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Sodium Current–Induced Release of Calcium from Cardiac Sarcoplasmic Reticulum

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The role of sodium-calcium exchange at the sarcolemma in the release of calcium from cardiac sarcoplasmic reticulum was investigated in voltage-clamped, isolated cardiac myocytes. In the absence of calcium entry through voltage-dependent calcium channels, membrane depolarization elicited release of calcium from ryanodine-sensitive internal stores. This process was dependent on sodium entry through tetrodotoxin-sensitive sodium channels. Calcium release under these conditions was also dependent on extracellular calcium concentration, suggesting a calcium-induced trigger release mechanism that involves calcium entry into the cell by sodium-calcium exchange. This sodium current–induced calcium release mechanism may explain, in part, the positive inotropic effects of cardiac glycosides and the negative inotropic effects of a variety of antiarrhythmic drugs that interact with cardiac sodium channels. In response to a transient rise of intracellular sodium, sodium-calcium exchange may promote calcium entry into cardiac cells and trigger sarcoplasmic calcium release during physiologic action potentials.

IN MAMMALIAN HEART MUSCLE, THE release of Ca^{2+} from the sarcoplasmic reticulum (SR) is important in the activation of contraction. In contrast to skeletal muscle, where Ca^{2+} release is directly controlled by membrane voltage through a charge-coupled release mechanism (*1*), Ca^{2+} release in the heart is generally believed to involve a Ca^{2+}-induced release mechanism (*2*). Sarcolemmal Ca^{2+} channels are thought to provide the primary source of Ca^{2+} for both the trigger for release of Ca^{2+} as well as the loading of Ca^{2+} into the SR (*3*). In studies (*4*) that have used Ca^{2+}-sensitive dyes to monitor SR Ca^{2+} release, it has not been possible to show Ca^{2+} release in the absence of Ca^{2+} entry through voltage-dependent Ca^{2+} channels. This had led to the suggestion (*5*) that Ca^{2+} influx through sarcolemmal Ca^{2+} channels is required for coupling membrane depolarization to SR Ca^{2+} release in heart muscle. However, intracellular Na^+ concentration is well known to be another important determinant of myocardial contractile strength (*6*). For example, Na^+ is implicated in the inotropic mechanism of action of digitalis glycosides, in which a rise of intracellular Na^+ is thought to augment Ca^{2+} entry into heart cells by promoting reverse-mode activity of the Na^+-Ca^{2+} exchanger (*7*). Although there is substantial evidence that Na^+-Ca^{2+} exchange is an important transport system for extruding Ca^{2+} (forward-mode activity) from cardiac cells (*8*), there is controversy whether this system promotes sarcolemmal Ca^{2+} entry during the action potentials and thereby contributes to excitation-contraction coupling under physiological conditions (*9*). These considerations prompted us to re-examine whether Ca^{2+} influx through voltage-dependent Ca^{2+} channels is mandatory for cardiac SR Ca^{2+} release. Our experiments show that Ca^{2+} influx through voltage-dependent Ca^{2+} channels is not a prerequisite for SR Ca^{2+} release. After pharmacological blockade of myocardial Ca^{2+} channels, SR Ca^{2+} release can be elicited in response to activation of tetrodotoxin (TTX)-sensitive Na^+ currents in an extracellular Ca^{2+}–dependent manner.

In isolated guinea pig myocytes, internally dialyzed with the Ca^{2+} indicator indo-1 (potassium salt) (*10*), action potentials elicited under current-clamp conditions were accompanied by a rapid increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Fig. 1A). The onset of this rise of $[Ca^{2+}]_i$ was delayed by approximately 15 to 30 ms after the upstroke of the action potential. During the action potential plateau, $[Ca^{2+}]_i$ either continued to slowly rise, was sustained, or slowly declined. Repolarization of the action potential resulted in the return of Ca^{2+} toward previous resting values. To test if Na^+ entry through voltage-dependent Na^+ channels might contribute to excitation-contraction coupling during the action potential, we examined the effects of the Na^+ channel antagonist TTX on the action potential Ca^{2+} transient. Exposure of cells to 5 μ*M* TTX significantly reduced the size of the Ca^{2+} transient (Fig. 1, A_1 and A_2). In this cell, TTX produced little, if any, change in the action potential plateau or duration, which makes it unlikely that the change observed in the Ca^{2+} transient was caused by diminished Ca^{2+} entry through Ca^{2+} channels during the plateau. The ability of TTX to reduce the amplitude of the Ca^{2+} transient is consistent with its known negative inotropic effects in the heart (*11*).

In other experiments, we voltage-clamped isolated myocytes (*12*) and directly examined the possibility that activation of Na^+ currents might contribute to the Na^+ transients recorded. Membrane holding potential was varied to examine the possible role of myocardial Na^+ currents (Fig. 1B). From a holding potential of −70 mV (near the cell's resting membrane potential), a 500-ms test pulse to 0 mV elicited an inward current that activated quickly and inactivated in two phases (Fig. 1, B_1). This inward current reflects the activation of two components: fast TTX-sensitive Na^+ channels and dihydropyridine-sensitive Ca^{2+} channels. During the test pulse, $[Ca^{2+}]_i$ rose rapidly from a resting value to reach a plateau slightly higher than 1 μ*M*. On return to the holding potential, $[Ca^{2+}]_i$ returned to a resting level, as observed in current-clamp experiments during the last phase of action potential repolarization. Na^+ currents were voltage-

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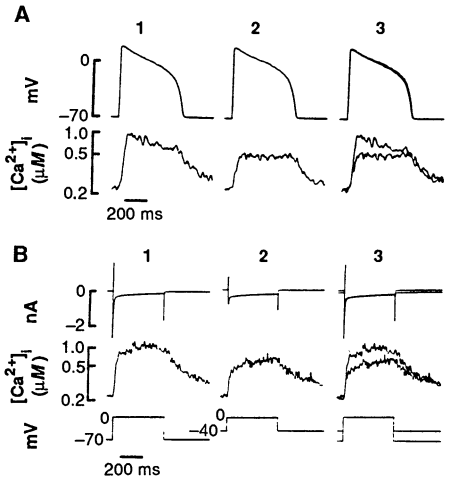

Fig. 1. (**A**) Effects of TTX on the action potential and Ca^{2+} transient. The action potential recorded in control conditions (A_1) was preceded by a train of five action potentials evoked at a frequency of 0.1 Hz to load the SR Ca^{2+} stores. During application of TTX (5 μM), action potentials were elicited every 30 s. The action potential and corresponding Ca^{2+} signals in A_2 were measured after 3 min of exposure to TTX (steady-state Ca^{2+} transient). Action potentials and Ca^{2+} transients from A_1 and A_2 are superimposed in A_3. (**B**) Effects of two different holding potentials on membrane current and amplitude of the Ca^{2+} signal evoked at 0 mV. The data in (A) and (B) are from different cells. A_1 and A_2 were obtained after a train of five conditioning test pulses to 0 mV and 500 ms in duration from a holding potential of −70 mV. Membrane currents and Ca^{2+} transients from B_1 and B_2 are superimposed in B_3. For (A) and (B), the cells were dialyzed with normal internal and external solutions containing K^+ (*12*), and the ratio (400 nm/500 nm) of the collected light signals was calibrated as described (*10*). Resting $[Ca^{2+}]_i$ under control conditions averaged 186 ± 14.7 nM (mean ± SEM, n = 20). The peak Ca^{2+} transient during the action potential averaged 1374 ± 360 nM (mean ± SEM, n = 15).

inactivated by changing the holding potential to −40 mV (Fig. 1, B_2). An identical test pulse to 0 mV elicited an inward current that was mainly composed of an L-type Ca^{2+} current. Under this condition, the Ca^{2+} transient elicited was significantly smaller than that elicited by a similar voltage-clamp pulse applied from −70 mV (Fig. 1, B_3). Similar results were obtained in five other cells. These observations suggest that activation of myocardial Na^+ currents during action potentials or when elicited by voltage-clamp depolarizations contribute to the generation of the Ca^{2+} transient. This conclusion is also consistent with earlier observations made in the same preparation (*4*) that show that the Ca^{2+} transient elicited by an action potential was usually larger in amplitude than that which could be evoked by maximal activation of L-type Ca^{2+} currents in voltage-clamped myocytes.

We then assessed whether Ca^{2+} transients could be elicited by voltage-clamp depolarizations in the absence of Ca^{2+} entry through voltage-dependent Ca^{2+} channels. Ca^{2+} currents were blocked by continuous exposure to a high concentration (5 μM) of the dihydropyridine Ca^{2+}-channel antagonist nisoldipine (Fig. 2A). From a holding potential of −80 mV, 500-ms test pulses to −40 mV were applied to the cell every 30 s. In the absence of Ca^{2+} current, a fast inward current was elicited. This inward current was followed by a rise of $[Ca^{2+}]_i$ that reached a peak and declined monotonically during the test pulse. Subsequent application of 30 μM TTX (1-min exposure) quickly abolished the inward current and Ca^{2+} transient. Similar observations have been made in nine other cells. These experiments show that in the absence of Ca^{2+} entry through Ca^{2+} channels, a TTX-sensitive Ca^{2+} transient was elicited by membrane depolarization. As concentrations of nisoldipine as high as 50 μM failed to inhibit this response, we conclude that the TTX-sensitive Ca^{2+} transients are linked to Na^+ entry through TTX-sensitive Na^+ channels.

The TTX-sensitive Ca^{2+} transients recorded in the presence of Ca^{2+} channel antagonists are most likely interpreted as being linked to Na^+ entry through sarcolemmal Na^+ channels. However, it might be argued that incomplete blockade of Ca^{2+} channels or loss of voltage control, which can be a significant problem in attempts to accurately clamp large Na^+ currents (*13*), might be responsible for the Ca^{2+} transients measured under these conditions. It is important to show that these phenomena can be observed when (i) sarcolemmal Ca^{2+} channel blockade is assured and (ii) adequate voltage control during the measurement of whole-cell Na^+ currents can be shown. The cell was internally dialyzed and externally superfused with solutions that contained high concentrations (10 μM) of the Ca^{2+}-channel antagonist D 600 (Fig. 2B). Similar concentrations produce complete blockade of L-type Ca^{2+} channels in cardiac cells and are more effective in cardiac cells when applied internally (*14*). To improve voltage control, we partially inactivated Na^+ currents by setting the holding potential near −60 mV. Using these precautions, we elicited fast inward currents of smaller amplitude (*15*) (Fig. 2, B_1 and B_3). The peak amplitude of the inward current was less than 2 nA, which is comparable to the amplitude of L-type Ca^{2+} currents measured in some cardiac cells. TTX (Fig. 2, B_2) (30 μM) abolished the inward current at all potentials, indicating that these currents can be attributed to the opening and closing of Na^+ channels. The lack of measurable inward current in the presence of TTX also

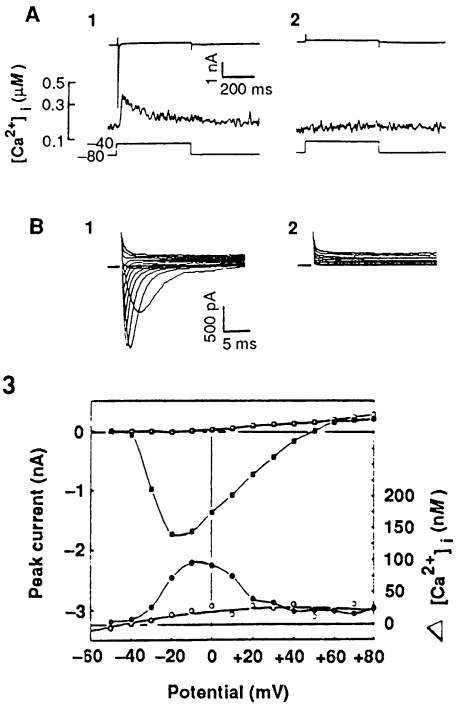

Fig. 2. Ca^{2+} transients in Cs^+-loaded cells in the absence of Ca^{2+} current. The data in (A) and (B) are from different cells. (**A**) The cell was continuously superfused with 5 μM nisoldipine. Step depolarizations (500 ms) to −40 mV from a holding potential of −80 mV were applied to the cell every 30 s. In the absence of TTX (A_1), this protocol elicited Na^+ currents and Ca^{2+} transients. Traces in A_2 were obtained after a 1-min application of 30 μM TTX. No conditioning protocol was used. (**B**) The cell was internally dialyzed and externally perfused with 10 μM D 600. Currents were elicited in the absence (B_1) and presence (B_2) of 30 μM TTX. Na^+ channels were partly inactivated by holding the membrane potential at −60 mV. Pulses (500 ms) from −50 to +80 mV were applied in 10-mV increments at 0.2 Hz. In B_3, peak inward currents (left ordinate; squares) and resulting changes in peak $[Ca^{2+}]_i$ from resting level (right ordinate; circles) were plotted as a function of pulse potential. Control, filled symbols; TTX (3 min), empty symbols. Resting $[Ca^{2+}]_i$ was 158 nM. A conditioning protocol was used as described in Fig. 4A. Resting $[Ca^{2+}]_i$ in nisoldipine-treated cells averaged 175 ± 14 nM (mean + SEM, n = 9).

shows that Ca^{2+} channels were effectively blocked by D 600 and makes it unlikely that there is some residual Ca^{2+} influx through T-type Ca^{2+} channels (*16*).

We measured the voltage dependence of the Na^+ currents and the Ca^{2+} transients before and after exposure to TTX. In the absence of drug, inward currents activated near −40 mV, peaked at −20 mV, and then declined and reversed near +50 mV, indicating reasonably good voltage control (*17*). The amplitude of the Ca^{2+} transient had a very similar voltage dependence. TTX abolished both the inward current and the Ca^{2+} transients in this cell and in four other cells under identical conditions. These experiments show that Na^+ current–dependent

Ca^{2+} transients can be elicited in cardiac myocytes in the absence of myocardial Ca^{2+} currents, and that these transients are not caused by the activation of a small fraction of unblocked Ca^{2+} channels or by the loss of adequate voltage control.

To examine the possible source of Ca^{2+} for these Na^+ current–dependent Ca^{2+} transients, we incubated some cells for 30 min with 30 μM ryanodine to deplete SR Ca^{2+} stores. In these cells, with negative holding potentials (−80 mV) and nisoldipine to block Ca^{2+} channels, the application of test pulses over the range −50 to +60 mV elicited TTX-sensitive inward currents, with little or no activation of voltage-dependent Ca^{2+} transients (Fig. 3A). The inability of Na^+ currents to evoke Ca^{2+} transients in ryanodine-treated cells suggests that the SR provides the Ca^{2+} source for Na^+ current–dependent Ca^{2+} transients. Although involvement of SR Ca^{2+} release seems certain, it is not clear how activation of Na^+ currents is coupled to the release of Ca^{2+} from the SR. Several hypotheses could explain such coupling: (i) release may be activated by a voltage-dependent step requiring opening of Na^+ channels; (ii) Na^+ ions may trigger release by direct activation of the SR Ca^{2+} channels; (iii) release may be stimulated by a Ca^{2+}-induced Ca^{2+} release mechanism (*2*) with Na^+-Ca^{2+} exchange causing influx of Ca^{2+} in response to a rise of intracellular Na^+ (reverse-mode activity). To discriminate between these possibilities, we designed a series of experiments to test if the Na^+ current–induced release process is dependent on extracellular Ca^{2+}.

In 2.5 mM extracellular Ca^{2+} (Fig. 3, B_1), the application of a 500-ms test pulse to −40 mV from a holding potential of −80 mV elicited Na^+ current–dependent Ca^{2+} transients as previously shown. The cell was then exposed to a nominally Ca^{2+}-free external solution in which Ca^{2+} was replaced by an equimolar concentration of Mg^{2+}. After a 5-min incubation in Ca^{2+}-free solution (during which time test pulses were not applied to avoid depletion of SR stores), a second test pulse to −40 mV was applied (Fig. 3, B_2). The Na^+ current elicited at this potential had a similar amplitude as that evoked in Ca^{2+}-containing solution; however, the Ca^{2+} transient was abolished. To test for the presence of a releasable SR Ca^{2+} store under these experimental conditions, we exposed the cell to caffeine in the same Ca^{2+}-free external solution. This compound promoted a very slow transient increase of $[Ca^{2+}]_i$, confirming the presence of a releasable Ca^{2+} store (Fig. 3, B_3). These results show that the coupling between activation of Na^+ channels and release of SR Ca^{2+} requires extracellular Ca^{2+}, implicating a Ca^{2+}-induced Ca^{2+} release mechanism that may involve Na-Ca exchange.

Our ability to show Na^+ current–induced Ca^{2+} release from SR in the absence of Ca^{2+} entry through Ca^{2+} channels was facilitated by a conditioning protocol that ensured a constant availability of Ca^{2+} in the SR (*4*) (Fig. 4A). A train of ten voltage-clamp pulses were applied to the cell before the application of the test pulse to −50 mV (*12*). Conditioning pulses from −50 to +60 mV were used because Ca^{2+} currents were blocked. There was little activation of Na^+ currents at these positive potentials (because of the reduced driving force for Na^+), and a small TTX-insensitive tonic increase in $[Ca^{2+}]_i$ occurred (Fig. 2, B_3), which has been attributed to Ca^{2+} influx caused by reverse-mode Na^+-Ca^{2+} exchange activity (*18*). We compared the membrane currents and Ca^{2+} transients (traces superimposed) elicited by the test depolarization to −50 mV in a nisoldipine-treated cell, in the absence of a conditioning protocol and after such a conditioning protocol (Fig. 4B). The Na^+ current–induced Ca^{2+} transient elicited after the conditioning protocol (labeled Cond) was significantly larger in amplitude than that elicited in the absence of the conditioning protocol. This suggests that application of the conditioning protocol to positive membrane potentials enables Ca^{2+} entry (via reverse-mode exchange activity), thereby increasing the size of the SR Ca^{2+} store, which then can be released in response to subsequent Na^+ current activation.

Recent voltage-clamp studies of isolated cardiac myocytes (*19*) have identified membrane currents associated with electrogenic Na^+-Ca^{2+} exchange activity. It might therefore be expected that similar exchange currents might be associated with Na^+ current–induced Ca^{2+} release if indeed Na^+-Ca^{2+} exchange is important in coupling Na^+ influx to SR Ca^{2+} release. However, the identification of such a small current associated with Ca^{2+} influx might be difficult because it would be expected to temporally overlap with and be obscured by the activation of Na^+ currents. We have, however, detected a small inward current that may be associated with electrogenic Ca^{2+} efflux by the exchanger. After the conditioning protocol, a larger Ca^{2+} transient was elicited and a small inward tail current was observed after the decay of the Na^+ current (Fig. 4B). This inward tail current resembles the inward creep currents that are activated in response to a rise of $[Ca^{2+}]_i$ and have previously been identified as Na^+-Ca^{2+} exchange currents in frog atrial cells and guinea pig ventricular myocytes (*18–20*).

Because Na^+ current–induced Ca^{2+} release requires extracellular Ca^{2+}, it seems unlikely that Na^+ ions are the direct trigger for Ca^{2+} release. This conclusion is consistent with the observation that Na^+ influx through Ca^{2+} channels in the absence of extracellular Ca^{2+} failed to elicit SR release in this preparation (*5*). Our results also imply that voltage-dependent Ca^{2+} release or tight coupling between Na^+ current acti-

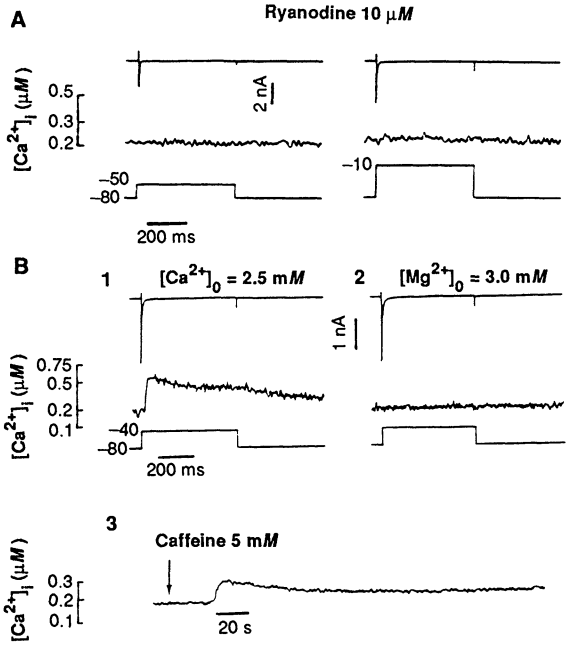

Fig. 3. (**A**) Effects of ryanodine on Na^+ current–induced Ca^{2+} transients. Nisoldipine (5 μM) was used to block Ca^{2+} currents. Cells were exposed to 10 μM ryanodine for 30 min. Voltage-clamp pulses (500 ms) from −70 to +60 mV were applied to this cell in 10-mV increments at 0.2 Hz. No conditioning protocol was used. (**B**) Test of the extracellular Ca^{2+} dependence. Nisoldipine (5 μM) was used to block Ca^{2+} currents. After a conditioning protocol (Fig. 4A), Na^+ current and associated Ca^{2+} transient were recorded during a test pulse to −40 mV from a holding level of −80 mV in the presence of a normal extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) (B_1). The perfusate was then switched to a medium in which Ca^{2+} was proportionately replaced by Mg^{2+} to maintain the total divalent cation concentration constant. During perfusion with this solution, no voltage-clamp pulses were applied to the cell. The traces shown in B_2 were obtained after a 5-min incubation in this nominally Ca^{2+}-free solution. Still in Ca^{2+}-free solution, the cell was subsequently exposed to 5 mM caffeine (B_3). The membrane potential was clamped at −80 mV during application of the compound. Similar results were observed in four other cells.

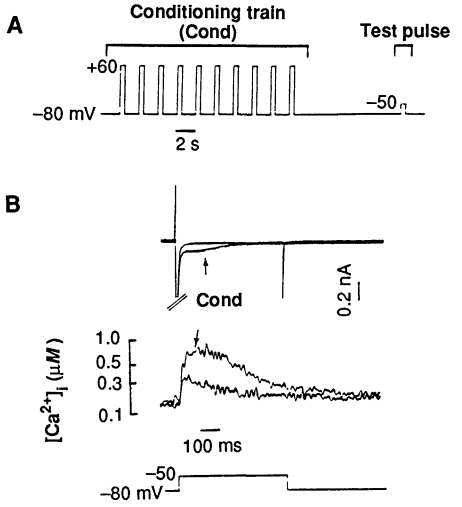

Fig. 4. Influence of a conditioning protocol on the magnitude of the Na^+ current–induced Ca^{2+} transient in a Cs^+-dialyzed cell. Nisoldipine (5 μM) was used to block Ca^{2+} currents. (**A**) A schematic diagram of the conditioning protocol used. A train of ten voltage-clamp steps (500 ms) was applied to +60 mV from a holding potential of −80 mV (frequency of 0.5 Hz). A delay of 5 s preceded a test pulse (500 ms) to −50 mV, which allowed full recovery of resting $[Ca^{2+}]_i$ to its original level. (**B**) Typical experiment showing the effects of loading the Ca^{2+} stores with this protocol. Current traces and Ca^{2+} signals before (2-min rest) and after conditioning (Cond) are superimposed. The current traces are shown at high gain and the peaks are not displayed. The amplitude of the inward currents before and after conditioning were 4.72 and 4.86 nA, respectively. In cells under similar experimental conditions, without a conditioning pulse train, a depolarization to −50 mV produced an average peak Ca^{2+} transient (difference from resting $[Ca^{2+}]_i$) of 135 ± 29 nM (mean ± SEM, n = 5). In cells in which the same test pulse was preceded by a conditioning pulse train, the average peak Ca^{2+} transient was 620 ± 141 nM (mean ± SEM, n = 9).

vation and SR Ca^{2+} release are rather unlikely. Our results support the participation of an electrogenic Na^+-Ca^{2+} exchange carrier in the coupling process. In this scheme, activation of myocardial Na^+ channels would produce a transient rise in intracellular Na^+ [sodium transient (*21*)] at a local site near the inner surface of the sarcolemma, which would shift the reversal potential of the Na^+-Ca^{2+} exchanger toward negative membrane potentials. This shift would thereby promote transient Ca^{2+} influx by the exchanger, which in turn would provide the Ca^{2+} trigger for SR release. Although a similar scheme has been suggested to explain digitalis inotropy (*7*), as well as the inotropic effects of changes in intracellular Na^+ concentration in cardiac muscle (*6*), our results show a direct link between Na^+ influx and SR Ca^{2+} release. Consistent with this scheme, it has been shown that in the absence of an increased intracellular Na^+ concentration, activation of Na^+-Ca^{2+} exchange fails to elicit a Ca^{2+} transient (*22*). These data suggest that Ca^{2+} influx mediated by Na^+-Ca^{2+} exchange (reverse mode), in addition to raising $[Ca^{2+}]_i$ to directly activate contractile proteins, can also provide a source of Ca^{2+} to trigger the release of additional Ca^{2+} from cardiac SR. Na^+ current–induced Ca^{2+} release may also explain in part the negative inotropic effects characteristic of a number of antiarrhythmic agents in the heart that have in common the ability to block myocardial Na^+ channels (*23*).

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10. Cells were enzymatically dispersed as previously described [J. R. Hume and A. Uehara, *J. Physiol. (London)* **368**, 525 (1985)]. Indo-1 (pentapotassium salt; 100 μM) was included in the pipette solution and dialyzed into the cell. The background autofluorescence was subtracted before gaining access to the cell interior. In whole-cell mode, loading of the dye was assessed by monitoring changes in fluorescence intensity. Voltage- or current-clamp protocols were usually initiated after 5 to 10 min of loading of the dye. The fluorescent probe was excited at 340 nm with a mercury arc lamp, and light emitted by the specimen (from a circular spot of 10 μm in diameter) was simultaneously measured at 400 and 500 nm by means of two matched photomultiplier tubes (Hamamatsu type R2560HA). The light collected from the optics of a Nikon diaphot inverted microscope was split by a series of dichroic mirrors and passed through narrow bandpass (10-nm) optical filters centered at 400 and 500 nm. The microfluorimeter used (Sycamore Scientific Corp., Santa Barbara, CA) was similar to that described elsewhere [G. A. Peeters, V. Hlady, J. H. B. Bridge, W. H. Barry, *Am. J. Physiol.* **253**, H1400 (1987)]. An in vitro calibration curve was constructed with the ratio of the intensities at 400 and 500 nm as described [G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **260**, 340 (1985)]. We used 40 mM EGTA and 5 mM $CaCl_2$ to determine R_{min} (mean 0.1033, n = 4) and R_{max} (mean 2.0224, n = 4) respectively. The calibration curve was constructed with the equation $[Ca^{2+}] = K_d\ [(R - R_{min})/(R_{max} - R)]\ F_0/F_s$, where k_d is the dissociation constant of indo-1 (250 nM at 37°C, pH 7.05), R is the ratio of the intensities at 400 and 500 nm, and F_0/F_s (1.9374) is the ratio of the maximum (R_{max}) and minimum (R_{min}) fluorescence intensities measured at 500 nm. Calculations of $[Ca^{2+}]_i$ by this method assume that the properties of indo-1 in the calibration solutions are similar to those of indo-1 in the cytoplasm [D. A. Williams, K. E. Fogarty, R. Y. Tsien, F. S. Fay, *Nature* **318**, 558 (1985)]. In a series of test experiments in which cells were loaded by the acetoxymethyl ester of indo-1, bleaching and evidence of significant buffering of $[Ca^{2+}]_i$ were frequently observed. However, bleaching and buffering were rarely observed in cells that were loaded with indo-1 (K^+ salt)–containing patch pipettes (assessed by examining the amplitude and time course of Ca^{2+} transients elicited by repetitive depolarizing pulses). Although we cannot eliminate the possibility that some degree of buffering of $[Ca^{2+}]_i$ by indo-1 occurred in these experiments, 100 μM is well below the concentrations required to show significant $[Ca^{2+}]_i$ buffering in lymphocytes [C. S. Owen, *Cell Calcium* **9**, 141 (1988)]. Membrane current or voltage, the voltage signals from the two photomultiplier tubes, and an analog ratio of the two fluorescent signals were digitized on-line during voltage- and current-clamp experiments by software run on an IBM-AT compatible computer. In current-clamp experiments, the data were digitized at a sampling rate of 1 kHz. During voltage-clamp protocols, two clock speeds were used; the first 50 ms were digitized at 50 kHz and the last 950 ms at 1 kHz.
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The Relationship Between Charge Movements Associated with I_{Ca} and $I_{Na\text{-}Ca}$ in Cardiac Myocytes

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Ventricular myocytes exhibit a nifedipine-sensitive inward calcium current (I_{Ca}) and contracture when they are voltage clamped from −40 to 0 millivolt in the presence of caffeine and in the absence of extracellular sodium. However, upon repolarization they fail to relax because neither the sarcoplasmic reticulum nor the sodium-calcium exchange can reduce intracellular calcium. Sudden application of extracellular sodium during the contracture (but after repolarization) causes immediate relaxation and activates a transient inward sodium-calcium exchange current ($I_{Na\text{-}Ca}$), whose peak slightly precedes mechanical relaxation. The total charge carried by the nifedipine-sensitive I_{Ca} is twice the total charge carried by the transient inward $I_{Na\text{-}Ca}$. Assuming an exchange stoichiometry of three sodium to one calcium, these results indicate that all the calcium entering the cell during the initial depolarization is extruded by the sodium-calcium exchange.

IN THE PRESENCE OF CAFFEINE, WHICH prevents Ca^{2+} sequestration by the sarcoplasmic reticulum (SR), mechanical relaxation of mammalian ventricular muscle is dependent on extracellular Na^+ (Na_o^+) (*1–3*). This Na_o^+-dependent relaxation is also voltage sensitive (*1–2*). Similarly, in the presence of ryanodine, which interferes with SR Ca^{2+} release, the decay of elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in guinea pig cells is controlled by voltage and is associated with an inward $I_{Na\text{-}Ca}$ tail (*4*). These observations indicate that a voltage-sensitive Na^+-Ca^{2+} exchange is capable of sufficient Ca^{2+} extrusion to cause relaxation. If this exchange is electrogenic, there should be an associated current that accompanies and slightly precedes mechanical relaxation and that decays as relaxation progresses and intracellular Ca^{2+} is reduced. Here we report the isolation of a current with these characteristics.

To isolate $I_{Na\text{-}Ca}$ we used a perfusion system (*5*) that rapidly increased Na_o^+ and activated $I_{Na\text{-}Ca}$ before the exchange could significantly dissipate ionic gradients responsible for its activation. This approach has already been applied to visual rods by Hodgkin *et al.* (*6*). Guinea pig ventricular myocytes were enzymatically isolated by a method similar to that described by us (*1*) and voltage clamped with the whole-cell disrupted patch technique (7) with a discontinuous single microelectrode voltage-clamp circuit (Axoclamp 2A, Axon Instruments, Burlingame, California). Microelectrodes (2 to 6 mΩ) were filled with dialyzing solution containing 131 m*M* CsCl, 0.2 m*M* $MgCl_2$, 3.0 m*M* Mg–adenosine triphosphate (ATP), 5.5 m*M* dextrose, and 10 m*M* Hepes; they also contained 20 μ*M* EGTA and no added Ca^{2+}. The *p*H was adjusted to 7.1 with CsOH. The total Cs^+ concentration was 140 m*M* and free Mg^{2+} was estimated to be 600 μ*M*. In experiments requiring extensive intracellular Ca^{2+} buffering, the pipette solution contained 14.0 m*M* EGTA. Contraction was measured as change in cell length with a video-based edge detector (*8*). The control external solution contained 145 m*M* LiCl, 0 m*M* KCl replaced with 4.4 m*M* LiCl, 2.7 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 11 m*M* dextrose, 10 m*M* caffeine, and 10 m*M* Hepes-LiOH, *p*H 7.4. Na^+ replaced Li^+ in the test solution. When necessary, nifedipine was used at a concentration of 10 μ*M*.

Cells were clamped at a holding potential of −40 mV in the control solution that contained no Na_o^+. An initial inward I_{Ca} was activated every 10 s (Fig. 1A) by depolarizing the cell to 0 mV for 2 s. This resulted in a contracture (Fig. 1D) that persisted after the cell was repolarized. In the presence of caffeine the contraction is attributable to Ca^{2+} entry via I_{Ca}. Five hundred milliseconds after the repolarization, Na_o^+ was applied very rapidly [half-time ($t_{1/2}$) ≅ 40 ms) (*5*). The Na_o^+ application

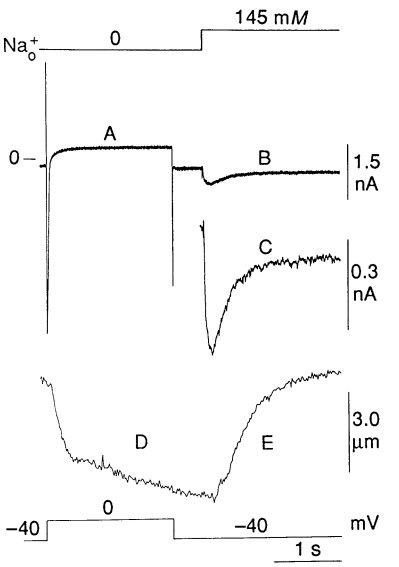

Fig. 1. Two-second voltage-clamp pulses in the absence of Na_o^+ and presence of 10.0 m*M* caffeine cause contraction. Rapid application of Na_o^+ 500 ms after repolarization causes relaxation. (**A**) I_{Ca} elicited by membrane depolarization. (**B**) The application of Na^+ produces putative transient inward $I_{Na\text{-}Ca}$. This current is displayed on an expanded scale in (**C**). (**D**) Contraction (cell shortening) activated by I_{Ca} recorded in (A). (**E**) After repolarization to −40 mV, relaxation does not occur until 500 ms after the clamp pulse when Na_o^+ is suddenly applied.

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