Sarcoplasmic reticulum and Na\(^+\)/Ca\(^{2+}\) exchanger function during early and late relaxation in ventricular myocytes

ATSUSHI YAO, HIROSHI MATSUI, KENNETH W. SPITZER, JOHN H. B. BRIDGE, AND WILLIAM H. BARRY

The Division of Cardiology and the Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah Health Sciences Center, Salt Lake City, Utah 84132

Yao, Atsushi, Hiroshi Matsui, Kenneth W. Spitzer, John H. B. Bridge, and William H. Barry. Sarcoplasmic reticulum and Na\(^+\)/Ca\(^{2+}\) exchanger function during early and late relaxation in ventricular myocytes. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2765–H2773, 1997.—The relative importance of the Na\(^+\)/Ca\(^{2+}\) exchanger in the initial and terminal phases of relaxation and the decline in the [Ca\(^{2+}\)]

transient was investigated in adult rabbit ventricular myocytes loaded with the Ca\(^{2+}\) indicator fluo 3. For electrically stimulated contractions, the peak intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) was 700 ± 87 nM and end-diastolic [Ca\(^{2+}\)] was 239 ± 30 nM (0.25 Hz, 37°C, 1.08 mM extracellular Ca\(^{2+}\) concentration; n = 14). Abrupt inhibition of Na\(^+\)/Ca\(^{2+}\) exchange was produced by removal of extracellular Na\(^+\) (KCl substitution) and Ca\(^{2+}\) [2 mM Ca\(^{2+}\)-free ethylene glycol-bis(\text{3-aminoethyl ether})-N,N,N,N'-tetraacetic acid] by means of a rapid switcher device (SW). Abrupt exposure to high K\(^+\) induced an action potential, although sufficient Ca\(^{2+}\) remained adjacent to the sarcolemma to induce a contraction (SW beat) and [Ca\(^{2+}\)] transient that were identical in amplitude to those induced by electrical stimulation (ES beat). The initial relaxation and decline in the [Ca\(^{2+}\)] transient was not significantly prolonged by abrupt elimination of the Na\(^+\)/Ca\(^{2+}\) exchanger, but the rate and extent of the terminal phase of the decline in the [Ca\(^{2+}\)] transient were significantly reduced. The first derivative of [Ca\(^{2+}\)] with respect to time versus [Ca\(^{2+}\)] during the decline of the [Ca\(^{2+}\)] transient attributable to sarcoplasmic reticulum (SR) function was estimated from the average SW transients, and that attributable to Na\(^+\)/Ca\(^{2+}\) exchange was estimated from the difference between SW and ES transients. By this analysis, the Na\(^+\)/Ca\(^{2+}\) exchanger produces 13% of the first half of the decline in [Ca\(^{2+}\)] and 45% of the second half of the decline. We conclude that abrupt inhibition of forward Na\(^+\)/Ca\(^{2+}\) exchange does not significantly affect the amplitude or the initial rate of decline of the [Ca\(^{2+}\)] transient and relaxation. However, its contribution to the reduction of [Ca\(^{2+}\)] becomes apparent late during the [Ca\(^{2+}\)] transient, when cytosolic [Ca\(^{2+}\)] has been reduced.

sodium/calcium exchange; fluo 3; relaxation; calcium ion transient

IT IS WIDELY RECOGNIZED that a variety of processes can contribute to regulation of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) in cardiac myocytes, including Ca\(^{2+}\) uptake by the sarcoplasmic reticulum (SR) Ca\(^{2+}\)-adenosine-triphosphatase (ATPase) and mitochondria, Ca\(^{2+}\) extrusion by the sarcosomial Ca\(^{2+}\)-ATPase and Na\(^+\)/Ca\(^{2+}\) exchange, and Ca\(^{2+}\) binding by intracellular proteins (6). Experiments performed by Bassani et al. (5) as well as work from our laboratory (3) have indicated that Ca\(^{2+}\) extrusion from the cell via the sarcosomial Ca\(^{2+}\)-ATPase is approximately an order of magnitude slower than that caused by the action of the SR Ca\(^{2+}\)-ATPase or the Na\(^+\)/Ca\(^{2+}\) exchanger. The sarcosomal Ca\(^{2+}\) pump is, therefore, unlikely to contribute significantly to the rapid decline in [Ca\(^{2+}\)] transient and abruptly disable Na\(^+\)/Ca\(^{2+}\) exchange in isolated rabbit ventricular myocytes. Relaxation and the decline of [Ca\(^{2+}\)] transient can then be compared in beats with and without Na\(^+\)/Ca\(^{2+}\) exchange, under conditions in which [Ca\(^{2+}\)] and SR Ca\(^{2+}\) loading are unchanged. Our results indicate that the effects of Ca\(^{2+}\) extrusion by Na\(^+\)/Ca\(^{2+}\) exchange on the decline of the [Ca\(^{2+}\)] transient are most apparent during the terminal phase of relaxation.

0363-6135/97 $5.00 Copyright © 1997 the American Physiological Society
METHODS

Dissociation of ventricular myocytes. Adult rabbit myocyte isolation was performed by a modification of a previously reported method (17). Hearts were removed from New Zealand White rabbits (2–2.5 kg) anesthetized with pentobarbital sodium (65 mg/kg iv). The heart was first immediately attached to an aortic cannula, and continuous retrograde coronary arterial perfusion at 37°C by pump (Masterflex; Cole-Parmer, Chicago, IL) was initiated at a coronary perfusion pressure of 60 mmHg. The heart was first perfused with nominally Ca2+-free modified Krebs-Ringer bicarbonate buffer solution for 5 min, immediately followed by 20 min of recirculating perfusion with the same solution containing 0.28 mg/ml collagenase (class I; Worthington Biochemicals, Freehold, NJ), 0.4 mg/ml hyaluronidase (type I-S; Sigma Chemical, St. Louis, MO), and 50 µM CaCl2. Both cell isolation solutions contained (in mM) 91.7 NaCl, 30 KCl, 1.2 MgSO4, 19 NaHCO3, 1.2 NaHPO4, 15 glucose, 20 taurine, and 0.5 adenosine and were gassed with 5% CO2-95% O2 (pH 7.40). The heart was then detached from the cannula, and the left ventricle was cut into small pieces in the same solution containing 50 µM CaCl2, 1% albumin, and 1 mg/ml insulin (Sigma). These pieces were incubated in another solution including 50 µM Ca2+, 0.28 mg/ml collagenase, and 0.0025% trypsin for 10 min. The cell suspensions were mixed with the same amount of inhibitor solution including 50 µM Ca2+, 0.0025% trypsin inhibitor (Sigma), and 12% fetal calf serum (FCS) and centrifuged at 250 revolutions/min for 5 min. The supernatant was discarded, and the cells were resuspended in minimum essential medium (GIBCO) containing 0.1% penicillin-streptomycin.

Solutions. The control superfusate for myocytes during physiological studies was a Na2-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered normal Tyrode solution containing (in mM) 118 NaCl, 3.7 KCl, 0.5 MgCl2, 1.08 CaCl2, 5.6 glucose, and 24 HEPES (free acid) titrated to pH 7.40 with NaOH. For rapid SW experiments (see METHODS), including 50 µM CaCl2, 1% albumin, and were gassed with 5% CO2-95% O2 (pH 7.40). The heart was then detached from the cannula, and the left ventricle was cut into small pieces in the same solution containing 50 µM CaCl2, 1% albumin, and 1 mg/ml insulin (Sigma). These pieces were incubated in another solution containing 50 µM Ca2+, 0.28 mg/ml collagenase, and 0.0025% trypsin for 10 min. The cell suspensions were mixed with the same amount of inhibitor solution including 50 µM Ca2+, 0.0025% trypsin inhibitor (Sigma), and 12% fetal calf serum (FCS) and centrifuged at 250 revolutions/min for 5 min. The supernatant was discarded, and the cells were resuspended in minimum essential medium (GIBCO) containing 0.1% penicillin-streptomycin.

Measurement of [Ca2+]i. The time course changes in [Ca2+]i was estimated with the Ca2+-sensitive fluorescent dye fluo 3. To prepare the fluo 3 loading solution, we mixed 10 ml of FCS with 234 µl of 25% Pluronic F127 (BASF; wt/wt in dimethyl sulfoxide) and sonicated the mixture. We then added 1,000 µl of fresh 1 mM fluo 3-acetoxymethyl ester (fluo 3-AM) to the solution. The cells were suspended in 9 ml of FCS-Pluronic mixture, sonicated to mix, and aliquoted into 50 200 µl samples that were stored frozen at −70°C in light-proof containers. This “loading stock” of 100 µM fluo 3-AM was diluted in physiological buffer to give 1 µM fluo 3-AM for cell loading. Myocytes on laminin-coated glass靠板s were exposed to 1 µM fluo 3-AM in physiological solution for 30 min at 30°C, washed in dye-free solution for an additional 30 min, and then perfused at 5 ml/min in a heated chamber (37°C) on the stage of an inverted epifluorescence microscope (Nikon Diaphot) for 4–10 min before experiments were begun. Probenecid (0.5 mM) was added to loading, wash, and perfusion solutions to inhibit the anion transporter and reduce the loss of dye from the cells.

Individual myocytes were paced at 0.25 Hz by means of bipolar platinum electrodes, with a 7-ms pulse at 1.5× threshold. Dye within cells was excited with light from a 100-W mercury-arc lamp (Nikon) through a 485-nm filter (DF 22, Omega), and fluo 3 fluorescence was detected at 530 nm (DF 30, Omega) with a photomultiplier tube (PMT; model 1897 AH, Hamamatsu) by means of an epifluorescence cube (505-nm DRLP dichroic, Omega). In most experiments, the fluo 3 fluorescence intensity was not calibrated but was recorded as arbitrary units above background (unloaded cell) fluorescence intensity. In some cells, calibrated [Ca2+]i values were measured by exposing cells to 10 µM ionomycin in the presence of 2 mM MnCl2. Fluorescence intensity at saturating Ca2+ (Fmax) was estimated as five times the fluorescence intensity at saturating Mn2+, and fluorescence intensity in the absence of Ca2+ (Fmin) as 1/40 Fmax as described by Kao et al. (19). [Ca2+]i was then calculated as Kd(Fmin)/(Fmax − F), where Kd is the dissociation constant given a value of 864 nM (23), after all F values were corrected for background fluorescence.

Measurement of contraction. The cells were illuminated with light from the standard microscope light source passed through a 700-nm band-pass filter. The magnified image of the contracting and relaxing myocyte was transmitted by a Pulnix video camera to a television monitor. Changes in cell length were measured with a two-edge video-based motion detector (Crescent Electronics, Salt Lake City, UT). The signal from the motion detector was calibrated to indicate changes in cell length in micrometers. Relaxation was quantified by measuring the time for the cells to return halfway from their contracted to their resting lengths and was expressed as relaxation half-time (t50). Simultaneous motion and fluo 3 [Ca2+]i transients could not be routinely measured with this system because of “spillover” of illumination light into the 530-nm fluo 3 fluorescence PMT. Therefore, studies of motion and [Ca2+]i were made using the same protocol in different cells. In a few instances, an intensified charge-coupled device camera (Gen II+, Stanford Photonics), which was more sensitive, was used to permit imaging of the cell at 700 nm, a low brightness that does not interfere with the 530-nm fluo 3 fluorescence measurements.

Electrophysiology measurements. Membrane potential was measured with suction pipettes using an Axoclamp 2B amplifier system (Axon Instruments, Foster City, CA) in bridge mode. Pipettes were made from borosilicate capillary tubing (Corning 7052) and had resistances of 2–4 MΩ. Suction pipettes were inserted in the cell membrane using a micromanipulator, and the pipettes were filled with a solution containing (in mM) 113 KCl, 5 or 10 NaCl, 0.5 MgCl2, 5 K+ATP, 10 HEPES, 5 dextrose, and 11 KOH (pH 7.5). Action potentials were elicited by intracellular injection of threshold constant current pulses (3 ms duration).

Solution switching. A solenoid-driven rapid solution SW was used to make fast changes of the extracellular solution surrounding a single myocyte (28). Two adjacent microstreams flowed simultaneously from neighboring square glass tubes (each 200 µm wide) separated by a 70-µm glass septum. A myocyte was positioned in the control stream so that activation of the solenoid moved the two streams laterally and suddenly exposed the cell to the test solution stream. Activation of the SW was controlled by a stimulation pulse 2 s in duration and delivered 4 s after the last of a train of eight 0.25-Hz electrically stimulated (ES) contractions.

Data analysis. Motion and [Ca2+]i data were digitized and analyzed using a Digidata 1200A analog-to-digital converter and Axoscope 1.0 (Axon Instruments) and Micro Origin 4.0 (Microcal) software running on a PC Innovation (West Valley City, UT) computer. Curve fitting was carried out using the Origin program. The statistical significance of differences in motion and [Ca2+]i, transient parameters were assessed using Student’s t-test or analysis of variance as appropriate.
RESULTS

Rapidity of SW-induced changes in perisarcolemmal K⁺ and Ca²⁺ concentrations. To define the role of the Na⁺/Ca²⁺ exchanger in the decline of [Ca²⁺], we sought to rapidly inactivate the "forward" Na⁺/Ca²⁺ exchange (Na⁺ in, Ca²⁺ out) by rapid removal of extracellular Na⁺ and to prevent Ca²⁺ influx by "reverse" exchange (Ca²⁺ in, Na⁺ out). We therefore measured the rate at which changes in external concentrations of Na⁺ and Ca²⁺ could be accomplished with a rapid SW. With this device, the bulk solution surrounding a myocyte can be exchanged within ~2 ms. However, an intact cardiac myocyte has unstimulated layers, which include an extensive T-tubule system. To estimate the time required for exchange of the univalent cation concentration adjacent to the sarcolemma, we performed the experiments illustrated in Fig. 1. In Fig. 1A, an isolated myocyte was abruptly exposed to an elevated KCl concentration (8.8 mM) while membrane potential was measured with a patch electrode. Exposure to high K⁺ caused the expected depolarization of membrane potential, which was virtually complete within 100 ms. In Fig. 1B, a cell was exposed to a 0 Na⁺-0 Ca²⁺ solution with a higher KCl concentration. For comparison, Fig. 1B shows an action potential evoked by an ES in normal external solution in the same cell. Exposure to high-K⁺ solution (SW activated) caused an action potential that was followed by a sustained depolarization to 0 mV. Compared with the electrically activated action potential, the SW-activated action potential had a slightly lower peak amplitude and a reduced plateau, probably secondary to the reduced influx of Na⁺ and Ca²⁺. On the basis of these results, we estimate that the univalent cation concentration adjacent to the sarcolemma can be changed abruptly with the SW device within 100 ms. Furthermore, it is apparent that exposure to a high-K⁺ solution by means of the SW can induce a rapid enough depolarization to elicit an action potential.

The rate of diffusion of divalent cations in the negatively charged glycocalyx surrounding the sarcolemma would be expected to be slower than for univalent cations. We therefore estimated the time required for alteration of the extracellular divalent cation concentration adjacent to the sarcolemma by using the rapid SW. As shown in Fig. 2, abrupt exposure to a Ca²⁺-free extracellular solution (2 mM EGTA) at varying times before ES of an isolated myocyte resulted in a decrease in the amplitude of the twitch. The magnitude of the decrease in amplitude was dependent on the time interval between the exposure of the myocyte to Ca²⁺-free extracellular solution and the subsequent ES. A similar result was obtained by observing the decline in the fluo 3 [Ca²⁺] transient. On the basis of these findings, we estimate that the extracellular Ca²⁺ concentration adjacent to the sarcolemma is reduced after a rapid switch to Ca²⁺-free EGTA solution with a time constant of ~100 ms in adult rabbit ventricular myocytes.

Comparison of rate of relaxation of SW-induced contractions with ES contractions. In view of results described in Rapidity of SW-induced changes in perisarcolemmal K⁺ and Ca²⁺ concentrations, it seemed likely that abrupt exposure to a high-K⁺, Ca²⁺-free EGTA solution would induce depolarization of the cell with resultant opening of the voltage-sensitive Ca²⁺ channels within ~50 ms when the extracellular Ca²⁺ concentration was still sufficient to provide adequate influx of Ca²⁺ via the L-type Ca²⁺ channel to trigger a contraction. However, the subsequent relaxation of the cell would occur in the effective absence of extracellular Na⁺ and Ca²⁺, thus inhibiting the function of the Na⁺/Ca²⁺ exchanger and preventing Ca²⁺ influx during the declining phase of the [Ca²⁺] transient. To test this possibility, we examined the effects of abrupt exposure to 140 mM K⁺ (0 Na⁺-0 Ca²⁺) in the presence of 2 mM

![Diagram](http://ajpheart.physiology.org/)
EGTA) on cell motion. An example is shown in Fig. 3A. In this protocol, the cell was paced at a rate of 0.25 Hz for 8 consecutive stimuli. This resulted in a stable level of contractile amplitude. After the eighth ES, the cell was activated by abrupt exposure to the 0 Na\(^+\)-0 Ca\(^{2+}\) solution (SW) and the contraction and relaxation were measured. The amplitude of the contraction induced by the SW was virtually identical to that induced by the preceding ES. The \(t_{1/2}\) of relaxation was slightly longer for the SW beats, but this increase did not reach statistical significance, as shown in the average results in Fig. 3B for 10 separate cells.

These experiments suggested that abrupt elimination of Na\(^+\)/Ca\(^{2+}\) exchanger function can be achieved with the rapid SW and that, in the presence of normal SR function, this has little effect on relaxation. To investigate corresponding alterations in the [Ca\(^{2+}\)] transient under these conditions, we measured fluo 3 fluorescence signals. An example of a simultaneously recorded fluo 3 fluorescence transient and cell motion is shown in Fig. 4. The fluo 3 fluorescence signal peaks well before the maximum shortening and declines well before the onset of relaxation. Figure 5 compares an ES- and SW-induced fluo 3 transient. As with the previously described motion measurements, the amplitude and \(t_{75}\) of decline of the fluo 3 transient were not significantly different for ES and SW beats (100 \(\pm\) 0\% and 305 \(\pm\) 19 ms vs. 98.2 \(\pm\) 3.1\% and 338 \(\pm\) 34 ms, respectively; \(n = 13\)). However, there was a significant prolongation of the \(t_{75}\) (time to 75\% decline of the fluo 3 transient; 530 \(\pm\) 32 and 727 \(\pm\) 70 ms, respectively; \(P < 0.01\)). Similar results were observed in myocytes paced at 1.0 Hz. These experiments suggested that, under...
expected, that the Na\(^+\)/Ca\(^{2+}\) exchange during terminal phase of [Ca\(^{2+}\)]i decline. To examine the relative contributions of SR and Na\(^+\)/Ca\(^{2+}\) exchange to the decline in [Ca\(^{2+}\)]i during different phases of relaxation, we measured end-diastolic (239 ± 30 nM) and peak systolic (700 ± 87 nM) calibrated [Ca\(^{2+}\)]i values in 14 cells studied under identical conditions (37°C, pacing at 0.25 Hz). These values are similar to those we have previously measured (17) in these cells with indo 1 under comparable conditions (37°C, 0.5-Hz stimulation). We used these average end-diastolic and peak systolic [Ca\(^{2+}\)]i values to convert the fluo 3 fluorescence traces of ES and SW signals averaged from 12 cells to [Ca\(^{2+}\)]i traces. The averaged [Ca\(^{2+}\)]i traces for ES and SW beats during the declining phase of the [Ca\(^{2+}\)]i transient, beginning at 90% of the peak of the ES transient, are displayed in Fig. 8A. The initial decline in the [Ca\(^{2+}\)]i is very similar for ES and SW beats, but there is a slowing of the terminal decline in [Ca\(^{2+}\)]i, in the SW beats, in which the Na\(^+\)/Ca\(^{2+}\) exchanger is abruptly inhibited. In addition, the diastolic level to which the [Ca\(^{2+}\)]i declined was higher in SW than ES beats. The difference in the ES and SW [Ca\(^{2+}\)]i transient declines (difference transient), which represents the component of [Ca\(^{2+}\)]i decline due to Na\(^+\)/Ca\(^{2+}\) exchange, is also apparent in Fig. 8A. We plotted the first derivative of [Ca\(^{2+}\)]i, with respect to time against [Ca\(^{2+}\)]i for SR function (SW transient) and for Na\(^+\)/Ca\(^{2+}\) exchanger function (differ-

**Fig. 5.** Example of a fluo 3 fluorescence transient induced by an ES vs. SW stimulus. Protocol was same as in Fig. 3. Amplitude and initial decline of fluo 3 transient were similar in ES and SW beats, but duration of peak was shortened and terminal phase of relaxation was prolonged in SW transient.

Effects of impaired SR function and Ca\(^{2+}\) binding on relaxation. We investigated the difference between the rate of relaxation in ES beats and SW beats in myocytes in which the SR function was impaired. We exposed adult rabbit myocytes to 500 nM thapsigargin for 15 min to inhibit SR Ca\(^{2+}\)-ATPase (18), and the effects are shown in Fig. 6. Exposure to thapsigargin caused a reduced rate of shortening and a prolongation in the rate of relaxation. However, when an SW contraction was initiated, essentially no relaxation was detected until the extracellular Na\(^+\) concentration was restored to normal at the end of the SW pulse. This indicates, as expected, that the Na\(^+\)/Ca\(^{2+}\) exchanger is effectively disabled by the SW protocol and that Ca\(^{2+}\) transport processes other than Na\(^+\)/Ca\(^{2+}\) exchange and SR function do not produce rapid relaxation in these cells.

Another process that must be considered an important factor in beat-to-beat relaxation is Ca\(^{2+}\) binding. Intracellular proteins bind and release ~95% of the Ca\(^{2+}\) entering the cell and released from the SR each contraction-relaxation cycle (1, 22, 27). We therefore investigated whether an increase in cellular Ca\(^{2+}\) buffering would alter the relative dependence of relaxation on Na\(^+\)/Ca\(^{2+}\) exchange and SR function. A marked increase in Ca\(^{2+}\) buffering was achieved by loading the cell with indo 1. This produced a marked degree of prolongation of the relaxation of ES beats (t\(_{1/2}\) = ~350 ms; Fig. 7). However, abrupt disabling of the Na\(^+\)/Ca\(^{2+}\) exchanger by means of the SW caused an insignificant delay in the rate of relaxation in indo 1-loaded myocytes. Thus, when relaxation is impaired secondary to increased Ca\(^{2+}\) buffering, the relative dependence of relaxation on SR and Na\(^+\)/Ca\(^{2+}\) exchanger function does not appear to be markedly altered.
ence transient) (Fig. 8C). There is a measurable contribution of Na\(^{+}\)/Ca\(^{2+}\) exchange to the decline in the [Ca\(^{2+}\)] transient. This contribution first becomes apparent \(\sim 50\) ms after the Ca\(^{2+}\) has declined to 90% of its peak value. The SR and Na\(^{+}\)/Ca\(^{2+}\) exchanger curves shown in Fig. 8C were extrapolated to peak systolic (700 nM) and end-diastolic (239 nM) [Ca\(^{2+}\)] values.

Subsequent integration of the areas under these curves revealed that the Na\(^{+}\)/Ca\(^{2+}\) exchanger contributes \(\sim 13\%\) to the first 50% decline and 45% to the last 50% of the decline of the total [Ca\(^{2+}\)] transient.

Forward and reverse Na\(^{+}\)/Ca\(^{2+}\) exchange during excitation-contraction coupling and relaxation in rabbit ventricular myocytes. Abrupt elimination of Na\(^{+}\)/Ca\(^{2+}\) exchange by the described SW protocol could decrease reverse as well as forward exchange during the plateau of the action potential and the peak of the [Ca\(^{2+}\)] transient. Thus the relatively small effect of elimination of Na\(^{+}\)/Ca\(^{2+}\) exchanger function on the initial decline in [Ca\(^{2+}\)] could reflect a reduction in Ca\(^{2+}\) influx by reverse exchange as well as indicating that Ca\(^{2+}\) extrusion by forward exchange is relatively unimportant during this phase of [Ca\(^{2+}\)] decline. To assess this possibility, we estimated the reversal potential for the Na\(^{+}\)/Ca\(^{2+}\) exchanger (\(E_{Na/Ca}\)) during a [Ca\(^{2+}\)] transient and action potential (Fig. 9). This analysis was performed for an intracellular Na\(^{+}\) concentration ([Na\(^{+}\)]\(_i\)) of 5 mM, which we have recently measured with sodium green and sodium-binding benzofuran isophthale in rabbit ventricular myocytes under these experimental conditions, and for an [Na\(^{+}\)] of 10 mM, allowing for a possible doubling of subsarcolemmal [Na\(^{+}\)] relative to cytosolic [Na\(^{+}\)] due to “fuzzy space”.

Fig. 8. A: composite ES and SW [Ca\(^{2+}\)] transients averaged from 12 records and plotted from 90% of peak value during [Ca\(^{2+}\)] decline. Difference transient shows difference between SW and ES traces, indicating component due to Na\(^{+}\)/Ca\(^{2+}\) exchange. B: solid lines overlying same transients in A indicate exponential fit of raw data. C: from curve-fit lines in B, absolute derivative of [Ca\(^{2+}\)] with respect to time (d[Ca\(^{2+}\)]/dt) was plotted for SW transient, indicating sarcoplasmic reticulum function, and for difference transient, indicating Na\(^{+}\)/Ca\(^{2+}\) exchanger function. See text for discussion.
abrupt exposure to 0 Na\(^+\) significantly because of the slower diffusion of Ca\(^{2+}\) to the terminal decline of the [Ca\(^{2+}\)] transient to the Ca\(^{2+}\) concentration ([Na\(^+\)] \(= 140\ mM\) and intracellular Na\(^+\) concentration ([Na\(^+\)]; 5 and 10 mM). Na\(^+/Ca\(^{2+}\) exchanger will operate in "reverse" mode when \(E_m > E_{NaCa}\). From this analysis, Na\(^+/Ca\(^{2+}\) exchanger functions almost exclusively in forward mode during decline of [Ca\(^{2+}\)] transient, and difference between \(E_{NaCa}\) and \(E_m\), which is driving force for Na\(^+/Ca\(^{2+}\) exchanger, is greatest during terminal phase of [Ca\(^{2+}\)] decline. Pipette [Na\(^+\)], was 5 mM and temperature was 32°C in this action potential recording.

**DISCUSSION**

Use of a rapid SW to induce a contraction and disable Na\(^+/Ca\(^{2+}\) exchanger. Our results clearly indicate that abrupt exposure to 0 Na\(^+\)-0 Ca\(^{2+}\) 140 mM K\(^+\) solution by means of a rapid SW can induce contractions and [Ca\(^{2+}\)] transients that are identical in amplitude to those caused by a preceding ES delivered after an identical diastolic interval. We believe this is because the rapid exposure to 140 mM K\(^+\) induces depolarization and opening of the L-type Ca\(^{2+}\) channel within a time interval (<50 ms) during which the [Ca\(^{2+}\)] adjacent to the Ca\(^{2+}\) channel has not yet declined significantly because of the slower diffusion of Ca\(^{2+}\) compared with K\(^+\), demonstrated in Figs. 1 and 2.

The subsequent relaxation of the contraction induced by this KCl depolarization appears to occur in the presence of a disabled Na\(^+/Ca\(^{2+}\) exchanger. This is predicted because the extracellular Na\(^+\) concentration has been reduced to zero and the cell is depolarized by continued exposure to 140 mM K\(^+\). We cannot exclude the possibility that the Na\(^+\) concentration within the T-tubule system is somewhat higher than zero because of the greater time required for exchange of T-tubule fluid compared with perisarcolemmal fluid. This could be a concern because of evidence suggesting that the Na\(^+/Ca\(^{2+}\) exchangers may be localized predominantly to T-tubule membranes (13). However, even if the Na\(^+\) concentration in the T-tubule system is somewhat higher than zero after exposure to the Na\(^+\)-free SW solution, it seems likely that the prolonged membrane depolarization induced by the high K\(^+\) would disable the Na\(^+/Ca\(^{2+}\) exchanger. Bridge et al. (10) have demonstrated that in isolated myocytes in which the SR is disabled by exposure to caffeine, relaxation is dependent on extracellular Na\(^+\) and is very sensitive to \(E_m\). This is due to the electrogenic nature of the Na\(^+/Ca\(^{2+}\) exchanger, and its operation in the forward direction (3 Na\(^+\) in, 1 Ca\(^{2+}\) out) is inhibited by membrane depolarization. The experiments in which the SR function was disabled by thapsigargin would appear to support this prediction. In these experiments, rapid relaxation was virtually eliminated in the rapid SW-induced contractions. This would not be observed if significant residual Na\(^+/Ca\(^{2+}\) exchanger function was present after the SW contraction. Thus our results suggest that exposure to the high-K\(^+\), low-Na\(^+\) solution by means of the rapid switcher does result in rapid and essentially complete disabling of forward Na\(^+/Ca\(^{2+}\) exchange. Presumably, under these circumstances the Na\(^+/Ca\(^{2+}\) exchanger reaches equilibrium and cannot extrude Ca\(^{2+}\) until an appropriate driving force is imposed by membrane repolarization.

Relative importance of SR and Na\(^+/Ca\(^{2+}\) exchanger function during initial and terminal relaxation. Bassani et al. (4) reported that inhibition of the Na\(^+/Ca\(^{2+}\) exchanger prolongs the overall time constant of the decline in the [Ca\(^{2+}\)] transient measured with indo 1 by as much as 45% in normal rabbit myocytes. In their experiments, the cells were first exposed to a 0 Na\(^+\)-0 Ca\(^{2+}\) solution for 5–7 min, and then Ca\(^{2+}\) was restored in the presence of 0 Na before ES. Our experiments, however, have shown only a small and statistically insignificant delay in the initial rate of relaxation and decline in [Ca\(^{2+}\)], when the Na\(^+/Ca\(^{2+}\) exchanger is abruptly disabled in adult rabbit ventricular myocytes. We believe this difference in results may be attributed to several factors. First, in our experiments we used a low concentration of the Ca\(^{2+}\) indicator fluo 3. This indicator has a higher \(K_d\) for Ca\(^{2+}\) than indo 1 (864 vs. 260 nM) and thus would be expected to buffer Ca\(^{2+}\) less markedly than indo 1, making it possibly more suitable for examining the rapid kinetics of initial Ca\(^{2+}\) decline. Our experiments with fluo 3 do indicate clearly that Na\(^+/Ca\(^{2+}\) exchange does contribute importantly to the rate of decline in [Ca\(^{2+}\)] from the midportion of the [Ca\(^{2+}\)] transient and appears to be necessary to reach end-diastolic [Ca\(^{2+}\)] levels. In the experiments of Bassani et al. (4), resting [Ca\(^{2+}\)] was also somewhat increased in the 0 Na\(^+\)-0 Ca\(^{2+}\) solution, although this
increase did not reach statistical significance. Second, in the experiments of Bassani et al. (4), simultaneous exposure to 0 Na⁺-0 Ca²⁺ solution could have resulted initially in some reversal of the Na⁺/Ca²⁺ exchange due to a difference in rate of removal of perisarclolemmal Ca²⁺ and Na⁺, with increased Ca²⁺ loading of the SR. Increased Ca²⁺ influx and loading of the SR can slow relaxation (24), and thus an increase in the degree of SR Ca²⁺ loading could have caused a slight slowing of relaxation in these experiments. The amplitude of contraction and the amplitude of the [Ca²⁺] transient was somewhat greater in the experiments of Bassani et al. (4) in myocytes stimulated in the presence of 0 Na⁺ and normal Ca²⁺ compared with that in myocytes stimulated under control conditions. This was attributed by Bassani et al. (4) to extrusion of Ca²⁺ by Na⁺/Ca²⁺ exchange during the peak of the [Ca²⁺] transient in a normal beat (see also Ref. 20). However, in our experiments, the amplitude of the [Ca²⁺] transient and the amplitude of the contraction were identical in ES beats and in beats in which the Na⁺/Ca²⁺ exchanger was abruptly disabled by the use of the SW. Because the extent of SR loading during ES and SW beats should be identical in our experiments, our findings suggest that Ca²⁺ extrusion by Na⁺/Ca²⁺ exchange does not significantly reduce the peak magnitude of the [Ca²⁺] transient during a normal beat. This conclusion is also supported by data shown in Fig. 9 and by the findings of Bouchard et al. (9) and is predicated by the dynamic model of the cardiac ventricular action potential formulated by Luo and Rudy (22).

The relative asymmetry of Na⁺/Ca²⁺ exchanger and SR function with respect to early and late relaxation may have functional importance. In the intact heart, the initial phase of relaxation is the isovolumic phase, the rapidity of which determines the interval between aortic valve closure and mitral valve opening. The terminal phase of ventricular relaxation occurs during early ventricular filling, after mitral valve opening. If our results in isolated myocytes can be extrapolated to the intact heart, we would predict that impaired Na⁺/Ca²⁺ exchanger function might affect early ventricular filling more markedly than isovolumic relaxation and thus contribute to hemodynamically significant diastolic dysfunction.

Potential value of SW method for assessment of SR function in intact myocytes. As previously demonstrated (3, 4, 10) in myocytes in which SR function has been pharmacologically impaired by exposure to caffeine or thapsigargin, the dependence on relaxation on Na⁺/Ca²⁺ exchanger function is much more marked. In these situations, abrupt disabling of the Na⁺/Ca²⁺ exchanger produces a dramatic prolongation of relaxation, as shown in Figs. 3 and 6. Our results also confirm previous reports by Noble and Powell (26) that Ca²⁺ indicators such as indo 1 can slow relaxation by buffering Ca²⁺. In rabbit myocytes well loaded with indo 1, a prolongation of relaxation comparable to that achieved with thapsigargin was achieved (t½ = 350 ms). However, in myocytes in which relaxation was prolonged because of abnormal Ca²⁺ buffering within the cell, abrupt disabling of the Na⁺/Ca²⁺ exchanger had a very minor effect on relaxation, as shown in Fig. 7. Thus the magnitude of the effect of abruptly disabling the Na⁺/Ca²⁺ exchanger on relaxation appears to depend on the cause of the slow relaxation.

We believe that these findings may be useful in allowing assessment of subtle abnormalities in SR function in isolated myocytes. Abrupt exposure of the myocytes to 0 Na⁺-0 Ca²⁺, high-K⁺ solution by means of the switcher should prolong relaxation if SR function is impaired. On the other hand, in cells in which the baseline relaxation in response to electrically stimulated contractions is prolonged due to Ca²⁺ buffering, abrupt elimination of the Na⁺/Ca²⁺ exchanger would not be expected to produce an impairment of initial relaxation or the decline in the [Ca²⁺] transient. This approach may be especially useful in determining the extent of SR dysfunction in isolated myocytes obtained from failing and hypertrophied myocardium in which Na⁺/Ca²⁺ exchanger function may be upregulated (14, 29).

The authors are grateful for the assistance of Pam Larson in the preparation of the manuscript and Drs. Massimiliano Zaniboni and Zhi Su for technical assistance. This work was supported by National Heart, Lung, and Blood Institute Grants HL-30478, HL-53773, HL-42873, and HL-34288. Address for reprint requests: W. H. Barry, Div. of Cardiology, Univ. of Utah Health Sciences Center, 50 N. Medical Dr., Salt Lake City, UT 84132.

Received 28 February 1997; accepted in final form 15 August 1997.

REFERENCES


