Negative Control Mechanism with Features of Adaptation Controls Ca^{2+} Release in Cardiac Myocytes

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ABSTRACT The central paradox of cardiac excitation-contraction coupling is that Ca^{2+}-induced Ca^{2+} release (CICR), an inherently self-regenerating process, is finely graded by surface membrane Ca^{2+} current (I_{Ca}). By using FPL 64176, a novel Ca^{2+} channel agonist that reduces inactivation of I_{Ca}, a rapid negative control mechanism was unmasked at the Ca^{2+} release level in isolated rat ventricular myocytes. This mechanism terminates CICR independently of the duration of trigger I_{Ca} and before the sarcoplasmic reticulum becomes depleted of Ca^{2+}. In its ability to be reactivated by incremental increases in trigger I_{Ca}, this mechanism differs from conventional inactivation/desensitization and is similar to the mechanism of increment detection or adaptation described for intracellular Ca^{2+} release channels. These results indicate that ryanodine receptor adaptation regulates Ca^{2+} release in cardiac muscle, accounting for or contributing to the graded nature of CICR and, additionally, permitting stores to reload at later times during Ca^{2+} entry.

INTRODUCTION

In mammalian heart, it is believed that calcium entering the cell via the L-type calcium channel (I_{Ca}) is the activator of the Ca^{2+} release channels of the sarcoplasmic reticulum (SR) (Fabiato and Fabiato, 1979; Cleemann and Morad, 1991; Sipido and Wier, 1991; Fabiato, 1983; Stern and Lakatta, 1992); by definition, this is calcium-induced Ca^{2+} release (CICR). Ca^{2+} released via this mechanism binds to troponin-C and initiates formation of actin-myosin cross-bridges, causing contraction. It is known that both Ca^{2+} release and resulting contraction are closely graded according to the amount of Ca^{2+} entry, with submaximal Ca^{2+} currents eliciting partial releases (Cleemann and Morad, 1991; Sipido and Wier, 1991; Cannell et al., 1987). This situation creates a paradox because of the expectation that CICR should exhibit strong positive feedback and lead to all-or-none Ca^{2+} release. Two kinds of mechanisms have been suggested to explain this dilemma. (i) Existence of morphological subdomains that separate trigger Ca^{2+} from the released Ca^{2+}. Ca^{2+} entering through the L-type Ca^{2+} channel might have preferential access to the activation site on the ryanodine receptor (RyR), with released Ca^{2+} exerting little positive feedback on further release (Stern, 1992; Gyorke and Palade, 1993). (ii) Existence of some negative control mechanism at the Ca^{2+} release level that counters the intrinsic positive feedback of CICR. Ca^{2+}-dependent inactivation of release has been demonstrated in skinned cardiac myocytes (Fabiato, 1985a) and in isolated SR vesicles (Chamberlain et al., 1984; Meissner and Henderson, 1987; Chu et al., 1993). Recently, it has been reported that cardiac SR Ca^{2+} release channels, incorporated in lipid bilayers and activated by photolysis of caged Ca^{2+}, undergo a unique kind of inactivation process characterized by the ability of apparently inactivated stores to be reactivated by higher [Ca^{2+}] (Gyorke and Fill, 1993).

The main difficulty in discriminating between these mechanisms at the whole cell level is that the Ca^{2+} current itself exhibits a rapid inactivation (Lee et al., 1985; Hadley and Hume, 1985; Cohen and Lederer, 1987), which obscures any inactivation process for Ca^{2+} release. To overcome this obstacle, we used FPL 64176, an L-type Ca^{2+} channel agonist that reduces I_{Ca} inactivation (Rampe and Lacerda, 1991). By slowing down I_{Ca} inactivation, we unmasked a separate, rapid ($\tau \sim 15$ ms), negative control mechanism, operating at the SR Ca^{2+} release level. This negative control mechanism is different from conventional inactivation/desensitization in that apparently inactivated release can be reactivated by a further increase in trigger Ca^{2+}. Thus, this study presents experimental evidence that RyR adaptation is a control mechanism for CICR in cardiac myocytes.

MATERIALS AND METHODS

Single ventricular myocytes were obtained from adult Sprague-Dawley rat hearts by enzymatic dissociation (Yazawa et al., 1990). The cells were loaded with fluo-3 by a 20-min incubation with 5 μM fluo-3 (acetoxymethyl ester form, Molecular Probes Inc, Eugene, OR) at room temperature. Membrane current were recorded using the whole-cell patch-clamp technique (Hamill et al., 1981). Pipettes had resistances of 3–5 MΩ when filled with internal solution containing 120 mM Cs-aspartate, 20 mM CsCl, 3 mM Na_2ATP, 3.5 mM $MgCl_2$, 5 mM HEPES, and 50 μM fluo-3 (pentaammonium salt, Molecular Probes) (pH 7.3). The external solution contained 140 mM NaCl, 3.0 mM CsCl, 0.5 mM $MgCl_2$, 1.0 mM $CaCl_2$, 10 mM HEPES, 0.25 mM NaH_2PO_4, 5.6 mM glucose, and 10 μM TTX (pH 7.3). Voltage pulses were applied from a holding potential of −50 mV at 30-s intervals. Current signals were filtered at 2 kHz and sampled at 4 kHz. Optical measurements were performed with an inverted microscope equipped with a photomultiplier tube. Light from a 150 W Xenon lamp was directed through a 485 nm excitation filter and reflected off a 505 nm dichroic mirror through a long working 20X fluor objective (Nikon). The emitted light was collected through the same objective and dichroic mirror and filtered with a 530 nm band pass filter (Omega Optical, Brattleboro, VT). Optical signals were filtered at 500 Hz, sampled at 4 kHz, and normalized to the resting fluorescence ($\Delta F/F$). All experiments were performed at room temperature (20–23°C).

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SR microsomes were isolated by differential centrifugation of dog heart homogenates by standard procedures (Tate et al., 1985). Ca^{2+} uptake and caffeine-induced Ca^{2+} release under control conditions and in the presence of 1 μM FPL 64176 were measured as described elsewhere (Dettbarn and Palade, 1993).

RESULTS AND DISCUSSION

Fig. 1 shows traces of inward Ca^{2+} current and intracellular Ca^{2+} transient recorded during a depolarizing pulse from −50 to +30 mV in an isolated rat myocyte loaded with the fluorescent Ca^{2+} indicator fluo-3. At 30 mV, the amplitude of I_{Ca} is decreased because of a reduced driving force for Ca^{2+}; consequently, the Ca^{2+} release is also submaximal. The dotted line shows the maximal Ca^{2+} transient recorded with a depolarizing pulse to 0 mV. In the upper part of the figure, the first derivative of Ca^{2+} transients is used as a measure of the rate of change in intracellular Ca^{2+}. In rat myocytes during Ca^{2+} transients, SR Ca^{2+} release far surpasses any other Ca^{2+} flux into or out of myoplasm, including flux through the L-type Ca^{2+} channels, SR pump flux (Wier et al., 1994), and Ca^{2+} removal by the Na^+/Ca^{2+} exchange (Bers et al., 1990). In our experiments as well, the rate of decline of the Ca^{2+} transient, a rough indicator of the rate of Ca^{2+} removal from the myoplasm (although some residual Ca^{2+} release after repolarization cannot be excluded) is about 10 times slower than the rate of rise of the Ca^{2+} transient. Thus, the decay of the total Ca^{2+}-change signal must result mainly from a reduction in the Ca^{2+} release and could not be accounted for by stimulation of an extrusion or sequestration mechanism. The early decay of release is not caused by depletion of Ca^{2+} from the SR, because only a fraction of the total intracellular Ca^{2+} is released at 30 mV. Because the rates of I_{Ca} inactivation and release decay exhibit similar kinetics, at least at this membrane potential (Fig. 1, *inset*), decay of release could be driven by inactivation of trigger I_{Ca}. Such an explanation would be consistent with preferential access models for CICR (Stern, 1992; Gyorke and Palade, 1993).

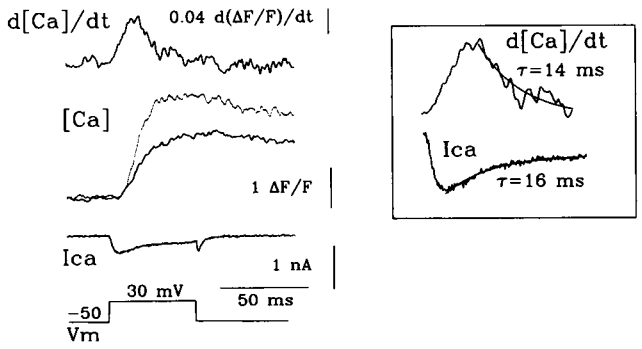

FIGURE 1 Changes of I_{Ca} and intracellular [Ca^{2+}] under control conditions. Traces of calcium current (I_{Ca}), intracellular Ca^{2+} transient ([Ca]), and first derivative of the Ca^{2+} transient (d[Ca]/dt) acquired simultaneously during a depolarizing pulse from −50 to 30 mV (V_M). The dotted line is the maximal Ca^{2+} transient recorded in the same cell during a depolarizing pulse to 0 mV. (*inset*) The descending phases of I_{Ca} and first derivative of the Ca^{2+} transient were best fit by a single exponential function (expanded scale). Time constants for decays of I_{Ca} and d($\Delta F/F$)/dt are 16 and 14 ms, respectively. Data are representative of 12 experiments.

To determine if release termination is indeed caused by inactivation of trigger I_{Ca} or if a separate inactivation mechanism exists for Ca^{2+} release, we used the Ca^{2+} channel agonist FPL 64176, which is known to reduce I_{Ca} inactivation (Rampe and Lacerda, 1991). Fig. 2 shows I_{Ca}, Ca^{2+} transient and the first derivative of the transient under the same conditions as in Fig. 1, but with 1 μM FPL 64176 in the external solution. As can be seen, inactivation of I_{Ca} is dramatically slowed down by the drug at this potential. Despite the slow I_{Ca} inactivation, submaximal release (at 30 mV) still exhibited a rapid decay (Fig 2, *inset*). In a total of five experiments, I_{Ca} at 30 mV decayed with time constants of 19.5 ± 3.3 and 167.9 ± 32.4 ms (Mean ± SD) under control conditions and in the presence of 1 μM FPL 64176, respectively. At the same time, decay of release, as determined by fitting exponentials to the descending phase of the first derivative traces, did not change significantly (14.9 ± 2.7 and 14.3 ± 1.2 ms, respectively). These experiments show that decay of release does not follow inactivation of I_{Ca}. Instead, the release mechanism itself exhibits a separate inactivation that terminates the release despite the continued flow of trigger I_{Ca} and before Ca^{2+} is depleted from the SR.

FPL 64176 also causes a dramatic increase in the size of tail I_{Ca} after repolarization (Fig. 2). This effect can be attributed to reduced I_{Ca} inactivation because tail currents result from an increase in Ca^{2+} influx through channels that have not inactivated during the pulse. The big tail current in the presence of the drug elicits a new large release from apparently inactivated stores (Fig. 2).

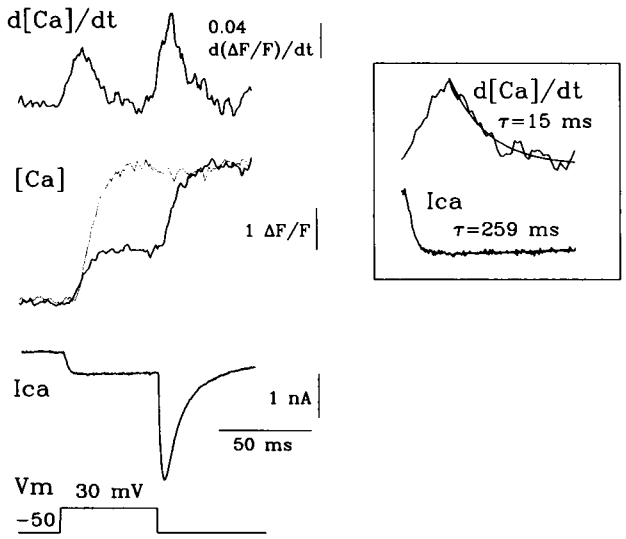

FIGURE 2 I_{Ca} and Ca^{2+} release termination in the presence of FPL 64176. Traces of calcium current (I_{Ca}), intracellular Ca^{2+} transients ([Ca]), and first derivative of the Ca^{2+} transients (d[Ca]/dt) acquired simultaneously during depolarizing pulses from −50 to 30 mV (V_M) 8 min after addition of 1 μM FPL 64176 to the bath solution. The dotted line is the maximal Ca^{2+} transient recorded in the same cell during a depolarizing pulse to 0 mV. The inset compares time courses of the descending phases of I_{Ca} and first derivatives of the Ca^{2+} transients. Although the rate of I_{Ca} inactivation is dramatically slowed down (τ = 259 ms), termination of release exhibits similar kinetics (τ = 15 ms) as under control conditions (Fig. 1). Data are representative of six experiments.

To demonstrate that most of the fluorescent signal is indeed caused by release of Ca^{2+} from the SR, in Fig. 3 we depleted Ca^{2+} stores using ryanodine. As can be seen from the trace in the presence of ryanodine, I_{Ca} itself makes only a very small contribution to the rate of rise of intracellular $[Ca^{2+}]$. Therefore, most of the fluorescence signal during the depolarizing pulse as well as upon repolarization is normally caused by CICR. FPL 64176 had no effect on Ca^{2+} uptake/release properties of the SR, as determined by Ca^{2+} uptake/release measurements in an isolated SR preparation as outlined in the Materials and Methods (not shown). Therefore, the results of our whole-cell measurements are not likely to be accounted for by FPL 64176 having a direct effect on Ca^{2+} uptake or release.

Secondary releases can be also observed under control conditions, with no drug in the solution, after depolarizing pulses to sufficiently high potentials (Cannell et al., 1987; Beuckelmann and Wier, 1988; Cleeman and Morad, 1991). Under normal conditions, however, I_{Ca} inactivates during the pulse nearly completely. Under these conditions, secondary releases could be explained by assuming that the release mechanism has no inactivation of its own but simply follows I_{Ca}, as predicted by local control models (Stern, 1992; Gyorke and Palade, 1993). Our experiments with FPL 64176 demonstrate that a negative control mechanism does exist for SR release, because Ca^{2+} liberation rapidly decays, whereas I_{Ca} is sustained essentially at the same level.

The ability of release to be reactivated by secondary increases in trigger I_{Ca} suggests that this negative feedback is not a conventional type of inactivation. Recently, it has been demonstrated that intracellular Ca^{2+} release channels including the inositol 1,4,5 trisphosphate (IP_3) receptor and RyR exhibit a unique adaptive behavior characterized by the ability of apparently inactivated stores to respond to higher doses of agonist (Muallem et al., 1989; Ferris et al., 1992; Gyorke and Fill, 1993). The mechanism of this phenomenon is not known, but for the cardiac RyR it appears to be a feature of individual release channels, as revealed by single-channel bilayer experiments (Gyorke and Fill, 1993). Although RyR adaptation in bilayer experiments was too slow (1 s) to account for decay of CICR in situ, our whole cell results suggest that the same process inside the cell, with all structural components of the E-C coupling machinery intact and metabolic factors present, proceeds at a much faster rate, actually terminating the release process. Recently, existence of a similar regulatory mechanism has been demonstrated in crayfish skeletal muscle (Gyorke and Palade, 1994), in which the process of excitation-contraction coupling also relays on Ca^{2+}-induced Ca^{2+} release (Gyorke and Palade, 1993). Taken together, these results indicate that CICR in muscle is controlled by a unique regulatory mechanism found in intracellular Ca^{2+} release channels including the ryanodine and IP_3 receptors. By countering the intrinsic positive feedback of CICR, this mechanism could account for or contribute to the graded nature of Ca^{2+} release in heart. In addition, by preventing release during later phases of the inward Ca^{2+} current, this mechanism would also enable stores to be reloaded after Ca^{2+} release. Thus, this mechanism could explain how the transsarcolemmal Ca^{2+} entry can both induce Ca^{2+} release from the SR and serve to load Ca^{2+} into the SR during a given beat (Fabiato, 1985b).

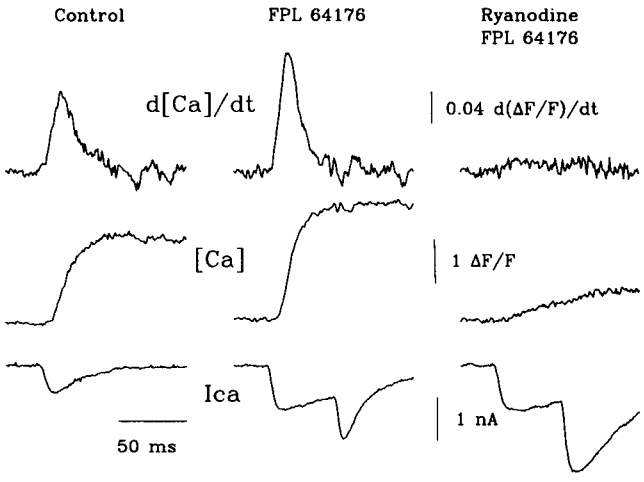

FIGURE 3 Effects of ryanodine on changes in I_{Ca} and $[Ca^{2+}]$ in the presence of FPL 64176. Traces of calcium currents (I_{Ca}), intracellular Ca^{2+} transients ([Ca]), and first derivatives of the Ca^{2+} transients (d[Ca]/dt) recorded during depolarizing pulses to 0 mV under control conditions (*left*), 2 min after addition of 1 μM FPL 64176 (*middle*), and 4 min after addition of 10 μM ryanodine (*right*). Data are representative of five experiments.

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