivation of the inactivating and noninactivating components of R_{rel} also appear to have the same kinetics. If a test pulse is terminated after 20 ms at the peak of R_{rel} before significant inactivation has occurred, the resulting time course of deactivation of R_{rel} is identical to that obtained after a 100 ms test pulse when inactivation is complete (data not shown). Thus, the SR calcium release channels responsible for both the activating and nonactivating components of R_{rel} close with the same kinetics.

(Note: we have given the impression that the two components of R_{rel} correspond to two distinct populations of SR calcium release channels, one which inactivates and one which does not. Although such a mechanism has been proposed (Rios and Pizarro, 1988), the analysis and model described here apply equally well to a single class of calcium release channels which partially inactivate. In this latter case, the effect of the prepulse is to put all of the channels into this inactivated state. The calcium-dependent inactivation model of Simon et al., (1991) was consistent with either of these two alternatives).

A structural basis for four gating subunits in a Hodgkin Huxley model for *E–C* coupling

The arrangement of the DHP and ryanodine receptors at the triad suggests one possible basis for the four gating particles. Freeze fracture studies of the triadic junction indicate that the ryanodine receptor in the SR membrane, which functions as the calcium release channel (Fleischer et al., 1985; Imagawa et al., 1987; Lai et al., 1988), forms a junctional complex with tetrads of DHP receptors in the *t*-tubule membrane which act as the voltage sensors for *E–C* coupling (Rios and Brum, 1987; Block et al., 1988). It is interesting to speculate that each DHP receptor in a tetrad corresponds to one of the four gating particles and that the calcium release channel opens only when all four DHP subunits are in their activated positions. If the gating of each subunit obeys a Boltzmann two-state model, then release would follow the fourth power of charge movement as suggested by our data.

An alternative possibility is that each subunit of the DHP tetrad gates one of the four identical subunits which make up the ryanodine receptor complex and that the Q^4 dependence is an inherent property of each DHP molecule. The gating properties of the DHP receptor are thought to reside in the α_1 subunit, which contains four internal repeats (Tanabe et al., 1991; Tanabe et al., 1987). Each repeat has an S4 segment which is highly conserved across several types of voltage-gated channels and is the likely voltage sensor. Indeed, the α_1 subunit of the DHP receptor is very similar to the α_1 subunit of the sodium channel which obeys Hodgkin-Huxley kinetics with $n = 3$ (Hodgkin and Huxley, 1952). This model requires that each ryanodine receptor be gated independently by a single DHP receptor yet there are four DHP receptors associated with each ryanodine receptor. However, the functional ryanodine receptor is actually a tetramer of four identical subunits, and hence it is possible that each subunit is gated by one of the four DHP receptors. This model is consistent with studies of the ryanodine receptor complex incorporated into lipid bilayers which indicate the presence of four equally spaced conductance levels (Kwok and Best, 1990; Lai et al., 1988). The Q^4 dependence would reflect the fact that each DHP subunit gates a single subconductance state of the ryanodine receptor independently of the rest. Our data do not allow for distinction of the two models presented.

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