A Slow Component of Intramembranous Charge Movement during Sarcoplasmic Reticulum Calcium Release in Frog Cut Muscle Fibers

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ABSTRACT Cut muscle fibers from *Rana temporaria* were mounted in a double Vaseline-gap chamber and equilibrated with an end-pool solution that contained 20 mM EGTA and 1.76 mM Ca (sarcomere length, 3.3–3.8 μm; temperature, 14–16°C). Sarcoplasmic reticulum (SR) Ca release, $\Delta[Ca_T]$, was estimated from changes in myoplasmic pH (Pape, P.C., D.-S. Jong, and W.K. Chandler. 1995. *J. Gen. Physiol.* 106:259–336). The maximal value of $\Delta[Ca_T]$ obtained during a depleting depolarization was assumed to equal the SR Ca content before stimulation, $[Ca_{SR}]_R$ (expressed as myoplasmic concentration). After a depolarization to −55 to −40 mV in fibers with $[Ca_{SR}]_R$ = 1,000–3,000 μM, currents from intramembranous charge movement, I_{cm}, showed an early I_β component. This was followed by an I_γ hump, which decayed within 50 ms to a small current that was maintained for as long as 500 ms. This slow current was probably a component of I_{cm} because the amount of OFF charge, measured after depolarizations of different durations, increased according to the amount of ON charge. I_{cm} was also measured after the SR had been depleted of most of its Ca, either by a depleting conditioning depolarization or by Ca removal from the end pools followed by a series of depleting depolarizations. The early I_β component was essentially unchanged by Ca depletion, the I_γ hump was increased (for $[Ca_{SR}]_R > 200$ μM), the slow component was eliminated, and the total amount of OFF charge was essentially unchanged. These results suggest that the slow component of ON I_{cm} is not movement of a new species of charge but is probably movement of Q_γ that is slowed by SR Ca release or some associated event such as the accompanying increase in myoplasmic free [Ca] that is expected to occur near the Ca release sites. The peak value of the apparent rate constant associated with this current, 2–4%/ms at pulse potentials between −48 and −40 mV, is decreased by half when $[Ca_{SR}]_R \cong$ 500–1,000 μM, which gives a peak rate of SR Ca release of ~5–10 μM/ms.

INTRODUCTION

Contraction in vertebrate skeletal muscle is activated by Ca, which is released from the sarcoplasmic reticulum (SR) into the myoplasm, where it can bind to the Ca-regulatory sites on troponin. During the past 30 years, many properties of SR Ca release have been elucidated from studies on intact, cut, and skinned fibers, on isolated triads and SR vesicles, and on single SR Ca channels incorporated into lipid bilayers, as reviewed by Ríos and Pizarro (1991), Schneider (1994), Meissner (1994), and Franzini-Armstrong and Jorgensen (1994). The studies in intact and cut fibers have shown that the rate of Ca release from the SR into the myoplasm is under the control of the voltage across the membranes of the transverse tubular system. With response times of only a few milliseconds, depolarization can turn on release and repolarization can turn it off. Two proteins, which are closely apposed at the triadic junction (Block et al., 1988), play important roles in this process: the dihydropyridine receptor (DHPR), which forms the voltage sensor in the tubular membrane (Ríos and Brum, 1987; Tanabe et al., 1988) and appears to underlie the Q_γ component of charge movement (Huang, 1990;

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Chen and Hui, 1991), and the ryanodine receptor (RyR), which forms the Ca channel in the SR membrane (Imagawa et al., 1987; Hymel et al., 1988; Lai et al., 1988).

Although tubular membrane potential plays the major role in the control of SR Ca release, it has become clear that other factors are also important. One such factor is myoplasmic Ca. Studies on skinned fibers have shown that Ca ions, when added to the solution bathing a fiber, can initiate SR Ca release (Endo et al., 1968; Ford and Podolsky, 1968), an effect usually called Ca-induced Ca release. On the other hand, after the SR has released sufficient Ca to complex the Ca-regulatory sites on troponin and thereby provide activation for contraction, the rate of release is decreased in a Ca-dependent manner, an effect usually called Ca inactivation of Ca release (Baylor et al., 1983; Simon et al., 1985; Schneider and Simon, 1988). These mechanisms provide positive and negative feedback between myoplasmic Ca and SR Ca release: under some conditions, Ca can open channels that are closed whereas, under other conditions, it can close channels that are open.

In addition to these effects of myoplasmic Ca on Ca movements through RyR Ca channels, myoplasmic Ca also appears to be able to influence intramembranous charge movement currents, I_{cm}, which arise from movements of DHPRs as they activate SR Ca channels. The first demonstration of such an effect was reported in a series of articles on voltage-clamped frog cut muscle fibers by Csernoch et al. (1991), García et al. (1991), Szücs et al. (1991), and Pizarro et al. (1991). These authors found that, during a step depolarization, I_{cm} consisted of two components, I_β and I_γ, as first described by Adrian and Peres (1977, 1979) in intact fibers and subsequently studied in several other laboratories. The I_β component increased rapidly after depolarization and then decayed with an approximately exponential time course. If the pulse potential was more positive than −60 to −50 mV, the I_β component was followed by a delayed I_γ "hump." Since maneuvers that reduced SR Ca release were found to reduce the I_γ hump, Pizarro et al. (1991) proposed that I_γ is a component of I_β that is caused by SR Ca release.

More recently, Jong et al. (1995*b*) studied I_{cm} in fibers in which SR Ca release had been eliminated by Ca depletion. To achieve this condition, cut fibers were equilibrated with Ca-free internal and external solutions; in addition, the internal solution contained 20 mM EGTA. After an equilibration period of at least an hour, the SR was depleted of any remaining readily releasable Ca by repeated depolarizations. In this Ca-depleted state, I_{cm} still consisted of distinct I_β and I_γ components, leading Jong et al. (1995*b*) to conclude that, contrary to the proposal of Pizarro et al. (1991), I_γ is not a component of I_β that is caused by SR Ca release. Rather, I_γ appears to represent a distinct species or transition(s) of charge movement.

Although I_γ humps can occur in the absence of SR Ca release, Jong et al. (1995*b*) found that their ON kinetics is accelerated by the presence of small amounts of Ca inside the SR (tens of micromolar referred to myoplasmic concentration), at least during pulses to −50 to −30 mV. They suggested that the acceleration of the ON kinetics of I_γ is produced by SR Ca release and the accompanying increase in free [Ca] that is expected to occur at myoplasmic sites near open RyR channels; the acceleratory effect is half maximal when the SR contains sufficient Ca for the peak rate of Ca release to be ~1 μM/ms (Jong et al., 1995*b*).

Thus, under appropriate conditions, Ca appears to be able to influence SR Ca release in three different ways: (*a*) Ca-induced Ca release, (*b*) Ca inactivation of Ca release, and (*c*) acceleration of the ON kinetics of I_γ. These represent positive (*a*) and negative (*b*) feedback between [Ca] and Ca movement through Ca channels of the RyR and positive feedback (*c*) between [Ca] and I_γ. Another combination, negative feedback between [Ca] and I_γ, is the subject of this article.

In the experiments described in this article, fibers were equilibrated with an internal solution that contained 20 mM EGTA plus 1.76 mM Ca so that the Ca content of the SR would be 1,000–3,000 μM (referred to myoplasmic concentration). After a small depolarization to near the mechanical threshold (for example, to −60 mV), I_{cm} consists of an early I_β component followed by a slow I_γ component, which can last as long as 100–300 ms (Adrian and Huang, 1984; Hui and Chandler, 1991; Jong et al., 1995*b*). If the pulse potential is made more positive (for example, to −55 to −40 mV), the I_β component is followed by the beginning of an I_γ hump. After the rate of SR Ca release increases to several micromolar per millisecond, however, a new feature is revealed: the amplitude of the hump is rapidly reduced and a slow component of current becomes apparent. This current, which can last as long as 500 ms, appears to be caused by intramembranous charge movement and, in particular, by I_γ. This delayed slowing of the ON kinetics of I_γ becomes apparent somewhat later than the acceleration of the ON kinetics described above, as reported by Jong et al. (1995*b*). As a result, after a step depolarization, the ON kinetics of I_γ first accelerates and then, after ~10 ms, slows down. Throughout this article, the term slow component of charge movement (or I_{cm} or I_γ) will be used to denote this delayed I_γ current that appears to be associated with SR Ca release.

A preliminary report of some of these results has been presented to the Biophysical Society (Pape et al., 1992).

METHODS

The experiments were carried out at 14–16°C on cut muscle fibers stretched to a sarcomere length of 3.3–3.8 μm and mounted in a double Vaseline-gap chamber. The only difference between the experimental procedure used here and that used by Jong et al. (1995*b*) is that, in the present study, all fibers were initially equilibrated with an end-pool solution that contained 1.76 mM Ca (see below) rather than one that was Ca-free. The methods used for the determination of intramembranous charge movement are described by Chandler and Hui (1990) and Hui and Chandler (1990, 1991). The methods used for the estimation of SR Ca release with EGTA-phenol red are described in Pape et al. (1995). Throughout this article, a 0.05-kHz digital Gaussian filter (Colquhoun and Sigworth, 1983) was used to filter the traces of SR Ca release. This had little effect on the time course of the rate of release; for example, in the $d\Delta[Ca_T]/dt$ traces in Figs. 1 and 2, the filter decreased the peak value by 3 and 5%, respectively, and increased the time-to-peak by 0.6 and 0.3 ms. The same filter was used for the I_{cm} traces before k_{app}, the apparent rate constant for charge movement, was calculated (see Figs. 12–14).

The composition of the end-pool solution was 45 mM Cs-glutamate, 20 mM Cs_2EGTA, 6.8 mM $MgSO_4$, 5.5 mM Cs_2-ATP, 20 mM Cs_2-creatine phosphate, 5 mM Cs_3-phospho(enol)pyruvate, and 5 mM 3-[N-morpholino]-propanesulfonic acid (MOPS). 1.76 mM Ca was added to the solutions that were used for the initial period of fiber equilibration; during the course of some experiments, as described in the text and figure legends, this solution was changed to one that contained 0 mM Ca. The pH was adjusted to 7.0 by the addition of CsOH, and the calculated concentration of free Mg was 1 mM (Pape et al., 1995). The composition of the central-pool solution was 110 mM TEA-gluconate, 10 mM $MgSO_4$, 1 μM tetrodotoxin, and 10 mM MOPS, pH = 7.1; it was nominally Ca-free. The holding potential was −90 mV.

The difference between the mean values of two sets of results was assessed with Student's two-tailed *t* test and considered to be significant if $P < 0.05$.

RESULTS

Slow Component of I_{test}-$I_{control}$ during a Voltage Pulse to −45 mV

Fig. 1 shows an example of the slow component of I_{test}-$I_{control}$ that was observed during voltage steps in the range −55 to −40 mV in fibers equilibrated with 20 mM EGTA plus 1.76 mM Ca. The top trace in *A* shows V_1, the voltage recorded in end pool 1, during an 800-ms pulse from −90 to −45 mV. The second trace shows I_{test}-$I_{control}$. I_{test} denotes the current recorded during the step to −45 mV. $I_{control}$ denotes the current that was constructed for the same voltage change from the average of eight records taken during control steps from −110 to −90 mV, applied just before the test pulse (Hui and Chandler, 1990). I_{test}-$I_{control}$ is expected to consist of I_{cm}, the current from intramembranous charge movement, plus any ionic component that would arise if the current-versus-voltage relation between −110 and −45 mV deviated from ohmic behavior. Possible contributions from Ca current across the surface and transverse tubular membranes have been eliminated by the use of a Ca-free external solution.

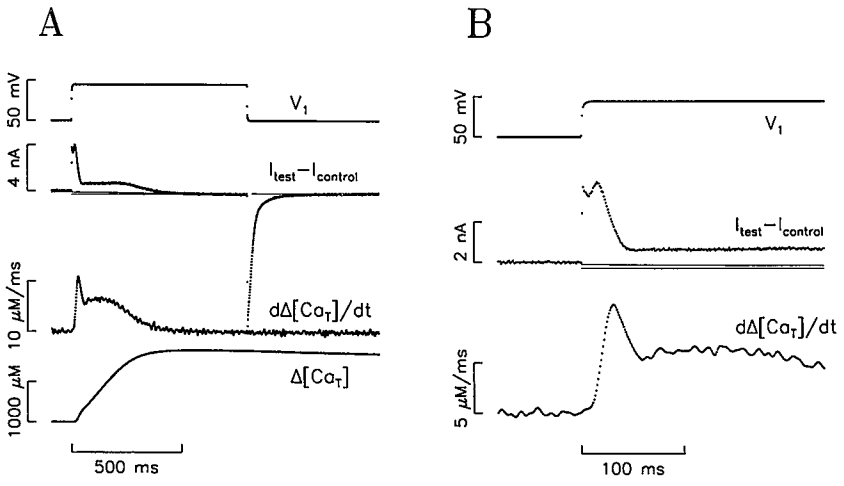

FIGURE 1. Currents from intramembranous charge movement and SR Ca release during an 800-ms depolarization to −45 mV. (*A*) The top trace shows V_1. The next trace shows I_{test}-$I_{control}$. The steady levels during and after the pulse, −0.29 and −0.22 nA, respectively, were estimated from the mean values during the final 48 ms of the corresponding segments of the trace; these are indicated by thin horizontal lines during and after the pulse. The values of ON and OFF Q_{cm} calculated on the basis of these steady state values of ionic current are 27.46 and −22.75 nC/μF, respectively. The upper line plotted during the pulse represents a least-squares fit of a sloping line through the final 48 ms of I_{test}-$I_{control}$ with the constraint that ON Q_{cm} = −OFF Q_{cm} = 22.75 nC/μF; the initial and final values of the line are −0.10 and −0.30 nA, respectively. The bottom two traces show $d\Delta[Ca_T]/dt$ and $\Delta[Ca_T]$, as labeled. (*B*) The initial segments of the V_1, I_{test}-$I_{control}$, and $d\Delta[Ca_T]/dt$ traces in *A* are plotted on an expanded time scale with increased gains for I_{test}-$I_{control}$ and $d\Delta[Ca_T]/dt$. Fiber reference, 412911; time after saponin treatment, 77 min; sarcomere length, 3.4 μm; fiber diameter, 128 μm; holding current, −33 nA; C_{app}, 0.01563 μF; r_i, 1.97 MΩ/cm; c_m, 0.216 μF/cm; concentration of phenol red at the optical site, 1.337 mM; estimated pH_R and free $[Ca]_R$, 6.869 and 0.066 μM, respectively; $[Ca_{SR}]_R$, 1,787 μM; interval of time between data points, 0.96 ms; temperature, 14°C. The end-pool solution contained 1.76 mM total Ca. In this and subsequent figures, C_{app} represents the apparent capacitance of the fiber, r_i represents the internal longitudinal resistance per unit length of fiber, and c_m represents the capacitance of the surface (including tubular) membranes per unit length of fiber; pH_R and $[Ca]_R$ represent the resting values of pH and myoplasmic free [Ca], respectively.

The thin straight lines show estimates of the ionic component, as described in a following section. This component is relatively small so that the time course of I_{test}-$I_{control}$ is determined mainly by I_{cm}.

After depolarization, I_{test}-$I_{control}$ increased to an early first peak followed by a dip and then a second peak, typical of the characteristics usually attributed to the I_β and I_γ components of I_{cm} (Adrian and Peres, 1977, 1979). These features are shown more clearly in Fig. 1 *B*, where the traces are plotted with expanded gain and time scale. Soon after the second peak, the current decreased fivefold to a small value, ~0.6 nA, that was maintained for ~200 ms before it started its gradual decline to a new steady level just below the prestimulus level (Fig. 1 *A*). This slow component appears to depend on the presence of Ca inside the SR, since it was never observed in fibers in which the SR had been depleted of its readily releasable Ca (see Figs. 5 and 9; Jong et al., 1995*b*). After repolarization, the inward tail of I_{test}-$I_{control}$ decayed rapidly to a value near the prestimulus level (Fig. 1 *A*).

The bottom trace in Fig. 1 *A* shows $\Delta[Ca_T]$, the estimated amount of Ca that had been released from the SR (expressed as myoplasmic concentration). After depolarization, $\Delta[Ca_T]$ progressively increased during the pulse and reached a plateau value of 1,787 μM after ~500 ms. After repolarization, $\Delta[Ca_T]$ remained elevated and decreased very slowly, as is typical of fibers equilibrated with 20 mM EGTA (Pape et al., 1995). The value of $[Ca_{SR}]_R$, the amount of readily releasable Ca that was inside the SR before stimulation (expressed as myoplasmic concentration), is assumed to be equal to the plateau value of $\Delta[Ca_T]$, 1,787 μM. This value is somewhat smaller than those reported by Pape et al. (1995) in fibers equilibrated with the same internal and external solutions. The reason is that, in the experiments reported in Pape et al. (1995), the duration of the recovery period between successive depleting depolarizations was 5 min instead of 2 min, so that the SR was able to reaccumulate more Ca from the myoplasm.

The third trace in Fig. 1 *A* and the bottom trace in Fig. 1 *B* show $d\Delta[Ca_T]/dt$, the estimated rate of SR Ca release. After depolarization, $d\Delta[Ca_T]/dt$ rapidly increased and reached a peak value of ~11 μM/ms. The time of peak $d\Delta[Ca_T]/dt$ was 15–20 ms after that of the I_{test}-$I_{control}$ trace. The $d\Delta[Ca_T]/dt$ trace then rapidly decreased to a local minimum of ~6 μM/ms. This decrease, which occurred at about the same rate as that of the I_{test}-$I_{control}$ trace, is similar to that observed in other experiments in fibers equilibrated with 20 mM EGTA and 1.76 mM Ca (Pape et al., 1995; Jong et al., 1995*a*) and attributed to Ca inactivation of Ca release (Baylor et al., 1983; Simon et al., 1985; Schneider and Simon, 1988; Simon et al., 1991; Jong et al., 1995a). After the early minimum, $d\Delta[Ca_T]/dt$ increased slightly and remained elevated for ~150 ms before eventually returning to zero. The return of $d\Delta[Ca_T]/dt$ to zero occurred at about the same time that I_{test}-$I_{control}$ decayed to its new steady level at the end of the pulse (Fig. 1 *A*).

In Some Experiments, a Local Minimum in I_{test}-$I_{control}$ Preceded the Slow Component

Fig. 2 shows a set of traces from another fiber, plotted and analyzed as in Fig. 1 (see legend for values of parameters). In this experiment, a local minimum (see arrow) occurred in the I_{test}-$I_{control}$ trace 10–15 ms after the peak in the $d\Delta[Ca_T]/dt$ trace. This minimum clearly separates the early rapid decrease in current and the prominent slow component. Such minima were observed in three of the 10 fibers used for the experiments reported in this article.

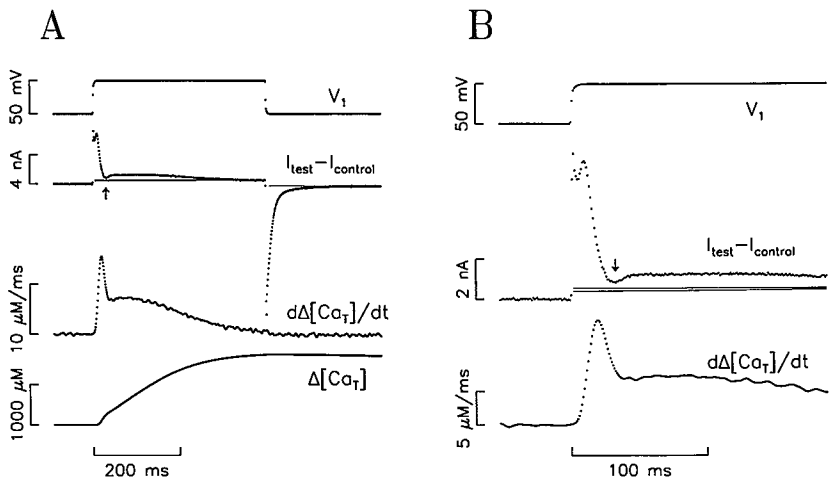

FIGURE 2. An inward dip, marked by arrows, in the slow component of charge movement current. Same format as Fig. 1, depolarization to −40 mV. The steady levels of I_{test}-$I_{control}$ during and after the pulse were 0.54 and −0.25 nA, respectively, which give ON Q_{cm} = 18.79 nC/μF and OFF Q_{cm} = −20.98 nC/μF. The lower line during the pulse gives ON Q_{cm} = −OFF Q_{cm} = 20.98 nC/μF; its initial and final values are 0.39 and 0.55 nA, respectively. Fiber reference, D18901; time after saponin treatment, 101 min; sarcomere length, 3.4 μm; fiber diameter, 111 μm; holding current, −47 nA; C_{app}, 0.01315 μF; r_i, 2.31 MΩ/cm; c_m, 0.177 μF/cm; concentration of phenol red at the optical site, 1.683 mM; estimated pH_R and free $[Ca]_R$, 6.896 and 0.059 μM, respectively; $[Ca_{SR}]_R$, 1,726 μM; interval of time between data points, 0.96 ms; temperature, 16°C. The end-pool solution contained 1.76 mM total Ca.

The local minimum in the I_{test}-$I_{control}$ trace in Fig. 2 is reminiscent of the negative phase in ON I_{cm} that was described by Shirokova et al. (1994). An important difference between their results and ours is that, in their experiments, ON I_{cm} actually became negative whereas, in our experiments, ON I_{test}-$I_{control}$, even at the minimum, was always more positive than the ionic current (thin straight lines, estimated as described in the next section). Thus, in our experiments, ON I_{cm} was always positive and never showed a negative phase. Although Huang (1994) and Hui and Chen (1994) also failed to find a negative phase in ON I_{cm}, their studies and ours do not rule out the possibility that such a negative phase might occur under other conditions, such as with slack length fibers and with small conditioning depolarizations applied before the test pulses (Shirokova et al., 1994).

In any event, according to the rationale presented in the Discussion, the presence of a local minimum in the I_{cm} trace suggests that the effect of SR Ca on the kinetics of I_{cm} is caused by Ca release or some associated event rather than by SR Ca content per se.

Estimates of the Ionic Component of I_{test}-$I_{control}$ and the Issue of ON-OFF Charge Equality

The estimate of I_{cm} from I_{test}-$I_{control}$ relies on the subtraction of the ionic component of current. For this purpose, Jong et al. (1995*b*) constructed an ionic current template from the prestimulus baseline and the steady state values of I_{test}-$I_{control}$ during and after the voltage pulse. The time course of ionic current was estimated from the ionic template by rounding the ON and OFF step changes according to the normalized voltage template (Hui and Chandler, 1990).

Fig. 1 *A* shows an ionic current template constructed in this manner. It is indicated by two horizontal lines, with a value of −0.29 nA during the pulse (lower line) and −0.22 nA after the pulse; the upper sloping line during the pulse is described below. The difference between I_{test}-$I_{control}$ and the ionic current determined with this template is taken to represent I_{cm}. The integral of I_{cm} gives Q_{cm}, the amount of charge that has moved. In this experiment, ON Q_{cm} = 27.46 nC/μF and OFF Q_{cm} = −22.75 nC/μF, suggesting that 4.71 nC/μF more charge moved during the ON of the pulse than during the OFF.

This difference between the values of ON and −OFF Q_{cm} in Fig. 1 *A* is larger than that obtained from fibers in which the SR had been depleted of Ca. With the same procedure used here to estimate Q_{cm} and at pulse potentials between −50 and −40 mV, Jong et al. (1995*b*) found that only one fiber out of a total of ten had an ON–OFF difference that exceeded 2.4 nC/μF in absolute value. In addition, the mean value of the ON–OFF difference from all 10 fibers was not significantly different from zero, suggesting that ON–OFF charge equality held in their experiments. If ON–OFF charge equality held in the experiment in Fig. 1, the ionic current must have varied with time during the pulse, after the pulse, or both.

To explore this possibility, a sloping straight line, $a + bt$, was used to represent the ionic current template during the pulse; a and b are constants and t represents time after the start of the voltage step. The constants a and b were determined from a least-squares fit to the final 48 ms of the ON segment of the I_{test}-$I_{control}$ trace with the constraint that ON Q_{cm} = −OFF Q_{cm} = 22.75 nC/μF. This line is plotted in Fig. 1, *A* and *B*, where, during the first part of the pulse, it lies slightly above the horizontal line. The initial and final values of the sloping line are −0.10 and −0.30 nA, respectively. Thus, ON–OFF charge equality can be satisfied with an ionic current template during the pulse that is only slightly different from the constant template constructed from the steady state value of I_{test}-$I_{control}$. If the ionic current template is represented accurately by the function $a + bt$, use of a constant template would introduce an error into the estimate of ON Q_{cm} that is approximately proportional to the product of b times pulse duration squared. This error would be expected to be greater in the experiments reported here than in most of those reported in Jong et al. (1995*b*) because longer pulse durations were usually used here so that the slow component of I_{test}-$I_{control}$ would be reliably resolved.

The general conclusion of this section is that, during depolarizations that are sufficiently long to measure the slow component of ON charge movement, any estimate of ON Q_{cm} is extremely sensitive to the ionic current template that is used to determine I_{cm} from I_{test}-$I_{control}$. For this reason, it is difficult to decide whether the values of ON and OFF Q_{cm} satisfy precise or only approximate ON–OFF charge equality. The next section and the experiments illustrated in Figs. 10 and 11 present additional evidence in favor of at least approximate ON–OFF charge equality.

Movement of the Slow Component of I_{test}-$I_{control}$ Is Associated with an Increase in the Absolute Value of OFF Q_{cm}

If the slow component of I_{test}-$I_{control}$ represents movement of intramembranous charge, it should be associated with an increase in the magnitude of OFF Q_{cm}. Figure 3 shows an experiment that was carried out to test this prediction. The top set of traces in *A* shows V_1 associated with 50-, 200-, 400-, 600-, 800-, and 1,400-ms depolarizations to −42 mV. The middle set of traces shows I_{test}-$I_{control}$ taken during both the depolarization and the period of repolarization to −90 mV, which lasted 600 ms. The bottom trace shows $d\Delta[Ca_T]/dt$ for the 1,400-ms depolarization. After the 600-ms period of repolarization that followed each pulse, a 350-ms pulse

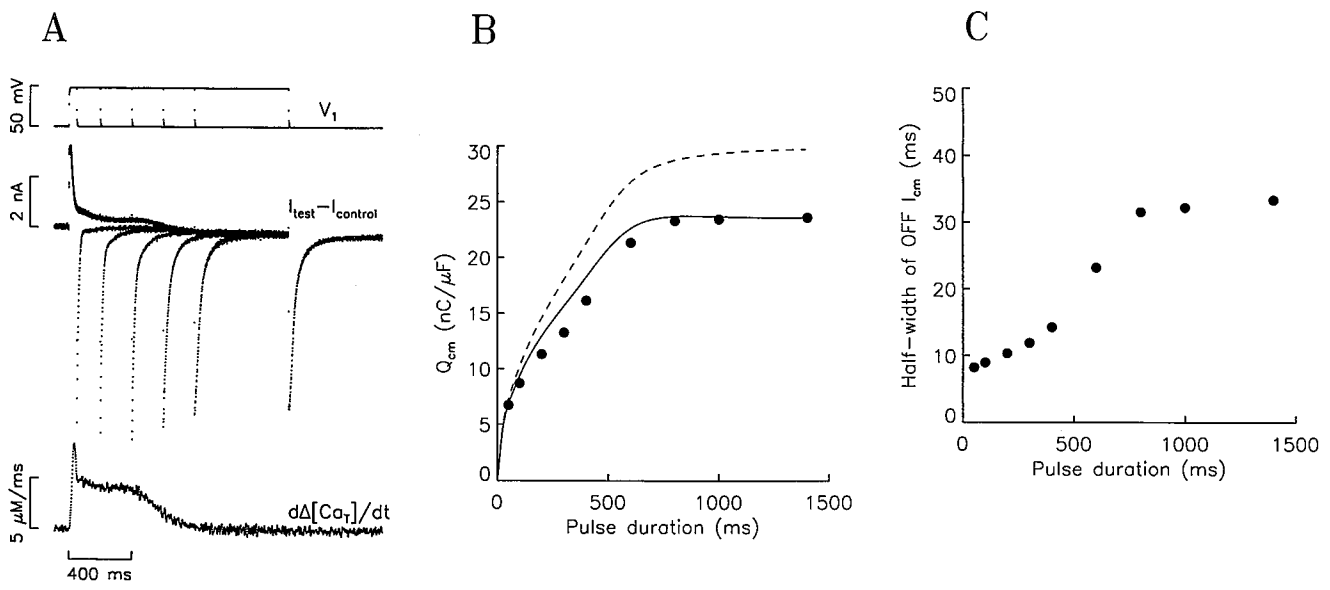

FIGURE 3. Charge movement and SR Ca release associated with depolarizations of different durations to −42 mV. (*A*) The three sets of superimposed traces show V_1, I_{test}-$I_{control}$, and $d\Delta[Ca_T]/dt$, respectively, associated with 50-, 200-, 400-, 600-, 800-, and 1,400-ms pulses. Each pulse was followed by a 600-ms repolarization to −90 mV and a 350-ms depolarization to −40 mV to deplete the SR of any remaining Ca; 5-min recovery periods were used between successive stimulations. (*B*) The filled circles show the absolute values of OFF Q_{cm}, obtained by integration of OFF I_{cm}, plotted as a function of pulse duration. The dashed curve shows the running integral of ON I_{cm} obtained with an ionic current template equal to −0.19 nA, given by the mean value of I_{test}-$I_{control}$ during the last 48 ms of the 1,400-ms pulse. The continuous curve shows the running integral of ON I_{cm} obtained with a sloping straight line for the ionic current template. The line was least-squares fitted to I_{test}-$I_{control}$ during the last 48 ms of the 1,400-ms pulse with the constraint that ON Q_{cm} = −OFF Q_{cm}; the initial and final (1,400 ms) values of the line are −0.05 and −0.19 nA, respectively. (*C*) The filled circles show the half-width of OFF I_{cm} plotted as a function of pulse duration. Fiber reference, 424911; sarcomere length, 3.3 μm; interval of time between data points, 0.96 ms; temperature, 15°C. First and last values: time after saponin treatment, 82–127 min; fiber diameter, 119–122 μm; holding current, −42 and −45 nA; C_{app}, 0.01510–0.01527 μF; r_i, 1.72–1.63 MΩ/cm; c_m, 0.208–0.210 μF/cm; concentration of phenol red at the optical site, 1.214–1.557 mM; estimated pH_R and free $[Ca]_R$, 6.844–6.835 and 0.074–0.077 μM, respectively; $[Ca_{SR}]_R$, 2,531–2,478 μM. The end-pool solution contained 1.76 mM total Ca.

to −40 mV (not shown) was used to deplete the SR of any remaining readily releasable Ca. Successive stimulations were separated by a 5-min recovery period to allow the SR to reaccumulate the released Ca (Pape et al., 1995). During the course of the experiment, which lasted 45 min, the value of $[Ca_{SR}]_R$ decreased by ~2%, from 2,531 to 2,478 μM.

In Fig. 3 *A*, the ON I_{test}-$I_{control}$ traces superimpose well, showing that the I_{test}-$I_{control}$ waveform was stable during the experiment. The slow component is clearly apparent, although its time course is somewhat different from that shown in Figs. 1 and 2; it shows an early slow decrease and then a longer duration plateau. These characteristics are similar to those of the $d\Delta[Ca_T]/dt$ signal, which is also somewhat different from the $d\Delta[Ca_T]/dt$ signals in Figs. 1 and 2. After repolarization, the time course of the OFF I_{test}-$I_{control}$ varied according to pulse duration. As the duration was progressively increased from 50 to 1,400 ms, the peak amplitude initially increased and then decreased, and a slow phase became progressively more pronounced.

The values of OFF I_{cm} were obtained from OFF I_{test}-$I_{control}$ by subtraction of the ionic component. This component was estimated from a constant ionic current template, determined from the final 48 ms of the 600 ms repolarization period, as described above in connection with Fig. 1. The filled circles in Fig. 3 *B* show the absolute values of OFF Q_{cm}, normalized by fiber capacitance, plotted as a function of pulse duration. In this experiment, the large, early phase of ON I_{cm} had been completed by the end of the shortest pulse, of duration 50 ms; thereafter, the ON I_{cm} consisted mainly of the slow component (Fig. 3 *A*). The absolute value of OFF Q_{cm} progressively increased with pulse duration from 6.7 nC/μF after the 50-ms pulse to 23.6 nC/μF after the longest pulse, of duration 1,400 ms (Fig. 3 *B*). The difference, 16.9 nC/μF, is associated with the slow component of ON I_{cm} and represents ~0.7 of the total amount of OFF Q_{cm} that was measured after the 1,400 ms depolarization (23.6 nC/μF).

ON I_{cm} in Fig. 3 *A* was estimated from the 1,400 ms ON I_{test}-$I_{control}$ with two different ionic current templates (not shown), as described in connection with Fig. 1. One template was a horizontal line at −0.19 nA, the mean value of I_{test}-$I_{control}$ during the final 48 ms of the 1,400-ms pulse. The dashed curve in Fig. 3 *B* shows

the corresponding running integral of I_{cm}. The value of ON Q_{cm} exceeded the absolute value of OFF Q_{cm} by an amount that progressively increased with pulse duration. With the 1,000- and 1,400-ms pulses, the difference was ~6 nC/μF.

The second ionic current template was a sloping straight line that was least-squares fitted through the final 48 ms of the I_{test}-$I_{control}$ trace, with the constraint that ON Q_{cm} = −OFF Q_{cm} = 23.6 nC/μF. The continuous curve in Fig. 3 *B* shows the corresponding running integral of I_{cm}. As expected, the agreement between ON and OFF charge is better with the sloping ionic current template than with the horizontal template. Nonetheless, the value of ON Q_{cm} was still larger than the absolute value of OFF Q_{cm} for pulse durations between 200 and 600 ms. The source of this difference could be a genuine inequality in ON and OFF Q_{cm}, experimental uncertainty, or an inaccuracy in the estimate of the ionic current. An example of the last possibility, which is considered in the Discussion, is that a Ca-induced outward current makes a small transient contribution to the ON ionic current that is not included in the sloping straight line template.

The main conclusion of this section is that the slow component of ON I_{test}-$I_{control}$ illustrated in Figs. 1–3 is correlated with increases in the absolute values of ON and OFF Q_{cm} that are similar in magnitude and time course. ON–OFF charge equality appears to be mostly, if not entirely, satisfied, consistent with the idea that the slow component of ON I_{test}-$I_{control}$ is primarily due to intramembranous charge movement.

The Duration of OFF I_{cm} Increases with Pulse Duration but Mainly After the SR Has Released Most of Its Ca

Fig. 3 *C* shows values of the half-width of OFF I_{cm} plotted as a function of pulse duration; half-width denotes the period of time that elapses from the time to half-peak on the rising phase of I_{cm} to the time to half-peak on the falling phase. The half-width increased from ~8 to ~12 ms when the duration was increased from 50 to 300 ms. The half-width then increased almost threefold, from ~12 to ~32 ms, when the duration was increased from 300 to 800 ms. With 1,000- and 1,400-ms pulses, the half-width increased only slightly, from ~32 to ~33 ms.

The most pronounced increase in the half-width of OFF I_{cm} occurred when the pulse duration was increased from 400 to 800 ms. This increase was associated with a decrease in the $d\Delta[Ca_T]/dt$ signal from its plateau level to nearly zero (Fig. 3 *A*). Two other experiments were carried out with varying duration pulses to −45 mV, and a similar correlation was found between the increase in the half-width of OFF I_{cm} and the decrease in the $d\Delta[Ca_T]/dt$ signal.

In a Ca-depleted fiber, the relation between the half-width of OFF I_{cm} and pulse duration is somewhat different from the sigmoid relation shown in Fig. 3 *C*; the value of OFF half-width increases approximately exponentially from a small initial value to a plateau value that is observed with long duration pulses (Fig. 8 in Jong et al., 1995*b*). Thus, without Ca inside the SR, the tendency for the OFF half-width to increase with pulse duration develops immediately (Fig. 8 in Jong et al., 1995*b*) whereas, with several hundred micromolar or more Ca inside the SR, the tendency is delayed until most of the Ca has been released (Fig. 3 *C* in this article).

The Slow Component of Charge Movement Appears to Activate Additional SR Ca Release

Since the amount of charge that is associated with the slow component of I_{cm} can be a substantial fraction of the total charge (e.g., 0.7 in Fig. 3), it was important to find out whether it is able to activate SR Ca release. The method used to assess this is shown in Fig. 4, which shows the ON segments of traces from the experiment illustrated in Fig. 1. The first three traces in Fig. 4 show V_1, I_{cm}, and Q_{cm} (the running integral of I_{cm}), respectively. The bottom trace shows the spatially averaged myoplasmic free Δ[Ca] signal; after depolarization, it increased to an early peak, decreased to a local minimum, and then increased to a steady level that was slightly larger than the early peak.

The fourth trace in Fig. 4 shows $d\Delta[Ca_T]/dt$. This has been corrected for SR Ca depletion by dividing the value of each experimental point by that of readily releasable Ca inside the SR, $[Ca_{SR}]$, and multiplying by 100 to give units of percent per millisecond; $[Ca_{SR}]$ is

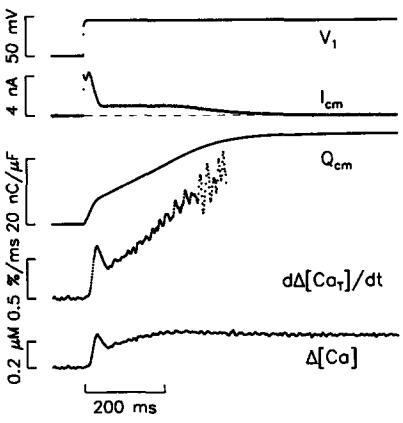

FIGURE 4. Comparison of the time course of ON Q_{cm} and $d\Delta[Ca_T]/dt$ corrected for SR Ca depletion, from the experiment illustrated in Fig. 1. The traces show, from top to bottom, V_1, I_{cm} (calculated with a constant ionic current template equal to −0.29 nA), Q_{cm}, $d\Delta[Ca_T]/dt$ corrected for SR Ca depletion (in units of %/ms), and Δ[Ca]; I_{cm} = 0 is indicated by a dashed line. Additional information is given in the legend of Fig. 1.

equal to $[Ca_{SR}]_R - \Delta[Ca_T]$ (Pape et al., 1995). If Ca flux through an open SR Ca channel is directly proportional to the value of $[Ca_{SR}]$, the value of $d\Delta[Ca_T]/dt$ (corrected for SR Ca depletion) should be proportional to the number of SR Ca channels that are open. After depolarization, the $d\Delta[Ca_T]/dt$ signal rapidly increased to a peak value and then rapidly decreased to a local minimum that was ~0.6 times as large. As mentioned above in connection with Fig. 1, this decrease in $d\Delta[Ca_T]/dt$, which occurred during a period in which Q_{cm} was increasing, is probably caused by Ca inactivation of Ca release. After the $d\Delta[Ca_T]/dt$ signal reached a local minimum, it progressively increased until the trace could no longer be reliably resolved because of the noise introduced by the depletion correction.

In Fig. 4, the increase in $d\Delta[Ca_T]/dt$ after the local minimum could have been produced by an increase in activation of Ca release, a decrease in Ca inactivation of Ca release, or both. During the first 150 ms after the minimum, however, it seems likely that the progressive, almost threefold increase in $d\Delta[Ca_T]/dt$ was primarily due to an increase in activation, since both spatially averaged free $\Delta[Ca]$ and $d\Delta[Ca_T]/dt$ (uncorrected for SR Ca depletion, Fig. 1) were greater than their respective values at the minimum. At later times, $d\Delta[Ca_T]/dt$ (corrected for SR Ca depletion, Fig. 4) continued to increase while the spatially averaged $\Delta[Ca]$ signal remained approximately constant (Fig. 4) and the $d\Delta[Ca_T]/dt$ signal (uncorrected for SR Ca depletion, Fig. 1) started to decrease. During this period, a decrease in Ca inactivation might have occurred and contributed to the increase in $d\Delta[Ca_T]/dt$ (corrected for SR Ca depletion, Fig. 4).

The features that have just been described for the traces in Figs. 1 and 4 were found in all of our experiments on the slow component of charge movement. The main conclusion from these experiments is that the slow component of charge movement appears to be able to activate SR Ca channels and that this progressive increase in activation produces a prolonged nonexponential time course of $d\Delta[Ca_T]/dt$ (uncorrected for depletion, Figs. 1–3).

The Charge Associated with the Slow Component of ON I_{cm} Is Probably Q_γ, Not a New Species of Charge

It is clearly important to find out whether the slow component of ON I_{cm} is a modified I_β or I_γ current or whether it is current from a new species of intramembranous charge. To distinguish between these possibilities, I_{cm} was measured in the same fiber at different voltages under three different conditions: with a large SR Ca content (condition I) and after SR Ca had been depleted by one of two procedures (conditions II and III). Fig. 5 *A* shows three pairs of traces that illustrate each of these conditions. Within each pair, the top and bottom traces show V_1 and $\Delta[Ca_T]$, respectively; the V_1 trace is thickened during the period when I_{cm} was measured.

In Fig. 5 *A*, condition I, I_{cm} was measured during the first depolarization, which lasted 800 ms, and during the following period of repolarization to −90 mV, which lasted 500 ms. Then, a second depolarization, 350 ms to −40 mV, was used to deplete the SR of any remaining Ca. In this example, the value of $\Delta[Ca_T]$ slowly increased during the first pulse, to −60 mV, to a value nearly half maximal. It remained relatively constant during the period of repolarization and reached a maximal plateau value of 1,590 μM during the second pulse.

Six I_{cm} traces taken under condition I are shown in Fig. 5 *B*, with voltages indicated (in mV). The ON I_{cm} trace at −60 mV shows an early I_β component followed by a small I_γ component that is similar to the one observed when the SR is Ca depleted (see corresponding traces in Fig. 5, *C* and *D*). There is little, if any, slow component of the kind illustrated in Figs. 1–4. Marked slow components are apparent in the next three traces, to −54, −51, and −48 mV. As the strength of the depolarization was increased, the amplitude of the slow component increased and its duration decreased. At −40 and −30 mV, the slow component merged with the early I_γ component.

During the experiment illustrated in Fig. 5 *B*, the value of $[Ca_{SR}]_R$ progressively decreased from 1,948 μM, when the first measurement was made, to 869 μM, when the last measurement was made 54 min later. The rather marked decrease in $[Ca_{SR}]_R$ during the experiment is a result of the relatively brief 2-min recovery period that was used between successive stimulations. This brief period was chosen so that the extensive series of stimulations required for this experiment (see the following) could be completed in a reasonable length of time. Even with these brief recovery intervals, the last stimulation was made almost 4 h after the fiber had undergone saponin treatment to render the end-pool segments permeable to small molecules and ions.

The traces in Fig. 5 *B*, condition I, were taken in alternation with those in Fig. 5 *C*, condition II. Under condition II (see middle pair of traces in Fig. 5 *A*), the pulses had the same amplitude and duration as under condition I except that the order was switched. As a result, almost all of the readily releasable Ca left the SR during the first pulse (350 ms to −40 mV) so that I_{cm} was measured in a depleted condition. In the experiment illustrated in Fig. 5 *C*, the value of $[Ca_{SR}]_R$ just before the second pulse varied between 16 and 43 μM. Although the traces of OFF I_{cm} in Fig. 5, *B* and *C*, are extremely similar, the ON components are different. In particular, although the usual I_β and I_γ components are apparent in Fig. 5 *C*, the slow component is absent.

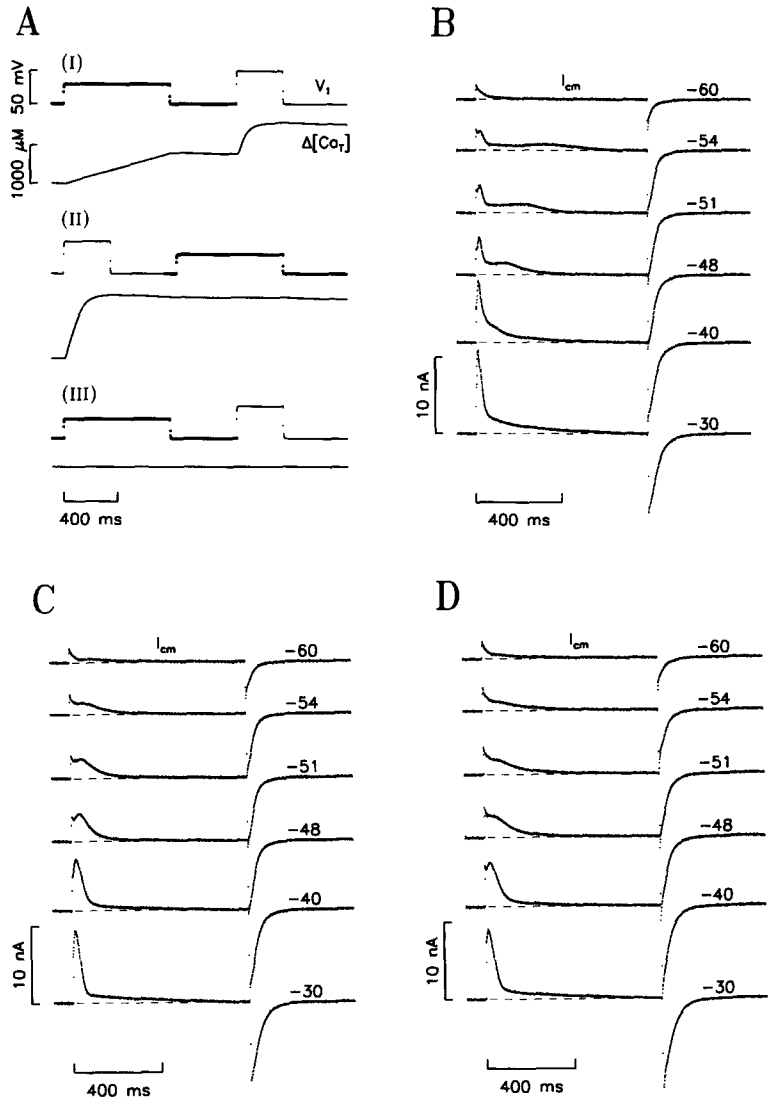

FIGURE 5. I_{cm} with different amplitude voltage pulses, recorded under three conditions. (*A*) Sample pairs of traces of V_1 (*upper*) and $\Delta[Ca_T]$ (*lower*) illustrate conditions (*I*), (*II*), and (*III*), respectively. (*B–D*) I_{cm} traces obtained under conditions I–III, respectively, are plotted during the intervals indicated by the thickened segments of the V_1 traces in *A*. Constant ionic current templates were used to estimate I_{cm} from I_{test}-$I_{control}$. *Dashed lines* show I_{cm} = 0 and numbers indicate the pulse voltage (in mV). Fiber reference, 410911; sarcomere length, 3.4 μm; interval of time between data points, 0.96 ms; temperature, 14°C. First and last values: time after saponin treatment, 63–232 min; fiber diameter, 142–131 μm; holding current, −23 and −34 nA; C_{app}, 0.01693–0.01788 μF; r_i, 1.84–1.87 MΩ/cm; c_m, 0.237–0.248 μF/cm; concentration of phenol red at the optical site, 0.879–2.323 mM; estimated pH_R, 6.951–6.857; estimated free $[Ca]_R$, 0.045–0.049 μM for conditions I and II and undetermined for condition III; $[Ca_{SR}]_R$, 869–1,948 μM before the first pulse for condition I, 16–43 μM before the second pulse for condition II, and ≤6 μM before the first pulse for condition III. The end-pool solution contained 1.76 mM total Ca in *B* and *C*, and 0 mM Ca in *D*. Additional information is given in the text.

After the measurements made under conditions I and II were completed 127 min after saponin treatment, the two end-pool solutions that had contained 1.76 mM Ca were changed to ones that were nominally Ca-free. After 79 min and 59 depleting depolarizations, a set of measurements was made under condition III (see bottom pair of traces in Fig. 5 *A*). The I_{cm} traces, shown in Fig. 5 *D*, are similar to those in Fig. 5 *C* except that the I_γ humps are somewhat less pronounced. This difference is probably because the value of $[Ca_{SR}]_R$ just before the measuring pulse was smaller in Fig. 5 *D*, ≤6 μM, than in Fig. 5 *C*, 16–43 μM, and consequently the accelerator effect of SR Ca observed at early times on the ON kinetics of I_{cm} was less pronounced (Jong et al., 1995*b*).

The I_{cm} traces in Fig. 5, *B–D*, were obtained from I_{test}-$I_{control}$ traces with constant ionic current templates. Because of the uncertainties associated with the estimate of this template during the pulse itself (see above and below), the estimates of OFF Q_{cm} are considered to be more reliable than those of ON Q_{cm}. The filled circles in Figs. 6 *A* and 6 *B* show the absolute values of OFF Q_{cm} obtained under condition I, with $[Ca_{SR}]_R$ = 869–1,948 μM, plotted as a function of V_1. The open symbols show the values obtained with the two methods of SR Ca depletion, diamonds under condition II (Fig. 6 *A*) and circles under condition III (Fig. 6 *B*).

In Fig. 6, the filled circles and open symbols are essentially the same except at −57 and −54 mV, where the values of the filled circles are larger by 1–4 nC/μF. It is difficult to tell whether this difference is genuine because it was not consistently observed in all of our experiments. Although the fiber used for Figs. 5 and 6 was the only fiber in which Q_{cm} vs *V* data were successfully obtained under all three conditions (I–III), data were obtained under conditions I and II in three other experiments. At voltages between −60 and −50 mV, the values of Q_{cm} were slightly larger under condition I than under condition II in one experiment (similar to the result in Fig. 6 *A*); the reverse was true in the other two experiments. Thus, within experimental error, the

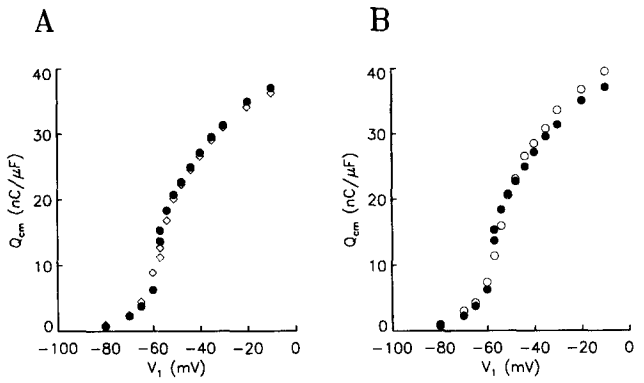

FIGURE 6. Q_{cm} vs V relations obtained under conditions I–III from the experiment in Fig. 5. The filled circles show absolute values of OFF Q_{cm} obtained under condition I, plotted as a function of pulse potential. The open symbols in *A* and *B* show values obtained under conditions II and III, respectively. Additional information is given in the legend of Fig. 5.

Q_{cm} vs V curves appear to be similar under conditions I and II.

The general conclusion from these four experiments is that the slow component of ON I_{cm}, and the corresponding component of OFF Q_{cm}, does not represent movement of a new species of intramembranous charge. Rather, the slow component appears to represent a change in the kinetics of the movement of the existing charge, presumably Q_β or Q_γ. Since the initial value of ON I_{cm} after a depolarization appears to be unaffected by SR Ca depletion (see also Figs. 8, 9, and 12), it seems reasonable to conclude that the kinetics of Q_β are unchanged by depletion. This leaves Q_γ as the likely candidate for the slow component of charge movement. The simplest interpretation of the results presented thus far is that SR Ca content or release or some associated event is able to markedly slow the ON kinetics of I_γ.

The Q_{cm} vs. V Relation in Fibers That Contain Ca Inside Their SR

The results in Fig. 6 suggest that the Q_{cm} vs. V relation is independent of the value of $[Ca_{SR}]_R$ between ~0 and 869–1,948 μM. To compare the properties of the Q_{cm} vs. V relation with $[Ca_{SR}]_R$ = 869–1,948 μM (obtained with 800-ms pulses) with those with $[Ca_{SR}]_R \cong 0$ mM reported by Jong et al. (1995*b*) (obtained with ≤400-ms pulses), the filled circles in Fig. 6 have been replotted in Fig. 7 *A* and least-squares fitted by the two-state Boltzmann distribution function,

$$Q = \frac{Q_{max}}{1 + \exp[-(V - \overline{V})/k]}. \quad (1)$$

V represents the voltage between the internal and external solutions, $\overline{V}$ is the value of V at which the charge is distributed equally between the two states at equilibrium, k is a voltage steepness factor, and Q_{max} represents the maximal amount of charge. The curve, which provides a poor fit to the data, includes contributions from currents that flow through the various pathways in a double Vaseline-gap experiment, as described by Hui and Chandler (1990). These gap corrections account for the foot in the curve at $V_1 < -60$ mV, the slight negative slope at $V_1 > -20$ mV, and the difference between the maximal value of the Q_{cm} data (in units of nC/μF) and the fitted value of q_{max}/c_m.

Table I *A* gives the fitted values of the Boltzmann parameters and other information from the experiment in Fig. 7 *A* and from three other experiments in which pulses of long duration, 700–800 ms, were used to ensure that all of the slow component of charge movement would be measured. Column 1 gives the fiber references. Columns 2 and 3 give, respectively, the times after saponin treatment and the values of $[Ca_{SR}]_R$ associated with the first and last measurements of Q_{cm}. Columns 4–6 give the values of the Boltzmann parameters (as indicated) that were obtained from least-squares fits of Eq. 1, with gap corrections, to the OFF Q_{cm} vs. V data. The mean values are $\overline{V}$ = −45.9 mV, k = 9.1 mV, and q_{max}/c_m = 66.6 nC/μF (q_{max}/c_m is equal to Q_{max} normalized by the apparent capacitance of the fiber, C_{app}).

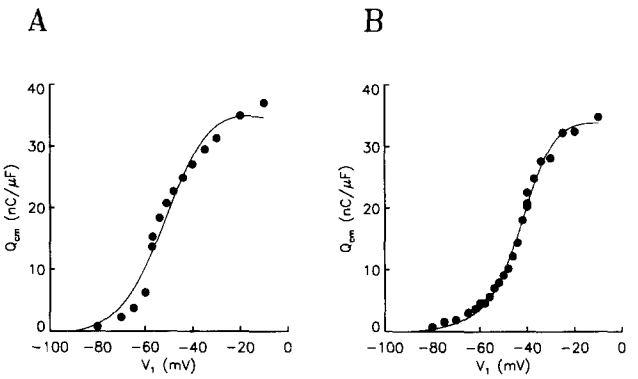

FIGURE 7. Q_{cm} vs V relations determined with depolarizing pulses of different duration. (*A*) The filled circles show the values of −OFF Q_{cm} from Fig. 6 (filled circles), obtained with 800-ms pulses. The curve shows the least-squares fit of Eq. 1, with gap corrections; $\overline{V}$ = −50.3 mV, k = 9.5 mV, and q_{max}/c_m = 46.6 nC/μF. The value of $r_e/(r_i + r_e)$ was 0.993. (*B*) The filled circles show the values of −OFF Q_{cm} from a different fiber, in which 400-ms pulses were used. The best fit of Eq. 1 was given by $\overline{V}$ = −41.9 mV, k = 6.7 mV, and q_{max}/c_m = 38.3 nC/μF. The value of $r_e/(r_i + r_e)$ was 0.982. Fiber reference, D21901; sarcomere length, 3.8 μm; temperature, 16°C. First and last values: time after saponin treatment, 76–116 min; fiber diameter, 109–104 μm; holding current, −45 and −49 nA; C_{app}, 0.01703–0.01693 μF; r_i, 2.33–2.47 MΩ/cm; c_m, 0.229–0.226 μF/cm; concentration of phenol red at the optical site, 1.179–1.793 mM; estimated pH_R and $[Ca]_R$, 6.669–6.521 and 0.166–0.326 μM, respectively; $[Ca_{SR}]_R$, 2,733–2,690 μM. The end-pool solution contained 1.76 mM total Ca.

TABLE I

Boltzmann Function Parameters Associated with Charge Movement

(1) Fiber	(2) Time after saponin	(3) $[Ca_{SR}]_R$	(4) $\overline{V}$	(5) k	(6) q_{max}/c_m	(7) *var*
	min	*μM*	*mV*	*mV*	*nC/μF*	*(nC/μF)²*
A: 700–800-ms pulses						
326911	69–145	2,800–1,571	−49.3	5.2	33.5	1.93
410911	63–121	1,948–783	−50.3	9.5	46.6	5.32
412911	203–253	1,176–488	−41.8	14.4	123.8	6.96
424911	132–232	2,384–2,128	−42.3	7.1	62.5	14.33
Mean			−45.9	9.1	66.6	7.14
SEM			2.2	2.0	20.0	2.62
B: 300–400-ms pulses						
D18901	71–121	2,087–1,801	−43.9	5.2	31.0	1.27
D21901	76–116	2,733–2,690	−41.9	6.7	38.3	0.90
403912	61–75	2,775–2,309	−49.7	5.4	23.3	0.35
Mean			−45.2	5.8	30.9	0.84
SEM			2.3	0.5	4.3	0.27

Column 1 gives the fiber references. Column 2 gives the initial and final values of the time after saponin treatment. Column 3 gives the initial and final values of $[Ca_{SR}]_R$. Columns 4–6 give the values of $\overline{V}$, k, and q_{max}/c_m that were obtained from the least-squares fits of Eq. 1, with gap corrections. Column 7 gives the values of the residual variance. The fibers in *A* and *B* were studied with 700–800-ms and 300–400-ms pulses, respectively. Sarcomere length, 3.3–3.8 μm; fiber diameter, 78–155 μm; holding current, −23 to −67 nA; r_i, 1.36–5.74 MΩ/cm; c_m, 0.111–0.237 μF/cm; $r_e/(r_i + r_e)$, 0.976–0.993; concentration of phenol red at the optical site, 0.879–2.436 mM; estimated pH_R and free $[Ca]_R$, 6.521–6.951 and 0.045–0.327 μM; temperature, 14°–16°C. The interval of time between successive stimulations varied between 2 and 5 min.

The corresponding mean values in Ca-depleted fibers, studied with the same external and internal solutions except that the internal solution was Ca-free, were $\overline{V}$ = −48.3 mV, k = 7.2 mV, and q_{max}/c_m = 33.5 nC/μF (Table II in Jong et al., 1995*b*). In the two sets of measurements, only the mean values of q_{max}/c_m are significantly different.

Column 7 in Table I *A* gives the values of the residual variance, *var*. This is defined as the sum of the squared differences between the experimental points and the theoretical curve, divided by the number of points minus 3 (the number of adjustable parameters in the Boltzmann function, Eq. 1); a small value of *var* indicates a good fit. The mean value of *var*, 7.14 (nC/μF)², indicates that Eq. 1 did not provide a good fit to the Q_{cm} vs. V data.

The Q_{cm} vs. V data in the experiments in Table I *A* are different from those in Ca-depleted fibers (Jong et al., 1995*b*) in two main ways: the data are not well fitted by Eq. 1 and, when they are fitted, the values of q_{max}/c_m are larger. Since the ON I_{cm} in Ca-depleted fibers does not contain a slow component, Jong et al. (1995*b*) usually used ≤400-ms pulses to obtain Q_{cm} vs. V data rather than 700–800-ms pulses as were used in Fig. 7 *A* and Table I *A*. It therefore was important to obtain Q_{cm} vs. V data with ≤400-ms pulses in fibers that contain Ca inside their SR.

Fig. 7 *B* shows Q_{cm} vs. V data from an experiment that was similar to the one in Fig. 7 *A* except that the data were obtained with 400-ms pulses. Equation 1 provides a better fit to the data in Fig. 7 *B* than to those in Fig. 7 *A*.

Table I *B* summarizes information from experiments with 300–400-ms pulses; the fiber references are different from those in Table I *A* because Q_{cm} vs. V data were not obtained with both 300–400-ms and 700–800-ms pulses in the same fiber. The values of *var* in column 7 are small, indicating that Eq. 1 provides a good fit to the Q_{cm} vs. V data. The mean values of the Boltzmann parameters in columns 4–6 are similar to those obtained in fibers after SR Ca depletion: $\overline{V}$ = −48.3 mV, k = 7.2 mV, and q_{max}/c_m = 33.5 nC/μF (Table II in Jong et al., 1995*b*). Only the mean values of k, 5.8 mV in Table I *B* and 7.2 mV in Jong et al. (1995*b*), are significantly different. The reason for this small difference is unknown. It may be, at least in part, because of incomplete movement of charge during a 300–400-ms pulse in the narrow range of potentials that gave a prolonged slow component of I_{cm}.

Although the corresponding mean values in columns 4–7 in Table I, *A* and *B*, are not significantly different, the individual and mean values in *A* and *B* show some interesting similarities and differences. Firstly, according to the values of *var* in column 7, Eq. 1 provides a better fit to Q_{cm} vs. V data obtained with 300–400-ms pulses (*B*) than to data obtained with 700–800-ms pulses (*A*). Secondly, the values of $\overline{V}$ are similar in *A* and *B*, indicating that the position of the Q_{cm} vs. V relation along the voltage axis is similar for 300–400-ms

and 700–800-ms pulses. Thirdly, the values of q_{max}/c_m are larger in *A* than in *B*.

The difference between the mean values of q_{max}/c_m, and perhaps the difference between the mean values of *var*, are mainly attributable to larger absolute values of OFF Q_{cm} after 700–800-ms pulses than after 300–400-ms pulses, especially after the most positive pulses used in our experiments. This suggests that the last half of a 700–800-ms strong depolarization is able to produce a change in the amount of charge moved or in ionic conductance that increases the estimated absolute value of OFF Q_{cm} and, consequently, the fitted value of q_{max}/c_m. Whatever the nature of this change, it appears to be independent of the value of $[Ca_{SR}]_R$ between ~0 and 869–1,948 μM since the open and filled symbols in Fig. 6, *A* and *B*, are similar. Perhaps a completely reliable estimate of the Q_{cm} vs. *V* relation in the fibers in Table I would have required long duration pulses (700–800-ms) at potentials between −55 and −40 mV, to insure complete movement of the slow component of charge, and shorter duration pulses (300–400 ms) at more positive potentials, to avoid the secondary increase in the estimate of −OFF Q_{cm}.

The main conclusion of this section is that, if the Q_{cm} vs. *V* relation is measured with pulses of duration ≤400 ms, the relation is little affected by increasing the value of $[Ca_{SR}]_R$, and consequently that of resting myoplasmic free [Ca], from zero to the values in the experiments in Table I. Overall, the Q_{cm} vs. *V* measurements described in this article and in Jong et al. (1995*b*) are consistent with the conclusion reached at the end of the preceding section, namely, that the slow component of charge movement seen at intermediate voltages (−55 to −40 mV) in fibers with $[Ca_{SR}]_R$ = 1,000–3,000 μM represents a change in the kinetics of I_γ with little, if any, change in the Q_γ vs. *V* relation.

The Time Course of the Slow Component of I_{cm} Can Be Changed by Altering $[Ca_{SR}]_R$ and the Change Is Reversible

Differences between the traces in Fig. 5 *B* and those in Fig. 5, *C* and *D*, suggest that the slow component of I_{cm} depends on SR Ca content in a reversible fashion. Additional evidence in support of this conclusion is presented in Fig. 8. Fig. 8, *A* and *B*, shows traces of I_{cm} and $d\Delta[Ca_T]/dt$, respectively, that were obtained during an 800-ms depolarization to −45 mV. Each depolarization was followed by a 600-ms repolarization to −90 mV and a 350-ms depolarization to −40 mV (not shown) to deplete the SR of any remaining Ca. The traces are plotted in chronological order from top to bottom, *a* to *f*.

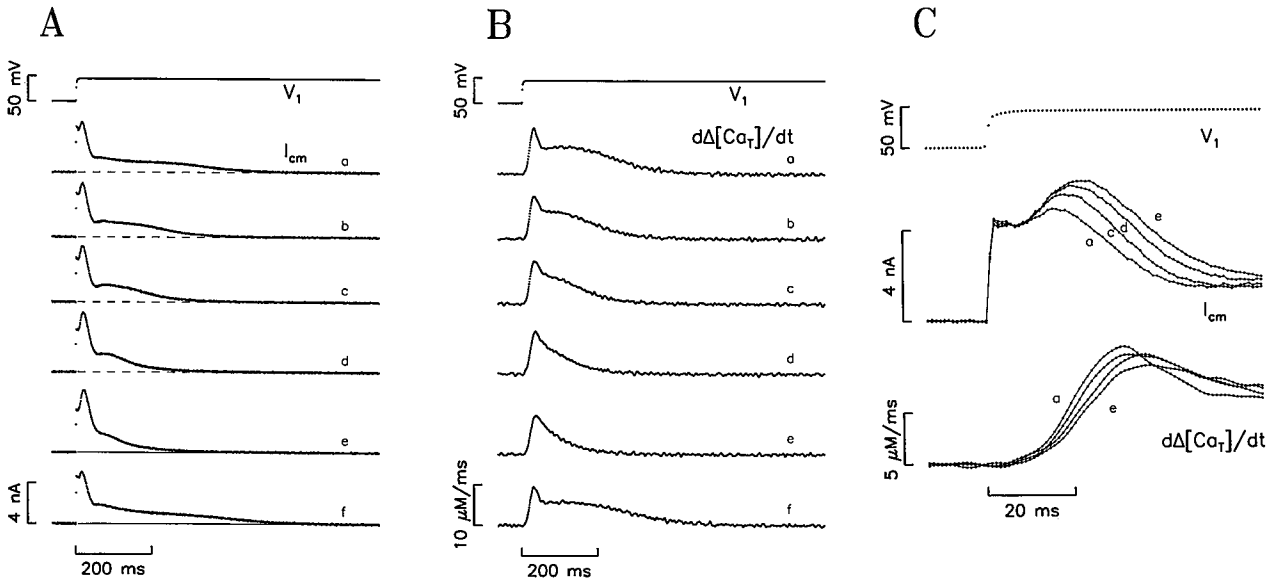

FIGURE 8. Effect of SR Ca content on charge movement currents and the rate of SR Ca release at −45 mV. (*A*) The top trace shows V_1. The other traces show I_{cm} calculated with constant ionic current templates; I_{cm} = 0 is indicated by dashed lines. The values of $[Ca_{SR}]_R$ were varied by the use of different recovery periods after the preceding depolarization; $[Ca_{SR}]_R$ = 1,841 μM for *a* (recovery period, 10 min), 1,449 μM for *b* (2.75 min), 1,251 μM for *c* (2 min), 987 μM for *d* (1.25 min), 842 μM for *e* (1 min), and 1,823 μM for *f* (10 min). The values of OFF Q_{cm} were −30.63 nC/μF (*a*), −31.59 nC/μF (*b*), −31.32 nC/μF (*c*), −30.90 nC/μF (*d*), −31.15 nC/μF (*e*), and −38.62 nC/μF (*f*). The order of the experiment was *a* to *f*. (*B*) Traces of $d\Delta[Ca_T]/dt$ from the same experiment. (*C*) V_1 and superimposed traces of I_{cm} and $d\Delta[Ca_T]/dt$ from *A* and *B* (*a*, *c*, *d*, and *e*), plotted on an expanded time scale; line segments connect sequential experimental I_{cm} and $d\Delta[Ca_T]/dt$ points. The same fiber that was used for Figs. 1 and 4. First and last values: time after saponin treatment, 129-169 min; fiber diameter, 132–136 μm; holding current, −37 and −41 nA; C_{app}, 0.01587–0.01595 μF; r_i, 1.83–1.66 MΩ/cm; c_m, 0.218–0.219 μF/cm; concentration of phenol red at the optical site, 1.752–1.859 mM; estimated pH_R and free $[Ca]_R$, 6.879–6.876 and 0.063–0.064 μM, respectively. The end-pool solution contained 1.76 mM total Ca. Additional information is given in the text and in the legend of Fig. 1.

From *a* to *e*, the value of $[Ca_{SR}]_R$ was progressively reduced from 1,841 to 842 μM by progressive decreases in the duration of the recovery period between successive stimulations, from 10 to 1 min (see legend). The recovery period was then progressively increased (traces not shown) and was 10 min before trace *f* was obtained, with $[Ca_{SR}]_R$ = 1,823 μM.

Values of OFF Q_{cm} were determined from OFF I_{cm} (not shown) and are given in the legend. They were relatively constant between −30.63 and −31.59 nC/μF from *a* to *e*. Thereafter, for reasons unknown, they progressively increased in absolute value to a value of −38.62 nC/μF for trace *f* (*open squares* from left to right in Fig. 10 *A* below); otherwise, the fiber was stable from *e* to *f*.

In Fig. 8 *A*, the initial amplitude of I_{cm} after depolarization was similar in all of the traces. This is shown more clearly in Fig. 8 *C*, where traces *a*, *c*, *d*, and *e* are plotted, superimposed, on expanded vertical and horizontal scales. The similarity of the initial time course of the traces is consistent with the idea that I_β was unaffected by reducing the value of $[Ca_{SR}]_R$ from 1,841 to 842 μM. After ~10 ms of depolarization, however, the I_{cm} traces began to diverge. As the value of $[Ca_{SR}]_R$ was decreased from *a* to *e*, the amplitude and time to peak of the I_γ hump progressively increased and, as shown in the bottom traces, the increase in $d\Delta[Ca_T]/dt$ became progressively delayed. The variation of the I_{cm} and $d\Delta[Ca_T]/dt$ traces with $[Ca_{SR}]_R$ suggests that the ON kinetics of I_{cm} was slowed by SR Ca and that the onset of the slowing was correlated with the value of $[Ca_{SR}]_R$ or with the onset of $d\Delta[Ca_T]/dt$. It is important to point out that, since the values of $[Ca_{SR}]_R$ encountered in this experiment were all substantially larger than 200–300 μM, the acceleration of the ON kinetics of I_γ by SR Ca release described by Jong et al. (1995*b*) should have been maximal in all of the traces.

In addition to the effect of $[Ca_{SR}]_R$ on the early time course of I_{cm} and $d\Delta[Ca_T]/dt$ (Fig. 8 *C*), the duration of the slow component of both I_{cm} and $d\Delta[Ca_T]/dt$ progressively decreased from *a* to *e* (Fig. 8, *A* and *B*). These effects of $[Ca_{SR}]_R$ were reversible, as shown by the similarity of traces *a* and *f* (Fig. 8, *A* and *B*), with $[Ca_{SR}]_R$ = 1,841 and 1,823 μM, respectively.

All of these results are consistent with the idea that SR Ca, in concentrations of several hundred micromolar or more (referred to myoplasm), is able to reversibly slow the ON kinetics of I_γ. If this effect is the result of SR Ca release or some associated event, as is argued in the Discussion, the traces in Fig. 8 *C* show that it is able to develop rapidly, within 5–10 ms after the beginning of SR Ca release.

In our experiments, 1 min was the shortest recovery period that could be used between successive stimulations; this time is determined primarily by the time required to take and process 8 CONTROL measurements and 1 TEST measurement. In the experiment in Fig. 8, a 1-min recovery period reduced the value of $[Ca_{SR}]_R$ to 842 μM (trace *e*). To study the effect of smaller values of $[Ca_{SR}]_R$ on I_{cm}, it was necessary to use the depletion protocol.

Fig. 9 shows results from one of our depletion experiments, on the fiber used for Figs. 5–6. As in all of the experiments reported here, the fiber was initially equilibrated with an end-pool solution that contained 1.76 mM total Ca. After a set of measurements was made under conditions I and II (Fig. 5, *B* and *C*), Ca was removed from the end pools (127 min after saponin treatment), and successive depolarizations were used to

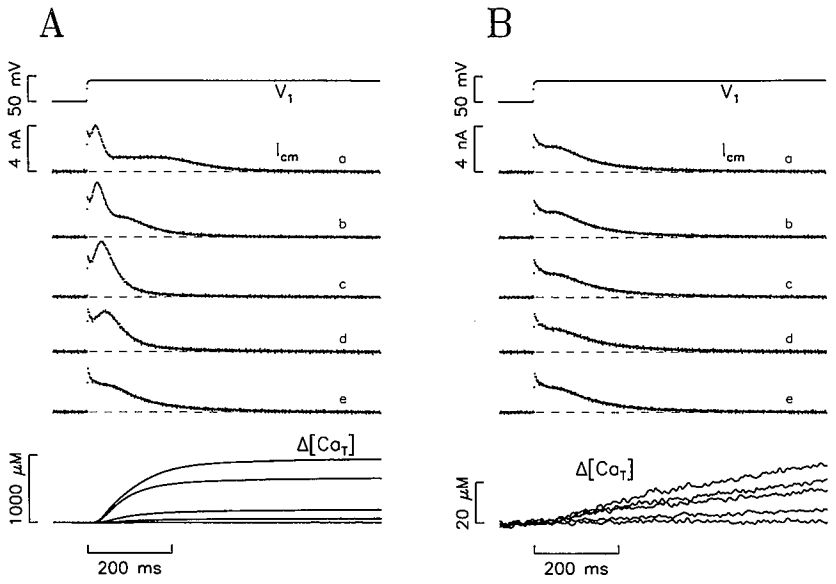

FIGURE 9. I_{cm} with different values of $[Ca_{SR}]_R$, obtained during the course of SR Ca depletion by successive depolarizations. Two 700-ms pulses to −48 mV were separated by a 450-ms period of repolarization to −90 mV. (*A*) The top trace shows V_1 during the initial part of the first pulse. The next five traces show I_{cm} determined with constant ionic current templates; I_{cm} = 0 is indicated by dashed lines. $[Ca_{SR}]_R$ = 949 μM in *a*, 662 μM in *b*, 194 μM in *c*, 64 μM in *d*, and 6 μM in *e*. The bottom superimposed traces show $\Delta[Ca_T]$. (*B*) Similar to *A* except that the traces were taken during the initial part of the second pulse. In both *A* and *B*, the amplitude of the $\Delta[Ca_T]$ traces progressively decreased from *a* to *e*. The same fiber as used for Figs. 5–6. First and last values: time after saponin treatment, 132–200 min; fiber diameter, 151–141 μm; holding current, −25 and −32 nA; C_{app}, 0.01688–0.01759 μF; r_i, 1.77–1.85 MΩ/cm; c_m, 0.236–0.244 μF/cm; concentration of phenol red at the optical site, 1.524–2.048 mM; estimated pH_R, 6.911–6.867. Additional information is given in the text and in the legend of Fig. 5.

deplete the SR of Ca. The pulse protocol that was selected for this purpose consisted of two 700-ms pulses to −48 mV, separated by a 450-ms period of repolarization to −90 mV. Fig. 9, *A* and *B*, shows records obtained during the first and second pulses, respectively. Within each panel, the top trace shows V_1. The next five traces show selected records of I_{cm} in chronological order from *a* to *e*. The bottom superimposed traces show $\Delta[Ca_T]$, plotted with different gains in *A* and *B*. In each panel, the amplitude of the $\Delta[Ca_T]$ traces progressively decreased from *a* to *e* (letters not shown).

In Fig. 9 *A*, as the value of $[Ca_{SR}]_R$ decreased from 949 (*a*) to 662 (*b*) to 194 (*c*) μM, the peak amplitude of I_{cm} increased, and the duration of its slow component decreased. This is similar to the results shown in Fig. 8 *A*, traces *a–e*, where the value of $[Ca_{SR}]_R$ decreased from 1,841 to 842 μM. When the value of $[Ca_{SR}]_R$ in Fig. 9 *A* was reduced from 194 (*c*) to 64 (*d*) and eventually to 6 (*e*) μM, the amplitude of the I_γ hump became progressively smaller and its duration became progressively longer, as described in Jong et al. (1995*b*). Additional information about the rather complex changes in the ON kinetics of I_{cm} in traces *a–e* is given below in connection with Fig. 12.

Fig. 9 *B* shows the I_{cm} traces that were obtained during the second pulse, after the first pulse had released most of the readily releasable Ca from the SR. All of the I_{cm} traces are similar to each other and to trace *e* in Fig. 9 *A*. This similarity shows that the condition of the fiber was stable during the course of the experiment.

In Fig. 9 *B*, the $\Delta[Ca_T]$ signals show small, approximately linear increases during the depolarization. In 11 experiments of this type carried out with a two-pulse protocol, small increases were observed twice during the second pulse, no changes were observed twice, and small decreases were observed seven times. Coincidentally, the largest changes in the absolute value of $\Delta[Ca_T]$ were those observed in the experiment in Fig. 9 *B*. Although such changes may arise from the reaction between Ca and EGTA and the associated change in myoplasmic pH, it is also possible that signals as small as these could reflect changes in pH that arise from other reactions.

The Effect of $[Ca_{SR}]_R$ on ON-OFF Charge Equality

Fig. 10 *A* shows the values of ON Q_{cm} (*diamonds*) and −OFF Q_{cm} (*squares*) plotted as a function of $[Ca_{SR}]_R$, from the experiment illustrated in Fig. 8. The letters *a–f* mark the points associated with the corresponding traces in Fig. 8 *A*. Filled symbols are used for the values from *a* to *e*, when the value of $[Ca_{SR}]_R$ was progressively decreased by the use of progressively shorter recovery periods between successive stimulations. The sequence was reversed after *e*, when the value of $[Ca_{SR}]_R$ was progressively increased by the use of progressively longer recovery periods. These data are represented by *open symbols*, with *f* marking the final point. From *a* to *e*, as the value of $[Ca_{SR}]_R$ was decreased, the value of −OFF Q_{cm} (*filled squares*) was relatively constant whereas the value of ON Q_{cm} (*filled diamonds*) decreased. From *e* to *f*, as the value of $[Ca_{SR}]_R$ was increased, both ON and −OFF Q_{cm} (*open symbols*) increased, although the increase in ON Q_{cm} (*open diamonds*) was more marked. These changes in Q_{cm}, which have been normalized by C_{app}, are not the result of changes in C_{app}, which varied less than 1% during the experiment (range, 0.01587–0.01601 μF).

Fig. 10 *B* shows the values of ON + OFF Q_{cm} plotted as a function of $[Ca_{SR}]_R$. *Filled triangles* show the values from *a* to *e*, and *open triangles* show the values after *e*. A positive (negative) value means that the absolute value of ON (OFF) Q_{cm} was greater than that of OFF (ON) Q_{cm}. The value of ON + OFF Q_{cm} was ~−3 nC/μF for the smallest values of $[Ca_{SR}]_R$ (*d* and *e*) and was 0–2 nC/μF for the largest values (*a* and *f*). The 3–5 nC/μF increase in ON + OFF Q_{cm} with increasing $[Ca_{SR}]_R$ could be a result of an increase in ON Q_{cm}, a decrease in OFF Q_{cm}, or both. The individual variations of ON and −OFF Q_{cm} with $[Ca_{SR}]_R$ in Fig. 10 *A* show that the

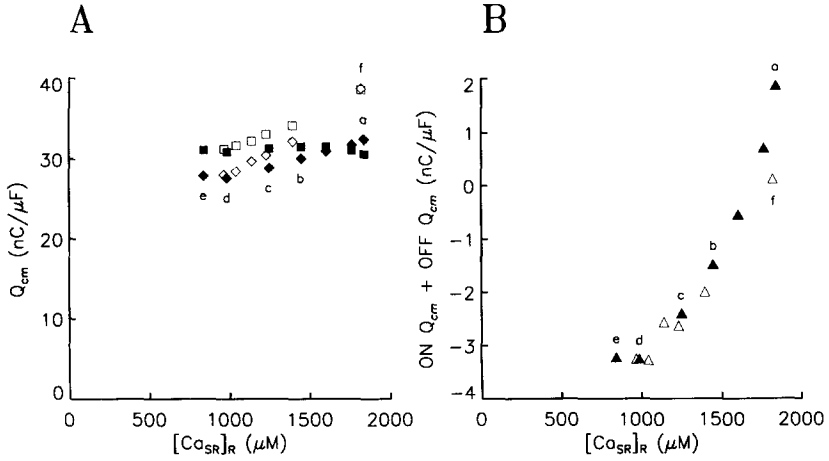

FIGURE 10. Effect of $[Ca_{SR}]_R$ on ON–OFF charge equality from the experiment in Fig. 8. (*A*) The *diamonds* and *squares* show the absolute values of ON and OFF Q_{cm}, respectively, plotted as a function of $[Ca_{SR}]_R$; constant ionic current templates were used for the estimates of Q_{cm}. (*B*) The *triangles* show the values of ON + OFF Q_{cm}, plotted as a function of $[Ca_{SR}]_R$. In both *A* and *B*, the letters *a–f* mark the points calculated from the traces in Fig. 8; *filled symbols* show the values obtained from *a* to *e* and open symbols show the values obtained after *e*.

values of ON Q_{cm} changed more than those of −OFF Q_{cm}. This is especially clear in the data from *a* to *e*, where the values of ON Q_{cm} decreased by almost 5 nC/μF (*filled diamonds*) whereas the values of −OFF Q_{cm} were relatively constant (*filled squares*).

Fig. 11 shows values of ON and −OFF Q_{cm} from the depletion experiment illustrated in Fig. 9. *A* and *B* show data from the first pulse (Fig. 9 *A*), and *C* and *D* show data from the second pulse (Fig. 9 *B*). The format of the figure is similar to that of Fig. 10 except that only open symbols have been used. In each panel, the abscissa represents the value of $[Ca_{SR}]_R$ before the first pulse.

Fig. 11 *A* shows that the value of −OFF Q_{cm} (*squares*) determined with the first pulse was relatively constant as the value of $[Ca_{SR}]_R$ decreased from 949 μM (*a*) to 6 μM (*e*, letter not shown). On the other hand, the value of ON Q_{cm} (*diamonds*) decreased as $[Ca_{SR}]_R$ decreased from 949 (*a*) to 300–400 μM and then remained relatively constant as $[Ca_{SR}]_R$ approached 0 μM. Figure 11 *B* shows that the value of ON Q_{cm} + OFF Q_{cm} decreased from ~4 to ~0 nC/μF when the value of $[Ca_{SR}]_R$ decreased from 949 μM (*a*) to 194 μM (*c*). This decrease is qualitatively similar to that shown in Fig. 10 *B*. With further decreases in the value of $[Ca_{SR}]_R$ in Fig. 11 *B*, from *c* to *d* to *e*, the value of ON Q_{cm} + OFF Q_{cm} increased ~1 nC/μF.

Fig. 11 *C* shows that the values of ON Q_{cm} (*diamonds*) and −OFF Q_{cm} (*squares*) determined with the second pulse were essentially constant during the experiment. Fig. 11 *D* shows that the values of ON Q_{cm} + OFF Q_{cm} were also essentially constant, with values that varied between 0.8 and 1.6 nC/μF. These results indicate that the condition of the fiber was stable during the experiment and that the changes in the value of ON Q_{cm} in Fig. 11 *A* and in the value of ON Q_{cm} + OFF Q_{cm} in Fig. 11 *B* were probably caused by the changes in the value of $[Ca_{SR}]_R$.

In Fig. 11 *D*, the mean value of ON Q_{cm} + OFF Q_{cm}, 1.23 nC/μF (SEM, 0.03 nC/μF), is significantly different from zero. For the reasons given in Jong et al. (1995*b*), this probably does not reflect a violation of ON–OFF charge equality. Jong et al. (1995*b*) measured ON Q_{cm} + OFF Q_{cm} in fibers in which the SR had been depleted of almost all of its readily releasable Ca. Many measurements of ON Q_{cm} + OFF Q_{cm} were made in

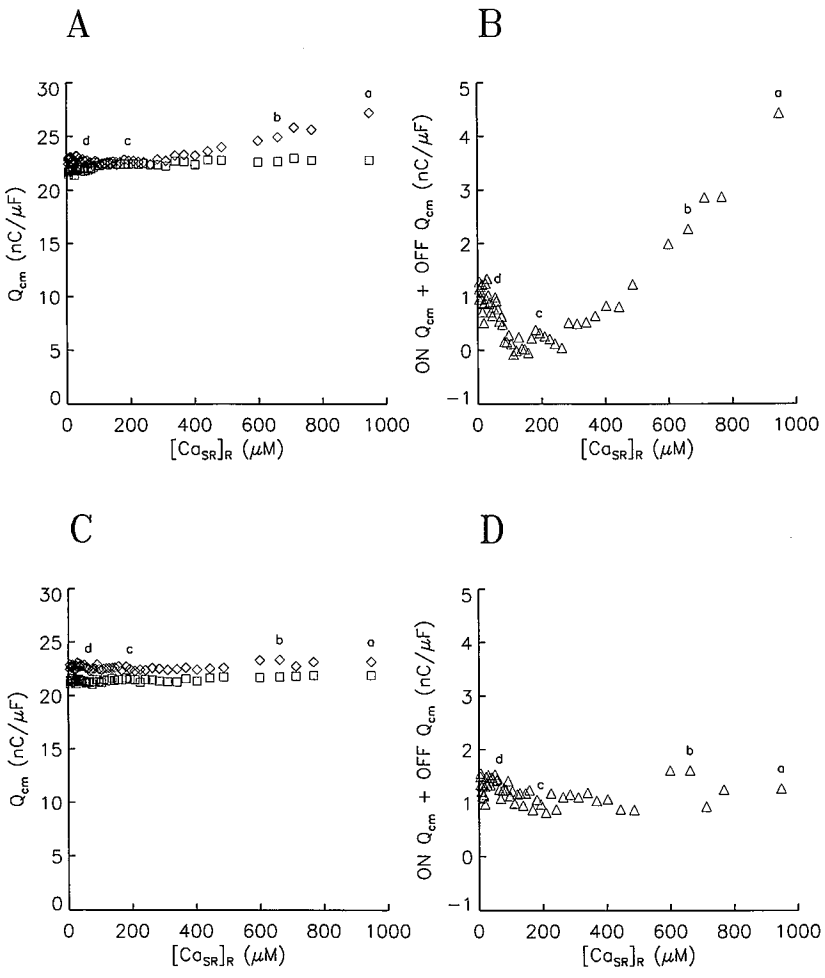

FIGURE 11. Effect of $[Ca_{SR}]_R$ on ON–OFF charge equality from the experiment in Fig. 9. (*A*, *B*) Values of Q_{cm} obtained from the traces in Fig. 9 *A* and other traces taken during the same experiment, plotted with the same format used for Fig. 10 except that only open symbols have been used. The letter *e*, corresponding to $[Ca_{SR}]_R$ = 6 μM, is not shown. (*C*, *D*) Similar to *A* and *B* except that the traces in Fig. 9 *B* and others from the same experiment were used for Q_{cm}.

each fiber and, in most of them, the mean value of ON Q_{cm} + OFF Q_{cm} was significantly different from zero, similar to the situation in Fig. 11 *D*. In each fiber in their study, the significant nonzero value of ON Q_{cm} + OFF Q_{cm} was considered to be caused by a consistent inaccuracy in the measurement of ON Q_{cm} + OFF Q_{cm} rather than by a violation of ON–OFF charge equality. The reason for thinking this is that, when the mean values of ON Q_{cm} + OFF Q_{cm} in all of their fibers were averaged, the resulting mean value of ON Q_{cm} + OFF Q_{cm} was not significantly different from zero.

The effect of $[Ca_{SR}]_R$ on ON–OFF charge equality was studied in a total of five fibers with pulse voltages that gave pronounced slow components of the ON I_{cm}, −48 to −40 mV. In three experiments, including the two in Figs. 10 *B* and 11 *B*, the value of ON Q_{cm} + OFF Q_{cm} increased with the value of $[Ca_{SR}]_R$. In the other two experiments, the value of ON Q_{cm} + OFF Q_{cm} showed little, if any, change with $[Ca_{SR}]_R$. The general conclusion is that, in some fibers, an increase in SR Ca content is able to increase the value of ON Q_{cm} + OFF Q_{cm}, probably by increasing the value of ON Q_{cm}. An increase in ON Q_{cm} could be caused by a genuine increase in the amount of intramembranous charge that moves during the pulse and does not return rapidly after repolarization. On the other hand, as described in the Discussion, it could be the result of a transient outward ionic current that makes a positive contribution to the estimate of ON Q_{cm} and little, if any, contribution to the estimate of OFF Q_{cm}.

The Effect of $[Ca_{SR}]_R$ on the Time Course of k_{app}

Fig. 12 shows signals associated with depolarizations to −48 mV during the depletion experiment in Fig. 9 *A*. The first set of superimposed traces shows three I_{cm} traces obtained with different values of $[Ca_{SR}]_R$: 194 μM in *c*, 64 μM in *d*, and 6 μM in *e*. For the first 10 ms after depolarization, all three traces are similar; they show a rapid increase followed by a small initial decrease. Thereafter, the traces diverge and the amplitude of the I_γ hump becomes progressively smaller from *c* to *e*. The duration of the hump becomes progressively longer, however, so that the value of ON Q_{cm} remains relatively constant, 22.74 nC/μF in *c*, 22.74 nC/μF in *d*, and 22.43 nC/μF in *e* (Fig. 11 *A*). These I_{cm} traces are similar to those in Fig. 2 in Jong et al. (1995*b*). The only difference in the protocol in the two experiments is that, in Fig. 12 *A*, the fiber was equilibrated first with an internal solution that contained 1.76 mM Ca and then with a Ca-free solution, whereas, in the experiment in Jong et al. (1995*b*), the fiber was equilibrated only with the Ca-free internal solution.

Jong et al. (1995*b*) described the ON kinetics of I_{cm} empirically in terms of k_{app}, the apparent rate constant for charge movement. Each value of k_{app} is obtained by dividing the value of ON I_{cm} by the amount of charge that has not yet moved, given by $Q_{cm}(\infty) - Q_{cm}$; $Q_{cm}(\infty)$ represents the amount of charge that is moved by a long-lasting pulse. The second set of superimposed traces in Fig. 12 *A* shows the calculated k_{app} traces, which were determined from the I_{cm} traces after they had been filtered with a 0.05-kHz digital Gaussian filter (Colquhoun and Sigworth, 1983) to reduce noise. The traces become progressively noisier after ~100 ms because the denominator in the expression for k_{app} becomes progressively smaller.

The third set of superimposed traces in Fig. 12 *A* shows $(k_{app}/k_{app,0} - 1)$, in which $k_{app,0}$ represents k_{app} in trace *e*, with $[Ca_{SR}]_R \cong 0$ μM. The value of $(k_{app}/k_{app,0} -$

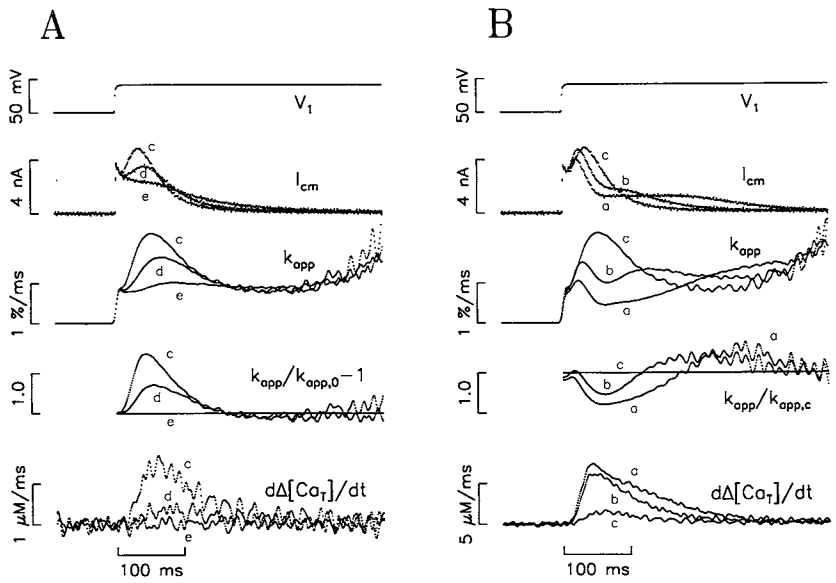

FIGURE 12. Effect of $[Ca_{SR}]_R$ on k_{app} from the experiment in Fig. 9. (*A*) The first set of superimposed traces shows I_{cm} traces *c*, *d*, and *e* from Fig. 9 *A*, as indicated. The next two sets of superimposed traces show k_{app} and $(k_{app}/k_{app,0} - 1)$, which were calculated from the I_{cm} traces after they had been filtered with a 0.05-kHz Gaussian digital filter; $k_{app,0}$ represents k_{app} from trace *e*. The bottom set of traces shows $d\Delta[Ca_T]/dt$. (*B*) The first two sets of superimposed traces are similar to those in *A* except that they were calculated from traces *a*, *b*, and *c* in Fig. 9 *A*. The bottom two sets of traces show $k_{app}/k_{app,c}$ and $d\Delta[Ca_T]/dt$; $k_{app,c}$ denotes k_{app} from trace *c*. See text for additional information.

1) provides an estimate of the acceleratory effect of SR Ca on k_{app}; a value of zero corresponds to no effect, a value of unity means that the value of k_{app} has been doubled with respect to $k_{app,0}$, and so forth. The traces in Fig. 12 *A* are very similar to those in Fig. 10 in Jong et al. (1995*b*). They show that, during the first 100–200 ms of depolarization, small values of $[Ca_{SR}]_R$ (or $d\Delta[Ca_T]/dt$) are associated with transient increases in $(k_{app}/k_{app,0} - 1)$ that have about the same time course as $d\Delta[Ca_T]/dt$, shown in the bottom set of traces.

Jong et al. (1995*b*) studied the effect of SR Ca content on $(k_{app}/k_{app,0} - 1)$ during step depolarizations to potentials between −50 and −30 mV. They found that the peak value of $(k_{app}/k_{app,0} - 1)$ increased with peak $d\Delta[Ca_T]/dt$ in a manner that could be described by a 1:1 binding equation. Between −50 and −30 mV, the half maximal value of $(k_{app}/k_{app,0} - 1)$ occurred at $d\Delta[Ca_T]/dt$ = 1.2–1.4 μM/ms (mean values). A similar analysis (not shown) was carried out on results from the experiment illustrated in Fig. 12 *A*. The value of $d\Delta[Ca_T]/dt$ for half maximal $(k_{app}/k_{app,0} - 1)$ was 0.85 μM/ms, which is similar to the values obtained by Jong et al. (1995*b*).

The first three sets of traces in Fig. 12 *B* are analogous to those in Fig. 12 *A* except that the value of $[Ca_{SR}]_R$ was larger: 949 μM in *a*, 662 μM in *b*, and 194 μM in *c*. The changes in the time course of k_{app} from *c* to *a* in Fig. 12 *B* show that a progressive increase in the value of $[Ca_{SR}]_R$ is correlated with a slowing of the ON kinetics of I_{cm} that becomes apparent at progressively earlier times. Trace *c* increased steadily to a peak that occurred at ~50 ms; it then decreased to a local minimum at 150–250 ms and eventually started to increase again. Trace *b* roughly coincided with trace *c* for ~20 ms, then deviated in the negative direction and reached a local minimum at 60–70 ms. Trace *a* also deviated from trace *c* but the deviation developed sooner than that of trace *b* and it was more pronounced.

The third set of superimposed traces in Fig. 12 *B* shows the three k_{app} traces divided, point by point, by the k_{app} trace labeled *c*, denoted by $k_{app,c}$; hence, the trace labeled *c* is constant and equal to unity. $k_{app}/k_{app,c}$ provides an estimate of the slowing effect of SR Ca on k_{app} relative to that associated with trace *c*. For the most part, traces *a* and *b* of $k_{app}/k_{app,c}$ lie below the unity baseline during the first 100–200 ms of depolarization, corresponding to the early decrease in k_{app}. At later times, they lie above the unity baseline, probably because k_{app} was increased by the acceleratory effect of SR Ca on k_{app} (Fig. 12 *A* and Jong et al., 1995*b*).

The bottom set of superimposed traces in Fig. 12 *B* shows $d\Delta[Ca_T]/dt$. If the ability of SR Ca to decrease k_{app} is mediated either by $d\Delta[Ca_T]/dt$ or by the increase that it produces in myoplasmic free [Ca] near the release sites, and if this decrease in k_{app} occurs without delay, k_{app} should reach a minimum at the same time that $d\Delta[Ca_T]/dt$ reaches a maximum. The minima in $k_{app}/k_{app,c}$ traces *a* and *b*, however, occurred 20–25 ms after the maxima in the corresponding $d\Delta[Ca_T]/dt$ traces.

An Estimate of the Half Inhibitory Effect of $[Ca_{SR}]_R$ or $d\Delta[Ca_T]/dt$ on k_{app}

Fig. 13 shows minimal values of $k_{app}/k_{app,c}$ from the experiment in Fig. 12 *B*, plotted as a function of $[Ca_{SR}]_R$ (*A*) and peak $d\Delta[Ca_T]/dt$ (*B*). They provide an estimate of the inhibitory effect of SR Ca on k_{app}. For values of $[Ca_{SR}]_R$ > 200–300 μM and values of $d\Delta[Ca_T]/dt$ > 2–3 μM/ms, the values of $k_{app}/k_{app,c}$ progressively decrease with increasing $[Ca_{SR}]_R$ and $d\Delta[Ca_T]/dt$. Half inhibition, which corresponds approximately to $k_{app}/k_{app,c}$ = 0.5, occurs at $[Ca_{SR}]_R \cong 600$ μM and $d\Delta[Ca_T]/dt \cong 6$ μM/ms.

A similar analysis was carried out with data from two other fibers in which the value of $[Ca_{SR}]_R$ was reduced to only ~300 μM. These fibers, studied with pulses to −47 and −40 mV, showed half inhibition when the value of $[Ca_{SR}]_R$ was ~800 and ~1,000 μM, respec-

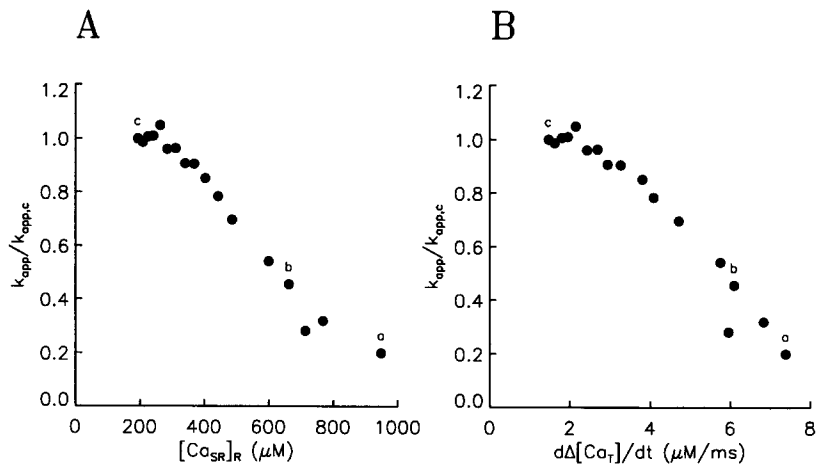

FIGURE 13. Minimal values of $k_{app}/k_{app,c}$ plotted as a function of $[Ca_{SR}]_R$ (*A*) and $d\Delta[Ca_T]/dt$ (*B*). The points marked *a*, *b*, and *c* were obtained from the $k_{app}/k_{app,c}$ traces in Fig. 12 *B* in the interval between 30 and 100 ms; the other points were obtained from other traces taken between *a* and *c*. The value of point *c* is unity by definition.

tively, and the value of $d\Delta[Ca_T]/dt$ was ~8 and ~11 μM/ms, respectively.

The general conclusion from these experiments is that progressive increases in the value of $[Ca_{SR}]_R$ above 200–300 μM and $d\Delta[Ca_T]/dt$ above 2–3 μM/ms are associated with progressive decreases in the ON kinetics of charge movement, most likely of I_γ. At pulse potentials between −48 and −40 mV, the inhibitory effect is half maximal when $[Ca_{SR}]_R \cong$ 500–1,000 μM and peak $d\Delta[Ca_T]/dt \cong$ 5–10 μM/ms.

Recovery from the Slow ON Kinetics of I_{cm} Appears to Be Several Times More Rapid than Recovery from Ca Inactivation of Ca Release

After a muscle fiber is depolarized and SR Ca release has started, two negative feedback processes can affect additional release: Ca can reduce Ca flux through SR release sites (Ca inactivation of Ca release, see above), and Ca can delay the opening of sites that have not yet been activated (slowing the ON kinetics of I_{cm}, this article). Increases in myoplasmic free [Ca] probably mediate both Ca inactivation of Ca release (Schneider and Simon, 1988) and the slowing of the ON kinetics of I_{cm} (see Discussion). Since both of these actions frequently occur concurrently, it is of interest to consider the possibility that they are under the control of the same myoplasmic Ca receptor. If this were the case, the same receptor would regulate the activity of two different proteins: the RyR, which forms the Ca channel that is sensitive to Ca inactivation of Ca release, and the DHPR, which is presumably responsible for I_{cm}.

There is already some evidence that the Ca receptor for Ca inactivation of Ca release is different from that for the slowing of the ON kinetics of I_{cm}. Jong et al. (1995*a*) showed that a 10–15-ms prepulse to −20 mV, followed by a brief period of repolarization, produced pronounced Ca inactivation of Ca release during a test pulse with little, if any, effect on the kinetics of ON I_{cm}. Since the slowing of the ON kinetics of I_{cm} appears to occur with a delay, a possible explanation consistent with the single receptor hypothesis is that the delay occurs after Ca binds to the receptor and that a prepulse duration of 10–15 ms is insufficient for the slowing effect to become apparent. If this were the case, another kind of experiment would be required to eliminate the possibility that the same Ca receptor regulates both Ca inactivation of Ca release and the slowing of the ON kinetics of I_{cm}.

To resolve this question, recovery from both the slowing of the ON kinetics of I_{cm} and Ca inactivation of Ca release were measured with a two-pulse method to find out whether they have the same time course. The first pulse was a 60-ms prepulse to −50 mV, which slowed the ON kinetics of I_{cm} and produced Ca inactivation of Ca release. After a variable period of repolarization to −90 mV, a second test pulse, also to −50 mV, was used to assess the amount of recovery of the two Ca-dependent processes. The top set of superimposed traces in Fig. 14 *A* shows V_1 during four trials, with recovery intervals of 10, 20, 40, and 100 ms. The middle and bottom sets of traces show I_{test}-$I_{control}$ and $d\Delta[Ca_T]/dt$, respectively.

Figure 14, *B* and *C*, shows the time course of recovery from the the slowing of the ON kinetics of I_{cm}. The *filled circles* in Fig. 14 *B* show the peak values of I_{test}-$I_{control}$ during the test pulse, normalized by the corresponding values during the prepulse, plotted as a function of the duration of repolarization. The continuous curve shows the least-squares fit of a decreasing exponential function plus a constant. The initial and final values of the curve are 0.42 and 0.97, respectively, and the time constant of the exponential function is 11.0 ms. Figure 14 *C* is similar to Fig. 14 *B* except that recovery is assessed in terms of peak values of k_{app} (traces not shown). The initial and final values of the fitted curve are 0.22 and 1.01, respectively, and the time constant of the exponential function is 5.8 ms. Thus, recovery from the slowing of the ON kinetics of I_{cm} appears to be rapid, with an apparent time constant of 11.0 ms for I_{test}-$I_{control}$ and 5.8 ms for k_{app}.

Figure 14 D shows the time course of recovery of $d\Delta[Ca_T]/dt$ from Ca inactivation of Ca release. The *filled circles* show the normalized peak values of test $d\Delta[Ca_T]/dt$, plotted against the duration of repolarization. The initial and final values of the fitted curve are 0.78 and 1.015, respectively, and the time constant of the exponential function is 61.8 ms. The value 61.8 ms lies within the range reported by Jong et al. (1995*a*), who studied recovery from Ca inactivation of Ca release under conditions that were essentially identical to those used here, except that their prepulses were briefer (duration of 10–15 ms) and more positive (to −20 mV).

The rapid recovery of the ON kinetics of I_{cm} in Fig. 14 is consistent with the experiment illustrated in Fig. 2, in which a local minimum occurred in the I_{cm} trace (see *arrow*) 10–15 ms after the peak in the $d\Delta[Ca_T]/dt$ trace. A plausible explanation is that the ability of SR Ca to slow the ON kinetics of I_{cm} is caused by SR Ca release or some associated event (see Discussion) but that the full effect requires 10–15 ms to develop. After I_{cm} had reached the local minimum in Fig. 2, it increased rather rapidly to a new quasi-steady level that was maintained for ~150 ms. The time constant associated with the final half of this rapid increase was 4–5 ms. If recovery from the local minimum in the I_{cm} trace reflects recovery of the ON kinetics of I_{cm} from the slowing effect of $d\Delta[Ca_T]/dt$, as seems possible, this experiment indicates that the time constant of recovery, 4–5 ms, was similar to the values of 11.0 and 5.8 ms that were determined in Fig. 14, *B* and *C*, respectively.

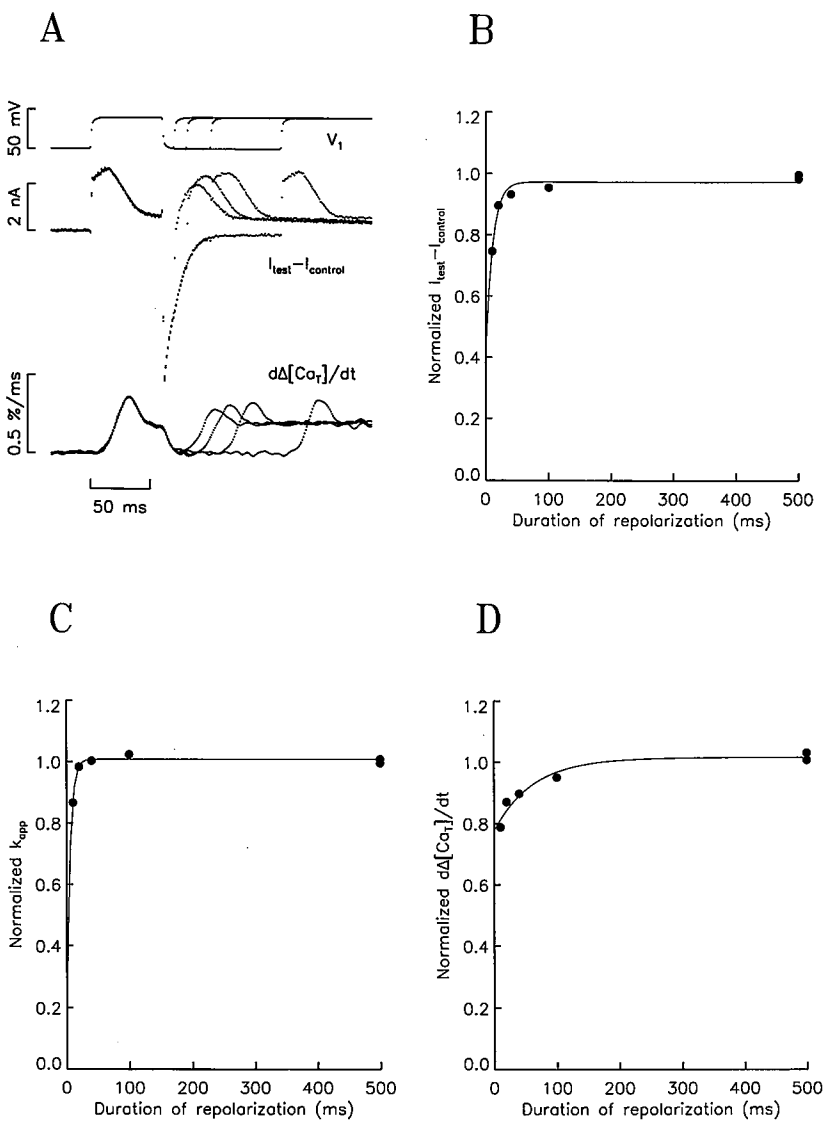

FIGURE 14. Recovery of the slow ON kinetics of I_{cm} and of Ca inactivation of Ca release during repolarization. The pulse protocol consisted of a 60-ms prepulse to −50 mV, a variable period of repolarization to −90 mV, and a 1,200-ms test pulse to −50 mV. (*A*) Three sets of superimposed traces show, from top to bottom, V_1, I_{test}-$I_{control}$, and $d\Delta[Ca_T]/dt$ associated with 10-, 20-, 40-, and 100-ms periods of repolarization. (*B*) *Filled symbols* show peak I_{test}-$I_{control}$ during the test pulse divided by the peak value during the prepulse, plotted as a function of the duration of repolarization. The curve shows a decreasing exponential function plus a constant that was least-squares fitted to the points; the initial and final values of the curve are 0.418 and 0.973, respectively, and the time constant of the exponential function is 11.0 ms. (*C, D*) Similar to B except that peak values of k_{app} and $d\Delta[Ca_T]/dt$, instead of I_{test}-$I_{control}$, were used. In *C*, the initial and final values of the fitted curve are 0.222 and 1.008, respectively, and the time constant is 5.8 ms. In *D*, the initial and final values of the fitted curve are 0.776 and 1.015 respectively, and the time constant is 61.8 ms. Fiber reference, 917911; sarcomere length, 3.4 μm; interval of time between data points, 0.96 ms; temperature, 14°C. First and last values: time after saponin treatment, 80–105 min; fiber diameter, 160–160 μm; holding current, −43 and −42 nA; C_{app}, 0.02005–0.01995 μF; r_i, 1.55–1.52 MΩ/cm; c_m, 0.277–0.275 μF/cm; OFF $Q_{cm}(\infty)$ after the test pulse, 18.31–18.95 nC/μF; concentration of phenol red at the optical site, 1.034–1.276 mM; estimated pH_R and free $[Ca]_R$, 6.864–6.856 and 0.068–0.070 μM, respectively; $[Ca_{SR}]_R$, 2,685–2,587 μM. The end-pool solution contained 1.76 mM total Ca.

Since I_{cm} probably arises from transitions of intramembranous charge particles between several states, it is unlikely that the state of the ON kinetics of I_{cm} at any moment in time can be described precisely by a single variable such as I_{test}-$I_{control}$ or k_{app}. In spite of this uncertainty, the results in Fig. 14 suggest that the recovery of the ON kinetics of I_{cm} from the slowing effect of $d\Delta[Ca_T]/dt$ is an order of magnitude more rapid than the recovery of peak $d\Delta[Ca_T]/dt$ from Ca inactivation of Ca release. This difference makes it unlikely that Ca inactivation of Ca release and the slowing of the ON kinetics of I_{cm} are controlled by the same myoplasmic Ca receptor.

DISCUSSION

The experiments reported in this article show that, when the SR contains at least several hundred micromolar Ca (expressed as myoplasmic concentration), a slow component of ON I_{cm} can be observed (Figs. 1–3). This component is most pronounced during depolarizations to −55 to −40 mV and can last as long as 500 ms at 14°–16°C (Fig. 5 *B*). At less positive potentials, the slow component is less pronounced, perhaps because the rate of SR Ca release is small and any effect that it might exert on the ON kinetics of I_{cm} would be difficult to resolve because I_{cm} is small. At more positive potentials, the durations of both I_{cm} and the rate of SR Ca release become progressively briefer and, consequently, the slow component becomes progressively briefer (Fig. 5 *B*).

Since, at all potentials, the slow component of ON I_{cm} disappears when the SR is depleted of Ca, the possibility arises that it might represent a new species of intramembranous charge that becomes apparent only when the SR contains at least several hundred micro-

molar Ca. This appears not to be the case, however, because the value of OFF Q_{cm} after a depleting depolarization is essentially the same with $[Ca_{SR}]_R$ = 1,000–3,000 μM as with $[Ca_{SR}]_R$ < 50 μM (Fig. 6). This independence of the value of OFF Q_{cm} on $[Ca_{SR}]_R$ makes it likely that the slow component of ON I_{cm} is due to a slowing of the kinetics of one of the known components of I_{cm}, I_β or I_γ. I_β seems an unlikely candidate, since the initial time course of ON I_{cm} is essentially the same with $[Ca_{SR}]_R$ = 1,000–3,000 μM as with $[Ca_{SR}]_R$ < 10 μM (Fig. 8 *C* and Fig. 12). By elimination, then, it seems likely that the slow component of ON I_{cm} is due to a slowing of the ON kinetics of I_γ. If this slowing effect is expressed in terms of an inhibition of k_{app} (Fig. 12 *B*), half inhibition occurs when $[Ca_{SR}]_R \cong$ 500–1,000 μM and peak $d\Delta[Ca_T]/dt \cong$ 5–10 μM/ms (Fig. 13), as assessed at pulse potentials between −48 and −40 mV.

In addition to slowing the ON kinetics of I_{cm}, SR Ca also appears to alter the OFF kinetics of I_{cm}; the increase in half-width of OFF I_{cm} that is observed with increasing pulse duration in a Ca-depleted fiber (Fig. 8 *C* in Jong et al., 1995*b*) is delayed in a non-depleted fiber until the SR has released almost all of its Ca (Fig. 3 and associated text).

The rest of the Discussion is concerned with four issues that arose during the course of our study.

Does ON-OFF Charge Equality Hold during the Slow Component of ON I_{cm}?

In two out of five experiments, the value of ON Q_{cm} + OFF Q_{cm} was essentially independent of $[Ca_{SR}]_R$ between 0 and 1,000–3,000 μM (not shown). In the three other experiments, however, the value of ON Q_{cm} + OFF Q_{cm} progressively increased with increasing $[Ca_{SR}]_R$ (Figs. 10 and 11). Since ON–OFF charge equality is expected to hold when the value of $[Ca_{SR}]_R$ is small, no more than a few hundred micromolar (Jong et al., 1995*b*), these increases in ON Q_{cm} + OFF Q_{cm} are probably due to the absolute value of ON Q_{cm} becoming greater than that of OFF Q_{cm}. Moreover, since there was a clear indication in at least portions of these three experiments that the value of OFF Q_{cm} was relatively independent of $[Ca_{SR}]_R$ (e.g., Fig. 11 *A* and the part of Fig. 10 *A* denoted by *filled symbols*), the increases in ON Q_{cm} + OFF Q_{cm} with $[Ca_{SR}]_R$ were probably the result of increases in the value of ON Q_{cm} with little, if any, change in the value of OFF Q_{cm}.

Although it is possible that SR Ca might be able to increase the value of ON Q_{cm} without any measurable effect on OFF Q_{cm}, it is important to consider the possibility that the estimate of ON Q_{cm} is contaminated by a component of ionic current that is observed only when the SR contains Ca. During SR Ca release, for example, an increase in myoplasmic free [Ca] might gate Ca-activated ionic channels in the surface or transverse tubular membranes. If such channels allow either Cs or gluconate ions to move down their respective electrochemical gradients, an outward current would develop from Cs movement from the internal to the external solution or gluconate movement in the opposite direction. If the gating by Ca were rapid, as is the case for maxi K channels in urinary bladder myocytes (Markwardt and Isenberg, 1992), the time course of the change in permeability would be expected to be similar to that of myoplasmic free [Ca] near the activation sites of the channels. In fibers equilibrated with 20 mM EGTA, changes in myoplasmic free [Ca] are expected to be confined to distances within a few hundred nanometers of the open SR Ca channels and to have a time course that is similar to that of the rate of Ca release (Pape et al., 1995). Thus, any outward current through rapidly gated Ca-activated channels would be expected to occur only during the period of SR Ca release. Such current could explain the results in Figs. 10 *B* and 11 *B*, since it would be expected to increase the estimate of ON Q_{cm} and to not affect the estimate of OFF Q_{cm}, at least after a long-lasting depleting depolarization. This idea is plausible, since Ca-activated K channels (Pallotta et al., 1981) and Ca-activated Cl channels (Hui and Chen, 1994) are known to be present in skeletal muscle fibers. For the idea to apply to our experiments, it would be necessary for the Ca-activated K channels to be slightly permeable to Cs ions or for the Ca-activated Cl channels to be slightly permeable to gluconate ions. As far as we are aware, it is not known whether this is the case in the Ca-activated channels in frog muscle fibers.

The tentative conclusion of this section is that ionic currents through Ca-activated channels might contaminate the estimate of ON Q_{cm} in experiments on fibers that contain at least several hundred micromolar Ca inside their SR. Such currents might account for the apparent inequality of ON–OFF charge in experiments such as those in Figs. 10 and 11.

Is the Slowing Effect of SR Ca on ON I_{cm} Due to SR Ca Content Per Se or to SR Ca Release or Some Associated Event?

In assessing the effects of SR Ca on I_{cm}, it is sometimes difficult to tell whether a particular effect is due to SR Ca content, to SR Ca release, or to some event associated with release such as an increase in myoplasmic free [Ca] near the SR Ca release sites. In the case of the acceleration of the ON kinetics of I_γ that is observed with small amounts of Ca inside the SR, Jong et al. (1995*b*) provided evidence that SR Ca release or some associated event plays the main causal role.

Two observations suggest that the slowing effect of SR Ca on ON I_{cm} is also caused by SR Ca release or some associated event. The first observation is that the

decrease in k_{app} does not occur immediately after depolarization, when the value of $[Ca_{SR}]$ is maximal, but develops later as the value of $[Ca_{SR}]$ is decreasing; the time course of the decrease in k_{app} is similar to that of $d\Delta[Ca_T]/dt$ but somewhat delayed (Figs. 8 *C* and 12 *B*). The second observation is that, in some experiments and under certain conditions, I_{cm} and k_{app} show prominent dips with local minima (Fig. 2 for dip in I_{cm}). Since the local minima occurred 10–15 ms after the peaks in the corresponding $d\Delta[Ca_T]/dt$ signals, the simplest interpretation is that the ON kinetics of I_{cm} is slowed by $d\Delta[Ca_T]/dt$, rather than $[Ca_{SR}]$, and that the slowing occurs with a delay of 10–15 ms.

SR Ca release can influence Ca-dependent processes in the myoplasm in two ways: by an increase in spatially averaged free [Ca] and by local increases in free [Ca] near the SR release sites. An increase in spatially averaged free [Ca] does not appear to be the cause of the slowing of the ON kinetics of I_{cm} because a depolarizing prepulse (condition II in Fig. 5) removes the slowing effect even though it increases the value of spatially averaged free [Ca], sometimes above the peak initial value that would be observed without the prepulse (Fig. 4). Local increases in myoplasmic free [Ca] near the SR release sites appear to be a more likely cause of the slowing effect. As mentioned above, in fibers equilibrated with 20 mM EGTA, such as those used in our experiments, such increases are expected to be restricted to distances within a few hundred nanometers of the release sites and to have an initial time course that is approximately proportional to that of $d\Delta[Ca_T]/dt$ with a proportionality constant that depends inversely on distance from the release sites (Pape et al., 1995).

A plausible hypothesis is that SR Ca release is able to slow the ON kinetics of I_{cm} by increasing the value of free [Ca] in the myoplasm near the release sites, perhaps near the DHPRs. The results in Figs. 2, 8 *C*, and 12 *B* suggest that this effect is not instantaneous but occurs with a delay (see above). The results in Figs. 2 and 12 *B* suggest that this delay is 10–15 and 20–25 ms, respectively, whereas those in Fig. 8 *C* suggest that it is no more than 5–10 ms. The reason for these apparent inconsistencies in the duration of the delay may be related to the complexity of the ON kinetics of I_{cm} and the possibility, as mentioned in the text discussion of Fig. 14, that the state of intramembranous charge cannot be described precisely by a single variable such as I_{cm} or k_{app}.

If the slowing of the ON kinetics of I_{cm} is caused by SR Ca release, as seems likely, maneuvers that decrease Ca inactivation of Ca release, and thereby increase the rate of Ca release, might be expected to accentuate the slowing effect. This might explain why the slow component of I_{cm} was so pronounced in the experiments reported in this article: Ca inactivation of Ca release appears to be decreased severalfold by equilibration of a fiber with 20 mM EGTA (Jong et al., 1995*a*).

Are the Effects of SR Ca on the Kinetics of I_{cm} Consistent with the Model of Q_γ, Case 3, Proposed by Jong et al. (1995b)?

An important question to consider is whether the results described in this article, about the effects of SR Ca release on the kinetics of I_{cm}, are consistent with the model of Q_γ proposed by Jong et al. (1995*b*), case 3. According to this model, the charge associated with Q_γ arises from groups of four interacting charge movement particles. Within a group, each particle can be in one of two states, resting and activating, with forward and backward rate constants that depend on the number of particles in the activating state. In general, as more particles enter the activating state, the forward rate constant becomes larger and the backward rate constant becomes smaller. These changes, which are produced by cooperative interactions among the charge movement particles, underlie the I_γ hump and the slowing of the OFF kinetics of I_{cm} that is observed with increasing pulse duration.

After depolarization of a fiber that contains at least several hundred micromolar Ca inside the SR, the I_{cm} trace shows an early I_γ hump before the severalfold reduction in amplitude of I_{cm} that defines the beginning of the slow component (Figs. 1 and 2). In terms of the model proposed by Jong et al. (1995*b*), the presence of an I_γ hump, although abbreviated, implies that cooperative interactions were able to occur initially among the charge movement particles and to increase the value of some of the forward rate constants. The subsequent severalfold reduction in I_{cm} indicates that the values of some of the rate constants were then reduced.

During the period of reduced ON kinetics of I_{cm}, while the SR was releasing Ca, the OFF kinetics remained relatively rapid and did not become slow until after the SR had released most of its Ca (Fig. 3 *C* and associated text). In terms of the model proposed by Jong et al. (1995*b*), this means that the decrease in the value of the backward rate constants that is attributed to cooperative interactions among charge movement particles did not become apparent until SR Ca release was almost over.

Thus, in terms of the model of Q_γ proposed by Jong et al. (1995*b*), case 3, the effect of SR Ca release on the forward and backward rate constants of charge movement is qualitatively similar to a reduction, possibly delayed, in the cooperative interactions among charge movement particles. It remains to be established, however, whether such cooperative interactions actually occur and whether their influence is reduced during SR Ca release. Even if this turns out to be the case, it is not clear that this explanation can account for all of the reduction in k_{app} that is observed experimentally,

such as the marked reduction in k_{app} in trace *a* in Fig. 12 *B*.

Possible Physiological Importance of the Slowing of the ON Kinetics of I_{cm} by SR Ca Release

Frog skeletal muscle has two negative feedback pathways between myoplasmic Ca and SR Ca release: Ca inactivation of Ca release (Baylor et al., 1983; Simon et al., 1985; Schneider and Simon, 1988) and slowing of the ON kinetics of I_{cm} by SR Ca (this article).

The physiological role of Ca inactivation of Ca release is to limit the amount of Ca that is released from the SR into the myoplasm and to thereby help prevent large increases in myoplasmic free [Ca] after stimulation. After a single action potential, for example, SR Ca channels are opened in response to depolarization of the membranes of the transverse tubules. When sufficient Ca has been released from the SR to complex most of the Ca-regulatory sites on troponin so that contraction can occur, the Ca channels are closed and release stops. Channel closure is probably caused by Ca inactivation of Ca release and by removal of activation by repolarization of the transverse tubular membranes. Although the relative importance of these two processes has not been determined, Ca inactivation of Ca release appears to play a role in limiting release elicited by a single action potential (Baylor and Hollingworth, 1988; Hollingworth et al., 1992; Pape et al., 1993).

If a fiber is stimulated to give a train of action potentials, the amount of Ca released by the second and subsequent action potentials is only 0.1–0.2 times that released by the first action potential (Baylor et al., 1983; Baylor and Hollingworth, 1988; Pape et al., 1993). This reduction of Ca release is usually attributed to the Ca inactivation of Ca release that is produced by the first stimulation and is maintained by subsequent stimulations (Baylor et al., 1983; Baylor and Hollingworth, 1988; Pape et al., 1993). The results presented here raise the possibility that this reduction is also aided by the slowing of the ON kinetics of I_{cm} by SR Ca. If the slowing is caused by SR Ca release or some associated event, as seems likely (see above), and if it develops with a delay of $\geq$5 ms, as also seems likely (at least at the potentials studied in our experiments, see above), it should have little effect on the activation of SR Ca release elicited by the first action potential. In muscle fibers equilibrated with 20 mM EGTA, the rate of recovery from the slowing effect is sufficiently rapid (Fig. 14) that activation of SR Ca release elicited by subsequent action potentials should also be little affected. On the other hand, under normal physiological conditions, in fibers with unmodified [Ca] transients, recovery from the slowing effect might not occur until the value of myoplasmic free [Ca] decreases to near the resting level, similar to the requirement for recovery from Ca inactivation of Ca release (Schneider and Simon, 1988). In this case, activation of SR Ca release by the second and subsequent action potentials in a train could be reduced by slowing of the ON kinetics of I_{cm}. If so, both Ca inactivation of Ca release and slowing of the ON kinetics of I_{cm} by SR Ca may be important for limiting SR Ca release elicited by normal repetitive stimulation.

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REFERENCES

Adrian, R. H., and C. L.-H. Huang. 1984. Experimental analysis of the relationship between charge components in skeletal muscle of *Rana temporaria*. *Journal of Physiology*. 353:419–434.

Adrian, R. H., and A. Peres. 1977. A 'gating' signal for the potassium channel? *Nature*. 267:800–804.

Adrian, R. H., and A. Peres. 1979. Charge movement and membrane capacity in frog muscle. *J. Physiol. (Lond.)*. 289:83–97.

Baylor, S. M., W. K. Chandler, and M. W. Marshall. 1983. Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from arsenazo III calcium transients. *J. Physiol. (Lond.)*. 344:625–666.

Baylor, S. M., and S. Hollingworth. 1988. Fura-2 calcium transients in frog skeletal muscle fibres. *J. Physiol. (Lond.)*. 403:151–192.

Block, B. A., T. Imagawa, K. P. Campbell, and C. Franzini-Armstrong. 1988. Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J. Cell Biol.* 107:2587–2600.

Chandler, W. K., and C. S. Hui. 1990. Membrane capacitance in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *J. Gen. Physiol.* 96:225–256.

Chen, W., and C. S. Hui. 1991. Differential blockage of charge movement components in frog cut twitch fibres by nifedipine. *J. Physiol. (Lond.)*. 444:579–603.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 191–263.

Csernoch, L., G. Pizarro, I. Uribe, M. Rodriguez, and E. Ríos. 1991.

Interfering with calcium release suppresses I_γ, the "hump" component of intramembranous charge movement in skeletal muscle. *J. Gen. Physiol.* 97:845–884.

Endo, M., M. Tanaka, and S. Ebashi. 1968. Release of calcium from sarcoplasmic reticulum in skinned fibers of the frog. *Proc. Int. Cong. Physiol. Sci.* 7:126.

Ford, L. E., and R. J. Podolsky. 1968. Force development and calcium movements in skinned muscle fibers. *Fed. Proc.* 27:375.

Franzini-Armstrong, C., and A. O. Jorgensen. 1994. Structure and development of E-C coupling units in skeletal muscle. *Annu. Rev. Physiol.* 56:509–534.

García, J., G. Pizarro, E. Ríos, and E. Stéfani. 1991. Effect of the calcium buffer EGTA on the "hump" component of charge movement in skeletal muscle. *J. Gen. Physiol.* 97:885–896.

Hollingworth, S., A. B. Harkins, N. Kurebayashi, M. Konishi, and S. M. Baylor. 1992. Excitation-contraction coupling in intact frog skeletal muscle fibers injected with mmolar concentrations of fura-2. *Biophys. J.* 63:224–234.

Huang, C. L.-H. 1990. Voltage-dependent block of charge movement components by nifedipine in frog skeletal muscle. *J. Gen. Physiol.* 96:535–557.

Huang, C. L.-H. 1994. Kinetic separation of charge movement components in intact frog skeletal muscle. *J. Physiol. (Lond.).* 481:357–369.

Hui, C. S., and W. K. Chandler. 1990. Intramembranous charge movement in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *J. Gen. Physiol.* 96:257–297.

Hui, C. S., and W. K. Chandler. 1991. Q_β and Q_γ components of intramembranous charge movement in frog cut twitch fibers. *J. Gen. Physiol.* 98:429–464.

Hui, C. S., and W. Chen. 1994. Evidence for the non-existence of a negative phase in the hump charge movement component (I_γ) in *Rana temporaria*. *J. Physiol. (Lond.).* 474:275–282.

Hymel, L., M. Inui, S. Fleischer, and H. Schindler. 1988. Purified ryanodine receptor of skeletal muscle sarcoplasmic reticulum forms Ca^{2+}-activated oligomeric Ca^{2+} channels in planar bilayers. *Proc. Natl. Acad. Sci., USA.* 85:441–445.

Imagawa, T., J. S. Smith, R. Coronado, and K. P. Campbell. 1987. Purified ryanodine receptor from skeletal muscle sarcoplasmic reticulum is the Ca^{2+} permeable pore of the calcium release channel. *J. Biol. Chem.*. 262:16636–16643.

Jong, D.-S., P. C. Pape, S. M. Baylor, and W. K. Chandler. 1995*a*. Calcium inactivation of calcium release in frog cut muscle fibers that contain millimolar EGTA or fura-2. *J. Gen. Physiol.* 106:337–388.

Jong, D.-S., P. C. Pape, and W. K. Chandler. 1995*b*. Effect of sarcoplasmic reticulum calcium depletion on intramembranous charge movement in frog cut muscle fibers. *J. Gen. Physiol.* 106: 659–704.

Lai, F. A., H. P. Erickson, E. Rousseau, Q.-Y. Liu, and G. Meissner. 1988. Purification and reconstruction of the calcium release channel from skeletal muscle. *Nature.* 331:315–319.

Markwardt, F., and G. Isenberg. 1992. Gating of maxi K^+ channels studied by Ca^{2+} concentration jumps in excised inside-out multichannel patches (myocytes from Guinea pig urinary bladder). *J. Gen. Physiol.* 99:841–862.

Meissner, G. 1994. Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. *Annu. Rev. Physiol.* 56: 485–508.

Pallotta, B. S., K. L. Magleby, and J. N. Barrett. 1981. Single channel recordings of Ca^{2+} activated K^+ currents in rat muscle cell culture. *Nature.* 293:471–474.

Pape, P. C., D.-S. Jong, and W. K. Chandler. 1992. Effects of sarcoplasmic reticulum (SR) calcium loading on intramembranous charge movement in frog cut muscle fibers. *Biophys. J.* 61:A130.

Pape, P. C., D.-S. Jong, and W. K. Chandler. 1995. Calcium release and its voltage dependence in frog cut muscle fibers equilibrated with 20 mM EGTA. *J. Gen. Physiol.* 106:259–336.

Pape, P. C., D.-S. Jong, W. K. Chandler, and S. M. Baylor. 1993. Effect of fura-2 on action-potential stimulated calcium release in cut twitch fibers from frog muscle. *J. Gen. Physiol.* 102:295–332.

Pizarro, G., L. Csernoch, I. Uribe, M. Rodriguez, and E. Ríos. 1991. The relationship between Q_γ and Ca release from the sarcoplasmic reticulum in skeletal muscle. *J. Gen. Physiol.* 97:913–947.

Ríos, E., and G. Brum. 1987. Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature.* 325:717–720.

Ríos, E., and G. Pizarro. 1991. Voltage sensor of excitation-contraction coupling in skeletal muscle. *Physiol. Rev.* 71:849–908.

Schneider, M. F. 1994. Control of calcium release in functioning skeletal muscle fibers. *Annu. Rev. Physiol.* 56:463–484.

Schneider, M. F., and B. J. Simon. 1988. Inactivation of calcium release from the sarcoplasmic reticulum in frog skeletal muscle. *J. Physiol. (Lond.).* 405:727–745.

Shirokova, N., G. Pizarro, and E. Ríos. 1994. A damped oscillation in the intramembranous charge movement and calcium release flux of frog skeletal muscle fibers. *J. Gen. Physiol.* 104:449–477.

Simon, B. J., M. G. Klein, and M. F. Schneider. 1991. Calcium dependence of inactivation of calcium release from the sarcoplasmic reticulum in skeletal muscle fibers. *J. Gen. Physiol.* 97:437–471.

Simon, B. J., M. F. Schneider, and G. Szücs. 1985. Inactivation of sarcoplasmic reticulum calcium release in frog skeletal muscle is mediated by calcium. *J. Gen. Physiol.* 86:36a.

Szücs, G., L. Csernoch, J. Magyar, and L. Kovács. 1991. Contraction threshold and the "hump" component of charge movement in frog skeletal muscle. *J. Gen. Physiol.* 97:897–911.

Tanabe, T., K. G. Beam, J. A. Powell, and S. Numa. 1988. Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature.* 336:134–139.