TIME COURSE OF ACTIVATION OF CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM IN SKELETAL MUSCLE

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ABSTRACT Myoplasmic free calcium transients were measured with antipyrylazo III in voltage clamped segments of frog skeletal muscle fibers and were used to calculate the rate of release (R_{rel}) of calcium from the sarcoplasmic reticulum. Intramembrane charge movement was measured for the same pulses in the same fibers. During a depolarizing pulse R_{rel} rose to an early peak and then decayed relatively rapidly but incompletely due to calcium-dependent inactivation (Schneider M.F., and B.J. Simon. 1988. *J. Physiol. (Lond.)*. 405:727–745). Two approaches were used to determine release activation independent of the effects of inactivation: (*a*) a mathematical correction based on the assumption that inactivation was a process occurring in parallel with and independently of activation; (*b*) an experimental procedure in which release was maximally inactivated by a large short prepulse and then the remaining noninactivatable component of release was monitored during a subsequent test pulse. Both procedures gave the same time course of activation of release. Release activation paralleled the time course of intramembrane charge movement but was delayed by a few milliseconds.

INTRODUCTION

A rise in free intracellular [Ca^{2+}] due to release of calcium from internal stores serves as the cytosolic messenger for regulation of a wide variety of functions in many types of cells (1–3). The primary signal for initiating calcium release varies among cells. In skeletal muscle calcium is stored within the sarcoplasmic reticulum (SR) and is released in response to electrical depolarization of the transverse tubular (TT) membrane, a component of the cell external membrane (4). TT control of SR calcium release is believed to begin with the voltage-dependent rearrangement of charged molecules within the TT membrane (5), which is detected electrically as intramembrane charge movement (6). During cell depolarization SR calcium release reaches an early peak but then declines (7, 8) due to a [Ca^{2+}]-dependent inactivation of release (9, 10).

In order to study the relationship between charge movement (Q) and release activation it is important to extract the activation time course from the observed release, which reflects both activation and inactivation. To this end, we report here the first determination of the release activation time course independent of the effects of inactivation and the first direct comparison of the kinetics of Q and release activation. For a variety of depolarizing pulses the time course of activation paralleled that of Q but was slightly delayed compared with Q. We also observed that both the inactivating and the noninactivating components of SR calcium release have the same time course of activation. Some aspects of this work have appeared in abstract form (11).

METHODS

A short segment of a single muscle fiber cut at both ends was isolated from a frog ileofibularis muscle and voltage clamped at a holding potential of −100 mV in a double vaseline gap chamber (12) using solutions designed to block most membrane ionic conductances (middle pool: 66.7 mM $[TEA]_2SO_4$, 20 mM $CoSO_4$, 7.5 mM $CaSO_4$, 5 mM Na Tris-maleate buffer, 10^{-7} g tetrodotoxin/ml; end pools: 102.5 mM Cs glutamate, 5.5 mM $MgCl_2$, 5 mM ATP, 1 g glucose/l, 4.5 mM Na Tris-maleate buffer, 13.2 mM Cs Tris-maleate buffer, 0.1 mM EGTA, 0.0082 mM added $CaCl_2$, 1 mM antipyrylazo III [AP III]; both pH 7.0). The calcium indicator dye AP III diffused into the fiber (12) and was used to monitor intracellular [Ca^{2+}] transients ($\Delta[Ca^{2+}]$). All procedures for electrical and optical measurements and for calculating [AP III], $\Delta[Ca^{2+}]$ and the rate of release of calcium from the SR (R_{rel}) were as previously described (13).

For determining the time course $Q(t)$ of intramembrane charge movement the capacitative transient after a 100 ms 20 mV hyperpolarization from the −100 mV holding potential was assumed to define the fiber linear capacitance. This transient was appropriately scaled and subtracted from the current transient at each edge of the depolarizing pulses (13). Ionic currents were then removed from the remaining current during each test pulse by fitting a straight sloping baseline to the points

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from 51 to 100 ms after the start of the test pulse (or from 26 to 50 ms for pulses to positive membrane potentials) and subtracting the baseline from the current throughout the test pulse. $Q(t)$ was calculated as the running integral of the remaining current.

RESULTS

Records a–c in Fig. 1 give intracellular [Ca^{2+}] transients ($\Delta[Ca^{2+}]$) obtained from a voltage clamped cut segment of a single-skeletal muscle fiber for test pulses to three different membrane potentials, each directly preceded by a 50-ms prepulse to −50 mV. The prepulse caused a barely detectable rise in [Ca^{2+}] in all three records, indicating that −50 mV was just beyond the threshold potential for activating release, but the predominant rise in [Ca^{2+}] occurred during each test pulse. Records d–f in Fig. 1 were calculated from the corresponding [Ca^{2+}] transients and give the rate of release (R_{rel}) of calcium from the SR during each test pulse. As previously described, R_{rel} reached an early peak during each test pulse but then declined markedly but incompletely during the 50 or 100 ms test pulse (7, 14). Previous results indicate that most of the decline in release during a pulse is due to a calcium-dependent partial inactivation of the SR calcium release system (9, 10) with only a minor contribution due to depletion of calcium from the SR (15).

The observed R_{rel} records could be mathematically corrected for the effects of inactivation by assuming that inactivation was an independent process that began at the start of each test pulse and followed a single exponential time course. In that case

$$R_{rel}(t) = A(t)\,[b + (1 - b)\exp(-t/\tau)], \qquad (1)$$

where $A(t)$ gives the time course of activation during the pulse, b is the noninactivatable fraction of release, τ is the time constant of inactivation, and t is the time from the start of the test pulse.

According to Eq. 1, once activation were complete $A(t)$ would be constant and $R_{rel}(t)$ would decline along a single exponential time course to $A(\infty)b$ with time constant τ. A single exponential plus a constant was therefore fit to the latter part of each R_{rel} record in Fig. 1 to obtain values of τ and b for that pulse and $A(t)$ during the pulse was calculated as $R_{rel}(t)/[b + (1 - b)\exp(-t/\tau)]$. The resulting records of $A(t)$ are shown as g–i of Fig. 1, with the vertical scale compressed compared with d–f. The uncorrected $R_{rel}(t)$ record is superimposed on each $A(t)$ record in Fig. 1.

Because the mathematical procedure used to correct R_{rel} for inactivation was somewhat arbitrary we devised an alternative experimental procedure for determining the release activation time course. The rationale was to first maximally inactivate release by raising [Ca^{2+}] (10) and then to monitor the time course of activation of the remaining noninactivatable component of calcium release. Records a and c in Fig. 2 show $\Delta[Ca^{2+}]$ and R_{rel} for the same pulse protocol as used in Fig. 1. Records b and d are for the same prepulse/test pulse combination, but now directly preceded by an extra 20 ms pulse to +50 mV (*bottom*). The extra pulse raised [Ca^{2+}] (Fig. 2 *b*), which remained elevated throughout the subsequent prepulse. The large increase in R_{rel} produced by the extra pulse (Fig. 2 *d*) turned off nearly completely by the end of the prepulse. Then, during the test pulse, R_{rel} increased essentially monotonically (Fig. 2 *d*) without exhibiting the early peak and marked decline characteristic of the test pulse R_{rel} record without the extra pulse (Fig. 2 *c*). If the elevation of [Ca^{2+}] produced by the extra pulse were sufficient to produce maximal inactivation, R_{rel} during the test pulse after the extra pulse would give the pure time course of activation of the remaining noninactivatable component of release.

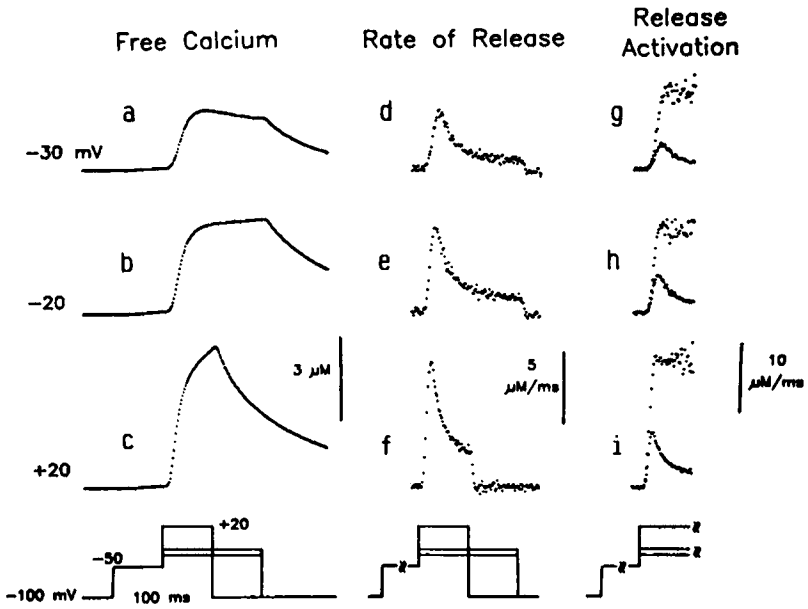

FIGURE 1 Activation of calcium release from the sarcoplasmic reticulum in a skeletal muscle fiber. (*a–c*) [Ca^{2+}] transients for test pulses to the indicated potentials, each immediately preceded by a prepulse to −50 mV (pulse protocol shown schematically below the records). (*d–f*) Rate of release of calcium from the SR during each test pulse (*bottom*) as calculated from the corresponding $\Delta[Ca^{2+}]$ records in *a–c*. R_{rel} was calculated assuming the fiber to have a linear instantaneous intrinsic calcium buffering capacity (E_1) (12) of 10. (*g–i*) Time course of the activation process $A(t)$ for SR calcium release calculated from each of the R_{rel} records in *d–f* according to Eq. 1. The peaked record below each $A(t)$ is R_{rel} from *d–f* replotted at the vertical scale of $A(t)$. The peak value of each observed R_{rel} was somewhat less than half the final level of $A(t)$, which is the release predicted to be attained in the absence of inactivation. Note that the vertical scale has been compressed by a factor of 2.3 in the right column compared with the middle column. Each data point is the average value of the signal over a 1 ms sampling period (13). Fiber 352, 95 μm diameter, 600–645 μM antipyrylazo III (7°C). Fiber stretched to eliminate movement but sarcomere length not measured.

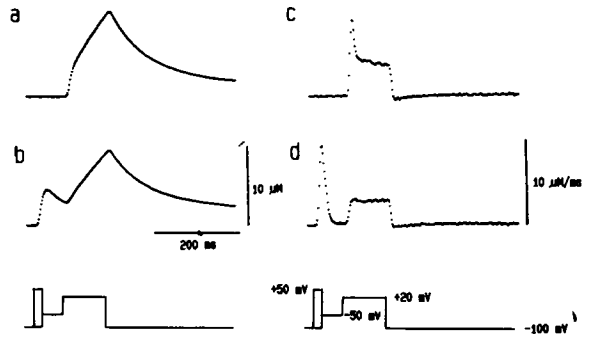

FIGURE 2 Protocol for determining the time course of activation of the noninactivatable component of SR calcium release. (*a* and *c*) $[Ca^{2+}]$ transient and R_{rel} for the same pulse protocol as used in Fig. 1. b, d, $\Delta[Ca^{2+}]$, and R_{rel} for the same pre and test pulse as in *a* and *c* but now immediately preceded by a 20 ms extra pulse to +50 mV (*bottom*). E_1 was assumed to equal 20 for calculation of R_{rel}. Each data point is the average value of the signal over a 2 ms sampling period. Fiber 343, 82 μm diameter, 3.9 μm/sarcomere, 380–394 μM AP III (7°C).

Fig. 3 compares the time course of activation of the noninactivatable component of release with the time course of activation of the total release. For each test pulse examined the time courses of activation of total and noninactivatable release were essentially the same. This finding indicates that the mathematical correction procedure used for Fig. 1 was probably valid. It also shows that if the inactivatable and noninactivatable components of release correspond to two different types of SR calcium channels (16, 17), then the two types of channels must have had the same activation time courses over the range of membrane potentials examined in Fig. 3.

Having developed a procedure for determining the time course of activation of SR calcium release it was of interest to examine the temporal relationship between intramembrane charge movement $Q(t)$ and release activation $A(t)$. Records *a–c* in Fig. 4 repeat the $A(t)$ records from Fig. 1 (*g–i*) and records d and e in Fig. 4 give $A(t)$ from another fiber using the same pulse protocol. Records *f–j* in Fig. 4 give $Q(t)$ from the same fibers for the same test pulses (indicated) as used for $A(t)$. $Q(t)$ was measured after the same 50 ms prepulse as used for determining $A(t)$. The right column in Fig. 4 presents superimposed records of $Q(t)$ and $A(t)$ during each of the test pulses. In all cases release activation was delayed by several milliseconds compared with charge movement. After this delay, release

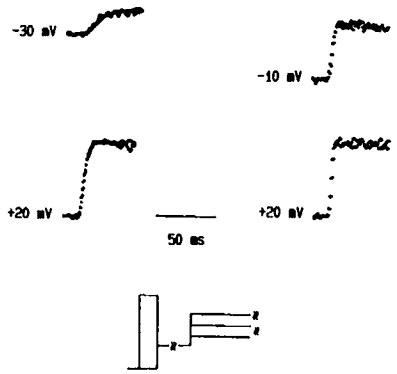

FIGURE 3 Comparison of the time courses of activation of the total SR calcium release and the noninactivatable component of SR calcium release. $A(t)$ was determined in two different fibers (*left and right*) for test pulses to the indicated potentials, each immediately preceded by a 50 ms prepulse to −50 mV (*bottom*). $A(t)$ is shown only for the period during and immediately preceding the test pulse (i.e., between the breaks in the pulse schematic). Noninactivatable release for each test pulse was determined as the release remaining after a 20 ms extra pulse to +50 mV directly preceeded the prepulse (pulse protocol of Fig. 2). Activation of total release for a test pulse to the same potential was determined from R_{rel} records without the extra pulse using the mathematical procedure of Fig. 1 to correct the observed release for inactivation. The records of total $A(t)$ obtained for each fiber for the pulse to +20 mV were arbitrarily scaled to the same steady-state value and the total $A(t)$ for the smaller pulse in each fiber was scaled by the same factor. Since release was still slightly activated before the test pulse after the extra pulse (Fig. 2), the baseline of each noninactivatable release record was set to zero and the record was then scaled to superimpose on the record of $A(t)$ for total release. (*Left*) Fiber 323, 80 μm diameter, 3.8 μm/sarcomere, 529–566 μM AP III (7.5°C). E_1 assumed to equal 10 for calculating R_{rel}. For this fiber the external solution contained 75 mM $(TEA)_2SO_4$, 5 mM $CsSO_4$, and no $CoSO_4$. (*Right*) Same fiber and conditions as Fig. 2.

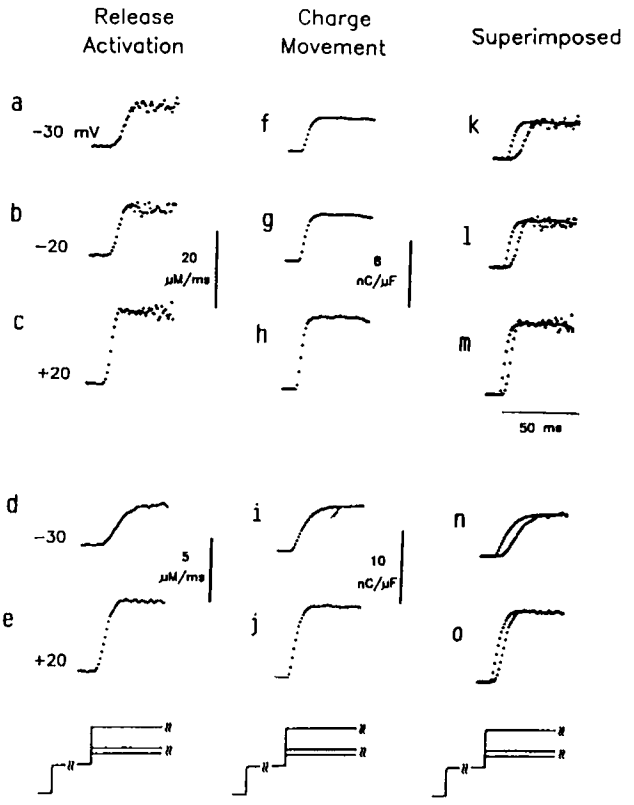

FIGURE 4 Comparison of the time courses of intramembrane charge movement and activation of SR calcium release. (*a–e*) $A(t)$ for test pulses to the indicated membrane potentials for the same pulse protocol (*bottom*) and calculation procedure as used in Fig. 1. (*f–j*) Charge movement time courses $Q(t)$ for test pulses to the same potentials and using the same pulse protocol as the corresponding $A(t)$ records in *a–e*. (*k–o*) Superimposed $Q(t)$ (earlier rise) and $A(t)$ records from columns on the left. (*Top*) Same fiber and conditions as Fig. 1. (*Bottom*) Same fiber and conditions as in Fig. 3, (*left*).

activation rose along a time course that roughly paralleled charge movement. The delay between charge movement and release activation decreased with increasing test pulse depolarization.

The $Q(t)$ records in Fig. 4 show only the charge moved during the test pulse even though the prepulses used for Fig. 4 moved considerable charge. We have omitted the prepulse charge from our comparison of $Q(t)$ and $A(t)$ because the prepulse charge cannot be a significant part of the final TT voltage-sensitive step leading to release activation since the prepulse itself produced almost no release activation. However, previous results indicate that the prepulse charge movement may result from a preliminary transition that must occur before the final release-controlling charge movement transition (13, 18, 19). In fact, the presence of a preliminary charge transition that precedes the charge movement step that actually controls release may account for part of the delay between the Q and release activation records in Fig. 4. The charge that moves first during the test pulses may have been predominantly charge from preliminary transitions that was not displaced by the prepulse. Movement of preliminary transition charge during the test pulse would not directly begin the release activation process but would be required before the subsequent occurrence of the final charge transition that actually controls release activation.

DISCUSSION

We have previously shown that inactivation of calcium release from the SR can be well described in terms of a single type of SR calcium channel that exhibits partial inactivation by a calcium-dependent process that occurs in parallel with activation (10). This model predicts that the time course of activation would be the same for the total and the noninactivatable components of release, as was observed here. Our model for inactivation consists of two steps, the first being calcium binding to a rapidly equilibrating high affinity site and the second being a slower transition of the calcium site complex to the inactivated state (10). With these properties, once $[Ca^{2+}]$ is slightly elevated essentially all sites are occupied and inactivation follows an exponential time course with a time constant determined by the rate constants of the second step. Thus, Eq. 1 would apply to this particular model for inactivation over all but the earliest part of each of the test pulses in Fig. 1. We have elected to use Eq. 1 to mathematically correct for release activation rather than using the more complete model just described in order to make the calculated release activation wave form as model-independent as possible. The mathematical correction procedure appears to be valid since our experimental method for determining release activation gave time courses essentially the same as those obtained with the mathematical method. An interesting corollary of our results is that both the inactivatable and noninactivatable components of SR calcium release follow the same time course of activation.

Having developed a procedure for determining the time course of the activation process for SR calcium release, we were for the first time able to compare the time course of the activation process with the time course of charge movement, the supposed voltage sensor for release. Since an appreciable fraction of the total charge movement is not directly involved in the final transition that actually controls opening of the SR channels (13), we attempted to compare release activation with the charge movement of the final transition. Thus, an important underlying aspect was the partial separation of the charge movement that was involved in the final transition from charge movement that arose from preliminary transitions of the voltage sensor for SR calcium release (13) or from other charge transitions unrelated to calcium release. Such partial separation is possible in skeletal muscle because the midpoint voltages of the final and preliminary transitions appear to differ by ~30 to 50 mV (13). The separation was achieved by directly preceding each test pulse by a prepulse to the voltage at which release just began to be activated. The prepulse should have moved much of the charge that is preliminary or unrelated to release activation while moving little of the charge of the final transition so that the charge remaining to be moved during the test pulse should have been predominantly charge of the final transition that actually activates release. Under these conditions we observed that during the test pulse the time course of release activation paralleled the remaining charge movement but was slightly delayed compared with the charge. The delay between charge movement and release activation could represent the time for TT to SR signal transmission to occur or could arise partially from the time required to move any remaining charge from preliminary transitions. Thus the observed delay should represent an upper limit on the time for TT to SR signal transmission, whether that transmission be by a diffusable chemical messenger, by a macromolecular mechanical coupling system, or by any other mechanism. We anticipate that subsequent studies will make progress toward defining the events occurring between charge movement and release activation.

This work was supported by research grants from the National Institutes of Health (R01-NS23346) and the Muscular Dystrophy Association. Dr. Simon was the recipient of a National Institutes of Health fellowship (IF32-AM07267).

Received for publication 13 May 1988 and in final form 22 August 1988.

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