THE RELEASE OF CALCIUM FROM THE SARCOPLASMIC RETICULUM LASTS AS LONG AS THE CALCIUM CURRENT IN CARDIAC MYOCYTES FROM RATTUS NORVEGICUS.

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The activation of mammalian cardiac muscle is thought to involve the release of Ca^{2+} from the sarcoplasmic reticulum, SR, in response to the influx of Ca^{2+} through the Ca^{2+} channel. This type of Ca^{2+}-induced Ca^{2+} release has been demonstrated in skinned cardiac fibers. Further evidence comes from intact cardiac cells where the intracellular Ca^{2+} activity, $[Ca^{2+}]_i$, and the cell shortening have a voltage dependence similar to that of the Ca^{2+} current, i_{Ca}. The mandatory role of Ca^{2+} influx in the release process is illustrated by the finding that depolarization of the surface membrane, opening of the Ca^{2+} channel and influx of Na^+ or Ba^{2+} are not sufficient to cause release. But if Ca^{2+} influx is required to start the release process, what terminates it? Once started it is conceivable that the release could sustain itself by positive feedback until the SR was effectively empty. Alternatively, the SR release could be interrupted by depolarization involving e.g. the closing of the sarcolemmal Ca^{2+} channel. Finally, the SR release might come to a halt once Ca^{2+} influx through the Ca^{2+} channel had stopped.

These possibilities were examined in voltage-clamped cardiac ventricular cells from the rat, Rattus norvegicus. Cells were dissociated by enzymatic digestion with trypsin and collagenase and were voltage clamped in the whole cell configuration. Fura-2 was used with dual wavelength excitation to measure $[Ca^{2+}]_i$. The patch clamp pipette had a resistance 2 to 5 Mohm and was filled with a dialyzing solution containing 120 mM CsCl, 5 mM magnesium adenosine triphosphate (MgATP), 0.1 mM adenosine 3;5'-monophosphate (cAMP), 0.4 mM Na_5fura-2 and 20 mM HEPES buffer titrated to pH 7.2 by addition of CsOH. The external solution contained 137 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, 0.01 mM tetrodotoxin (TTX) and 10 mM HEPES buffer at pH 7.4. All experiments were performed at room temperature (20 to 25 °C).

Figure 1 (A: left panels) shows results from an experiment where release of Ca^{2+} from the SR was initiated by depolarization to 0 mV from a holding potential of -40 mV. The membrane was then repolarized to the holding potential 2, 5 and 8 ms later (2,5,8) at times the inward Ca^{2+} current (i_m) was prominent or it was held depolarized for a longer period until the inward current subsided (L). The early repolarization terminated both the inward current and the development of the intracellular Ca^{2+} transient (Ca_i). This shows that the Ca^{2+} release does not necessarily run to completion but can be stopped at any time during its development .

The experiment illustrated in the middle panels (B) was performed to test if the interruption of the rising phase of the Ca^{2+} transient required repolarization. A fully developed intracellular Ca^{2+} transient (L) is compared to two transients where the voltage clamp pulse was terminated either by repolarization to the holding potential (R) or by further depolarization to 100 mV (D) i.e. past the reversal potential for i_{Ca}. The two procedures are equally effective in aborting the Ca^{2+} release suggesting that it is the cessation of the Ca^{2+} influx and not the change in membrane potential which terminates the release process.

It might be argued that the closing of the Ca^{2+} channel is required to stop the Ca^{2+} release from the SR. We explored this point by testing if the Ca^{2+} channel is open at +100 mV even though, in the absence of a driving force, it does not lead to Ca^{2+} influx. The right panels compares two Ca^{2+} transients

developing at 0 mV. One was produced by depolarization (D) from the holding potential (-40 mV) while the other was produced by repolarization (R) to the holding potential from a prepulse at 100 mV. It is seen that the tail transient produced by repolarization develops with significantly shorter delay than the transient produced by depolarization. This suggests that the development of the Ca^{2+} transient produced by the depolarizing pulse is delayed by the time required to open the Ca^{2+} channel while the repolarizing pulse occurs at a time when the Ca^{2+} channel is already open, and it confirms the notion that the Ca^{2+} channel is open at +100 mV.

These results indicate that the release of Ca^{2+} from the sarcoplasmic reticulum does not sustain itself by a positive feed back mechanism in which Ca^{2+} released from the SR induces further release by Ca^{2+} induced Ca^{2+} release. Rather the release process depends on the continuous influx of Ca^{2+} through the Ca^{2+} channel and it is stopped if this influx is stopped independent of whether the membrane is repolarized or the Ca^{2+} channel is closed.

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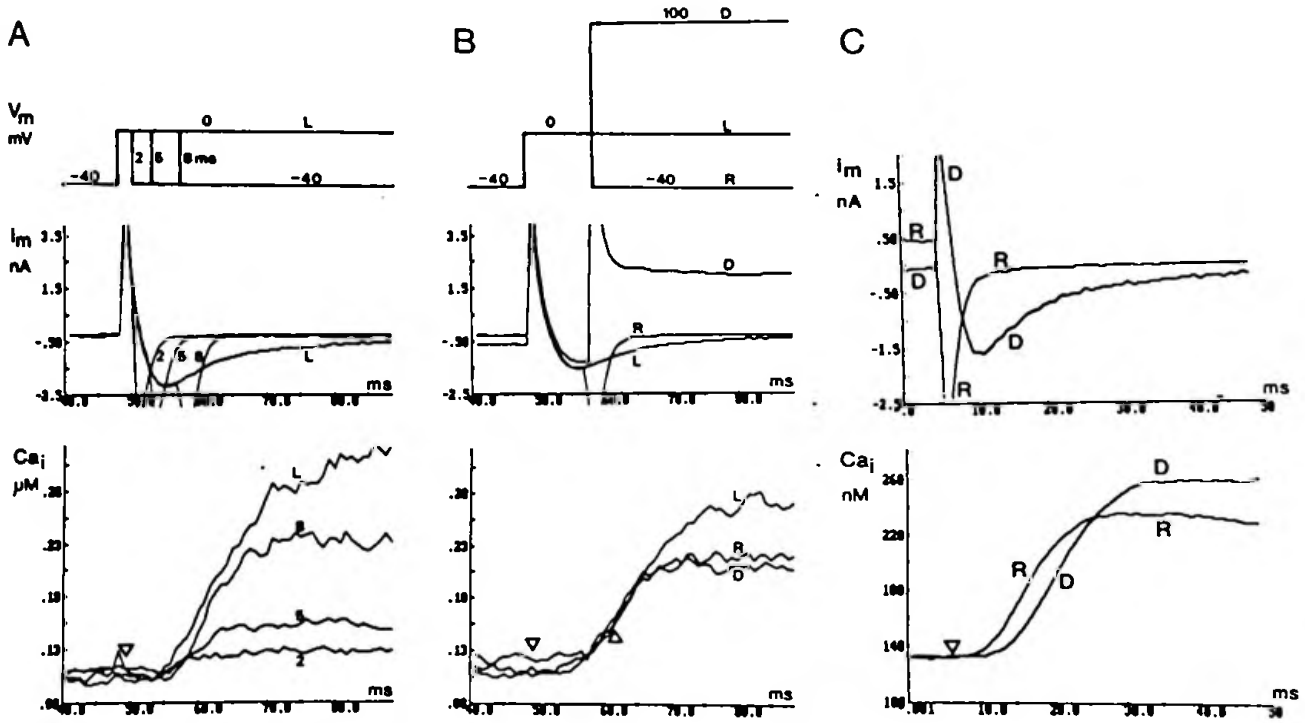

Figure 1. Membrane current (i_m) and intracellular Ca^{2+} transient (Ca_i) produced by depolarizing and repolarizing clamp pulses. The voltage clamp protocols used in the left and middle panels are shown schematically at the top. The right panels compares the effects depolarization (D) from -40 mV to 0 mV to the effect of repolarization (R) from +100 mV to -40 mV.