

**Na\(^+\)-Ca\(^{2+}\) Exchange Activity Is Localized in the T-Tubules of Rat Ventricular Myocytes**

Z. Yang, C. Pascarel, D.S. Steele, K. Komukai, F. Brette, C.H. Orchard

**Abstract**—Detubulation of rat ventricular myocytes has been used to investigate the role of the t-tubules in Ca\(^{2+}\) cycling during excitation-contraction coupling in rat ventricular myocytes. Ca\(^{2+}\) was monitored using fluo-3 and confocal microscopy. In control myocytes, electrical stimulation caused a spatially uniform increase in intracellular [Ca\(^{2+}\)] across the cell width. After detubulation, [Ca\(^{2+}\)] rose initially at the cell periphery and then propagated into the center of the cell. Application of caffeine to control myocytes resulted in a rapid and uniform increase of intracellular [Ca\(^{2+}\)]; the distribution and amplitude of this increase was the same in detubulated myocytes, although its decline was slower. On application of caffeine to control cells, there was a large, rapid, and transient rise in extracellular [Ca\(^{2+}\)] as Ca\(^{2+}\) was extruded from the cell; this rise was significantly smaller in detubulated cells, and the remaining increase was blocked by the sarcolemmal Ca\(^{2+}\) ATPase inhibitor carboxyeosin. The treatment used to produce detubulation had no significant effect on Ca\(^{2+}\) efflux in atrial cells, which lack t-tubules. Detubulation of ventricular myocytes also resulted in loss of Na\(^+-\)Ca\(^{2+}\) exchange current, although the density of the fast Na\(^+\) current was unaltered. It is concluded that Na\(^+-\)Ca\(^{2+}\) exchange function, and hence Ca\(^{2+}\) efflux by this mechanism, is concentrated in the t-tubules, and that the concentration of Ca\(^{2+}\) flux pathways in the t-tubules is important in producing a uniform increase in intracellular Ca\(^{2+}\) on stimulation. (Circ Res. 2002;91:315-322.)

**Key Words:** t-tubules ■ calcium ■ sodium-calcium exchanger

Transverse (t-) tubules are invaginations of the cell membrane that are found in skeletal and cardiac muscle. In cardiac ventricular myocytes they occur perpendicular to the longitudinal axis of the cell at intervals of \(\approx 2\ \mu m\) and extend into the cell both laterally and longitudinally. Immunohistochemical studies have shown that many of the proteins involved in excitation-contraction coupling are concentrated at the t-tubules, suggesting an important role for the t-tubules in Ca\(^{2+}\) cycling during excitation-contraction coupling. For example, L-type Ca\(^{2+}\) channels are concentrated in the t-tubular membrane, in close proximity to the Ca\(^{2+}\) release channels (ryanodine receptors; RyR) of the junctional sarcoplasmic reticulum (SR).

Detubulation of cardiac cells enables the functional consequences of this protein distribution to be investigated. Detubulation of rat ventricular myocytes results in a \(\approx 25\%\) decrease in cell surface area, which is accompanied by a \(\approx 75\%\) decrease in the amplitude of the L-type Ca\(^{2+}\) current, and a decrease in the amplitude of the Ca\(^{2+}\) transient. This is consistent with the immunolabeling data that show the L-type Ca\(^{2+}\) channels concentrated in the t-tubular membrane.

In the present study, we have used detubulation to investigate further the role of the t-tubules in Ca\(^{2+}\) cycling during excitation-contraction coupling in rat ventricular myocytes. We have investigated the hypothesis that the t-tubules underlie the rapid and homogeneous Ca\(^{2+}\) release observed in cardiac ventricular myocytes (eg, Kawai et al\(^{6}\)). We have also investigated the functional distribution of the Na\(^+-\)-Ca\(^{2+}\) exchanger between the surface and t-tubule membranes. This exchanger is the major route of Ca\(^{2+}\) efflux from cardiac cells, and can also act as a Ca\(^{2+}\) influx pathway; it has been suggested that Ca\(^{2+}\) entering the cell by this route might, under some circumstances, act as a trigger for Ca\(^{2+}\) release from the SR. Determining the location of the exchange protein is, therefore, important for our understanding of Ca\(^{2+}\) flux in the cardiac cell. However the distribution of the exchanger is unclear from immunolabeling studies. Some studies show concentration of the exchange protein in the t-tubule membrane, whereas others show a more uniform distribution of the exchanger between the surface and t-tubule membranes.

**Materials and Methods**

**Cell Isolation**

Adult Wistar rats (\(\approx 250\ g\); Central Biomedical Services, Leeds, UK) were stunned and then killed by cervical dislocation, in accordance with the UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act of 1986. Ventricular myocytes were isolated as described previously.

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Osmotic Shock Treatment of Ventricular Myocytes With Formamide

To induce osmotic shock, and hence detubulation, 1.5 mol/L formamide was added to the cell perfusate for 15 minutes, before returning to control solution, as described previously.6

Confocal Imaging

Confocal imaging was performed using a laser-scanning unit (Bio-Rad Microradiance 2000) attached to a Nikon Diaphot inverted microscope. The X-Y resolution of the system was 0.45 μm, as measured from the point-spread function of fluorescent microspheres (diameter 0.175 μm). The aperture size was set to the size of the Airy disc to optimize z-axis resolution. Fluo-3 was excited with the 488-nm line of an argon ion laser and fluorescence measured at >515 nm. Line scan images were acquired at 6 ms or 2 ms intervals across the width of the cell. To reduce possible laser damage, the position of the line was changed after 2 to 3 scan sequences. Images were analyzed using IDL (Research Systems Inc) and Laserpix (Bio-Rad) software.

Monitoring Intracellular Ca2+ (Ca2+) Distribution

Myocytes were loaded with the fluorescent Ca2+ indicator fluo-3 (Molecular Probes) by incubation with its AM form (5 μmol/L for 6 minutes).

Monitoring Ca2+ Efflux

Myocytes were initially bathed in a solution containing (in mmol/L) NaCl 113, KCl 5, MgSO4 1, Na2HPO4 1, HEPES 10, Na acetate 20, glucose 5.5 (pH 7.4, 310 mOsm/L). The cells were superfused with a solution containing (in mmol/L) NaCl 113, MgCl2 2, CaCl2 1, CoCl2 2, HEPES 10, and Glucose 5.5 (pH 7.4, adjusted with CsOH); calculated free [Ca2+]i was reduced to ~100 nmol/L (solution as above except 0 Ca2+ and 0.4 mmol/L EGTA) for 100 seconds. The cell was then perfused for 60 seconds with solution containing 10 μmol/L Fluo-3 and 0.05 mmol/L EGTA before rapid application of, and perfusion with, solution containing 20 mmol/L caffeine. In early experiments, 5 μmol/L thapsigargin was also used during the 60 seconds before application of caffeine, to inhibit Ca2+ reuptake by the SR. However, this had little effect on the response to caffeine and was not used subsequently.

Current Measurements

Na+-Ca2+ exchange current (I Na/Ca) was monitored using the whole-cell (ruptured patch) voltage clamp technique as described previously.12 The pipette solution contained (in mmol/L) CsCl 110, NaCl 20, TEACl 20, MgCl2 0.4, HEPES 10, BAPTA 5, CaCl2 1, and glucose 5 (pH 7.2, adjusted with CsOH); calculated free [Ca2+]i 95 nmol/L. The cells were superfused with a solution containing (in mmol/L) NaCl 140, CsCl 4, MgCl2 1, HEPES 5, Glucose 10, and CaCl2 2.5 (pH 7.4, adjusted with NaOH). This solution also contained 20 μmol/L strophanthidin to block the Na+/K+ pump and 10 μmol/L nifedipine to block L-type calcium current (I Ca). The BAPTA within the pipette buffered Ca2+ sufficiently to stop contraction; control experiments showed that Ca2+ and K+ currents were absent in these solutions.

Sodium current (I Na) was also monitored using the whole-cell (ruptured patch) voltage clamp technique with low resistance electrodes (1 to 2 MΩ). The pipette solution contained (in mmol/L) CsCl 130, MgCl2 2, Na2ATP 5, Na2GTP 0.5, CaCl2 0.5, HEPES 10, and EGTA 5 (pH 7.2, adjusted with CsOH). The cells were superfused with a solution containing (in mmol/L) NaCl 20, CsCl 110, TEACl 10, MgCl2 2, CaCl2 1, CoCl2 2, HEPES 2, and Glucose 5.5 (pH 7.4, adjusted with CsOH).

Membrane currents were recorded using an Axopatch 1D voltage clamp amplifier, controlled by Vclamp software (Cambridge Electronic Design) running on an Elonex PC433 computer, via a CED1401 A/D interface. Data were digitized (at 2 kHz during recording of I Na/Ca and 20 kHz during recording of I Na) via this interface and stored on the hard disk of the computer. The protocols are described in the relevant section of the Results. A straight line was fitted to the central, linear portion of the I-V relation of I Na/Ca; this line adequately described the majority of the I-V relationship, and its slope was used as a simple method of characterizing I Na/Ca.

The rapid solution changes made on the confocal microscope could only be performed at room temperature without inducing large temperature transients. To ensure consistency, all experiments were, therefore, performed at room temperature.

Chemicals

All solutions were prepared using ultrapure water supplied by a Milli-Q system (Millipore). All solution constituents were reagent grade and purchased from Sigma.

Statistical Analysis

Data are expressed as mean ± SEM of n cells; paired or unpaired t tests were used as appropriate. A value of P < 0.05 was taken as statistically significant.

Results

Effect of Formamide Treatment on T-Tubule Structure and Membrane Capacitance

Treatment with formamide resulted in loss of the t-tubules, visualized using the dye di-8-ANNEPS, as reported previously6 (not shown). Membrane capacitance was determined from capacity transients at the beginning of 10-mV hyperpolarizing pulses from a holding potential of ~80 mV. Capacitance decreased significantly (P < 0.05, n = 25) from 193 ± 41 pF in control cells to 143 ± 34 pF in detubulated cells. This decrease (25.9%) is similar to that reported previously.6 Assuming that 80% of the treated cells are detubulated, these data suggest that the decrease in cell capacitance in the cells that are detubulated is 32.4%, close to previous estimates of the percentage of the cell membrane in the t-tubules (33%).1

Effect of Detubulation on Intracellular Ca2+ Distribution

Previous work has shown that I Ca is concentrated in the t-tubules.6 Detubulation would therefore be expected to disrupt normal synchronous Ca2+ release. Confocal microscopy was used to image the spatial distribution of Ca2+ in control and detubulated cells. Figure 1A shows a line scan image of a control cell, loaded with fluo-3, during electrical stimulation at 0.5 Hz, bathing [Ca2+]i close to the cell membrane can still be observed. The SR inhibitor ryanodine had a similar effect (not shown). Thus, it appears that the slow propagation of Ca2+ into the cell after detubulation is due to Ca2+-induced Ca2+ release (CICR) from the SR after Ca2+ influx at the cell surface. It is unlikely that altered RyR function or distribution contributes to the inhomogeneous Ca2+ release observed after detubulation because the rate of propagation of spontaneous Ca2+ waves, which depends on RyR distribution and function, was similar in control and detubulated cells, and the amplitude and distribution of the rise of Ca2+ produced by caffeine, which acts by
opening RyR, was the same in control and detubulated cells (see next section).

**Effect of Detubulation on Ca\(^{2+}\) Extrusion**

Figure 2 shows confocal images of cardiac cells bathed in solution containing fluo-3 to monitor extracellular Ca\(^{2+}\) (Ca\(_o\); see Materials and Methods). Under these conditions, application of 20 mmol/L caffeine enabled Ca\(^{2+}\) efflux from the cell to be monitored. Figure 2A shows that in a control cell, application of caffeine resulted in a rapid and marked rise of Ca\(_o\) over ~1 second, as Ca\(^{2+}\) was extruded from the cell. Ca\(_o\) subsequently slowly declined to baseline as the extruded Ca\(^{2+}\) was washed away in the perfusing solution. Figure 2B shows that in a detubulated cell under identical conditions the rise of Ca\(_o\) was markedly reduced: peak fluorescence/basal fluorescence (F/Fo) increased to 5.73±0.46 in control cells (n=8), and to 2.28±0.22 in detubulated cells (n=7; P<0.05). These data suggest, therefore, that loss of the t-tubules results in a decrease in Ca\(^{2+}\) efflux that is greater than the ~30% loss of cell membrane (see "Effect of Formamide . . ."), and therefore, that Ca\(^{2+}\) efflux pathways are concentrated in the t-tubules.

Na\(^{+}\)-Ca\(^{2+}\) exchange and sarcolemmal Ca\(^{2+}\) ATPase are the two main extrusion pathways in rat ventricular cells.\(^7,8\) We investigated, therefore, the effect of the sarcolemmal Ca\(^{2+}\) ATPase inhibitor carboxyoeosin on the residual Ca\(^{2+}\) efflux in detubulated cells. Figure 2C shows Ca\(_o\) during caffeine application in a representative detubulated cell in the presence of carboxyoeosin, showing that inhibition of the ATPase abolished the residual rise of Ca\(_o\) (n=3).

Figure 3 shows that electrical stimulation of detubulated cells produced a smaller intracellular Ca\(^{2+}\) transient than in control cells, as reported previously,\(^6\) but that the increase of Ca\(_i\) produced by caffeine had a similar amplitude and uniform distribution in control and detubulated cells (F/Fo was 3.01±0.22 in control cells, n=10, 3.03±0.20 in detubulated cells, n=11; NS), but that the decline of the caffeine-induced rise of Ca\(_i\) was prolonged in the detubulated cells: the time for a 75% decrease from peak increased from 2.57±0.23 (n=10) to 5.56±0.04 seconds (n=11; P<0.001). Thus, the decreased Ca\(^{2+}\) efflux observed in detubulated cells is not due to a decrease in the rise in Ca\(_i\) produced by caffeine.
To ensure that the decreased efflux observed after formamide treatment was not due to a direct effect of formamide on the proteins involved, the effect of formamide treatment on Ca\(^{2+}\) extrusion was monitored in atrial cells, which lack t-tubules. Formamide treatment had no significant effect on the rise of Ca\(_a\), that occurred on application of caffeine to these cells: F/F\(_o\) increased to 3.44 ± 0.66 in control cells (n = 6) and to 3.22 ± 0.21 in formamide-treated cells (n = 6; NS; not shown). Thus, it appears that in ventricular cells Na\(^+\)-Ca\(^{2+}\) exchange flux is concentrated in the t-tubules, and that the residual Ca\(^{2+}\) flux after detubulation is due to Ca\(^{2+}\) ATPase activity on the surface membrane. To investigate this further, I\(_{Na/Ca}\) was monitored.

**Effect of Detubulation on I\(_{Na/Ca}\)**

I\(_{Na/Ca}\) was measured in control and detubulated cells using a descending voltage clamp ramp protocol as described previously\(^{12}\): from a holding potential of −40 mV, the cell membrane was depolarized to +50 mV and held at this voltage for 100 ms, to allow inactivation of rapidly inactivating currents, before being hyperpolarized to −120 mV over 2 seconds. Ramps were applied at 0.4 Hz. This was repeated in the presence of 5 mmol/L nickel, to block the exchanger. The difference current (between the absence and presence of nickel) was taken as I\(_{Na/Ca}\) (see Convery and Hancox\(^{12}\) and Discussion).

Figure 4A shows the mean current density-voltage relationship for the current recorded during the descending voltage ramp before and after application of Ni, and the difference current, in control cells. The slope of each current-voltage ramp before and after application of Ni, and the slope of the relation was calculated to enable comparison of the difference current, in control cells. The slope of each current-voltage ramp before and after application of Ni, and the relationship for the current recorded during the descending voltage clamp ramp protocol as described previously\(^{12}\): from a holding potential of −40 mV, the cell membrane was depolarized to +50 mV and held at this voltage for 100 ms, to allow inactivation of rapidly inactivating currents, before being hyperpolarized to −120 mV over 2 seconds. Ramps were applied at 0.4 Hz. This was repeated in the presence of 5 mmol/L nickel, to block the exchanger. The difference current (between the absence and presence of nickel) was taken as I\(_{Na/Ca}\) (see Convery and Hancox\(^{12}\) and Discussion).

Figure 4A shows the mean current density-voltage relationship for the current recorded during the descending voltage ramp before and after application of Ni, and the difference current, in control cells. The slope of each current-voltage ramp before and after application of Ni, and the slope of the relationship for the current recorded during the descending voltage ramp protocol and the current density-voltage relationship in control and detubulated cells, showing that current density was not significantly different from the control current density in the normal cells. Although the identity of the Ni insensitive current in the detubulated cells was also not significantly different from the control current density in the normal cells. Although the identity of the Ni insensitive current in the detubulated cells was also not significantly different from the control current density in the normal cells. Although the identity of the Ni insensitive current in the detubulated cells was also not significantly different from the control current density in the normal cells.
the cell membrane); a decrease in current density after detubulation suggests that the current was concentrated in the t-tubule membrane.

The technique used to monitor $I_{\text{Na/Ca}}$ has been described previously. Blockers were used to inhibit other currents so that Ni-sensitive current, as in previous studies, is taken to represent $I_{\text{Na/Ca}}$. Block of the exchanger might alter local Ca, thus altering Ca$^{2+}$-activated currents. However, Choi et al have shown that even when Ca was increased using caffeine (ie, to levels higher than those in the present study, in which Ca was buffered with BAPTA), no resulting current was observed in rat ventricular myocytes when the exchanger was inhibited by Ni or by the absence of bathing Na$^+$ and Ca$^{2+}$, making this unlikely.

$I_{\text{Ca}}$ was monitored using the whole-cell patch clamp technique with low-resistance (1 to 2 MΩ) electrodes, and using low extracellular [Na$^+$] (20 mmol/L) and normal/high intracellular [Na$^+$] (11 mmol/L) to reduce the Na$^+$ gradient and, hence, current. However, despite this, and although the time to peak of the current was relatively fast, it seems possible that voltage control was lost during measurement of $I_{\text{Ca}}$. This problem would be common to control and detubulated cells, and the amplitude and time course of the current were not significantly different in the two groups.

Spatial Distribution of Ca$^+$ During Electrical Stimulation

In control cells, Ca$^{2+}$ increased homogeneously across the cell on electrical stimulation. However, in formamide-treated cells, Ca$^{2+}$ initially rose close to the cell membrane and then propagated into the cell interior, consistent with detubulation: the initial rise close to the surface membrane is similar to that reported in atrial and Purkinje cells, which lack t-tubules. This propagation was inhibited by thapsigargin and ryanodine, suggesting that it is due to CICR as in atrial cells but in contrast to Purkinje cells, in which the inward spread of Ca$^{2+}$ appears to be due principally to buffered diffusion of Ca$^{2+}$. Thus, it appears that in detubulated cells the initial rise is due to Ca$^{2+}$ influx across the surface cell membrane, and the propagation to CICR from the SR. Because Na$^-$Ca$^{2+}$ exchange activity appears to be concentrated in the t-tubules (see next section), it is likely that Ca$^{2+}$ influx via Ca$^{2+}$ channels in the surface membrane causes the initial rise of Ca. Consistent with this idea, the rise was increased by the Ca$^{2+}$ channel agonist BayK (not shown).

In agreement with previous work showing that Ca$^{2+}$ sparks arise predominantly at the t-tubules, the present data suggest that the t-tubules play an important role in excitation-contraction coupling because the high concentration of Ca$^{2+}$ influx pathways in the t-tubule membrane, $I_{\text{Ca}}$, and $I_{\text{Na/Ca}}$ (see next section), allows synchronized CICR from the adjacent ryanodine receptors and thus synchronized Ca$^{2+}$ release throughout the cell.

Distribution of the Na$^+$-Ca$^{2+}$ Exchanger Between Cell Surface and T-Tubule Membrane

Measurement of Ca, during application of caffeine to control cells showed a marked and rapid rise of Ca as Ca$^{2+}$ was extruded from the cell. Although the amplitude of the rise of
Ca produced by caffeine was not significantly different in control and detubulated cells, the rise of $C_{a}$ was significantly smaller in detubulated cells, suggesting that the $Ca^{2+}$ efflux pathways are concentrated in the t-tubules. In the presence of carboxyeosin, even the residual $Ca^{2+}$ efflux was abolished, suggesting that it was due to $Ca^{2+}$ ATPase activity. These data suggest, therefore, that $Ca^{2+}$ extrusion via $Na^{+}$-$Ca^{2+}$ exchange occurs exclusively in the t-tubules. Consistent with this, measurement of $I_{Na}$ showed that detubulation abolished $I_{NaCa}$.

It is unlikely that the observed effects can be ascribed to direct effects of formamide, because formamide treatment had no effect on $Ca^{2+}$ efflux from atrial cells, which lack t-tubules but possess $Na^{+}$-$Ca^{2+}$ exchange, and which undergo similar size and volume changes as ventricular cells in response to formamide and its withdrawal (Brette and Orchard, unpublished observation, 2001). In addition, measurements were made after removal of formamide, so that osmotic effects on the exchanger would be absent. It is also unlikely that the effects of formamide are secondary to effects on the cytoskeleton, because formamide treatment had no apparent effect on cytoskeleton structure, assessed by labeling with monoclonal antibody to $\beta$-tubulin (Brette and Orchard, unpublished observations, 2002).

The simplest explanation of the present data is that the exchange protein is localized to the t-tubules, consistent with a recent immunohistochemical study using rat ventricular myocytes. An alternative explanation is that the exchange protein is uniformly distributed, but only the protein located in the t-tubules is active during the protocols used in the present investigation. This might occur, for example, if $Na^{+}$ entering the cell via $I_{Na}$ is used by the exchanger, but access of this $Na^{+}$ to the exchanger is different in the t-tubules and at the surface membrane. This appears unlikely because (1) $I_{Na}$ appears to be uniformly distributed (as seen in this study); (2) there appears to be little colocalization of the two proteins; (3) during measurement of $I_{NaCa}$, $I_{Na}$ will be triggered on returning to $-40$ mV from $-120$ mV at the end of the voltage ramp, so that time is available for intracellular diffusion of $Na^{+}$ between $I_{Na}$ being triggered and $I_{NaCa}$ being monitored.

Although it has previously been shown that there is a high concentration of $Ca^{2+}$ channels in the t-tubules, the $Ca^{2+}$ channel blocker nifedipine was used in the present study during measurement of $I_{NaCa}$ to inhibit interference by $Ca^{2+}$ influx by this route. Thus, ion flux on $Na^{+}$-$Ca^{2+}$ exchange is localized in the t-tubules, probably because the exchange protein is concentrated in the t-tubules, although it remains possible that protein function does not mirror protein distribution because of other (unknown) local regulation.

It is also of interest that $I_{Na}$ appears to be uniformly distributed across the cell membrane; Scriven et al showed, using immunolabeling, that the L-type $Ca^{2+}$ channel, $Na^{+}$-$Ca^{2+}$ exchanger, and $Na^{+}$ channel appear to be concentrated in the t-tubules. However, only the $Na^{+}$ channel showed significant presence on the sarcolemma between the z-lines, consistent with a more uniform distribution of this protein.

**Functional Consequences of the Observed $I_{NaCa}$ Distribution**

$Na^{+}$-$Ca^{2+}$ exchange is the major $Ca^{2+}$ efflux pathway in rat ventricular myocytes. Because $Ca^{2+}$ efflux via the exchanger is localized to the t-tubules, they play an important role in $Ca^{2+}$ efflux in normal cells. It is, however, worth considering the determinants of the decline of the electrically stimulated, and caffeine-induced, $Ca^{2+}$ transients in detubulated cells. The decline of the electrically stimulated $Ca^{2+}$ transient in detubulated cells is presumably due to $Ca^{2+}$ uptake by the SR, $Ca^{2+}$ diffusion into adjacent regions of the cell because of the inhomogeneous distribution of $Ca_{s}$, and $Ca^{2+}$ efflux via the sarcolemmal $Ca^{2+}$ ATPase.

The amplitude of the caffeine-induced rise of $Ca_{s}$ was unchanged by detubulation, although its decline was slower. The maintained amplitude is, perhaps, surprising, given that $I_{Ca}$ and $I_{NaCa}$ are concentrated in the t-tubules. However, $Ca^{2+}$ efflux was lower in the detubulated cells. Because $Ca^{2+}$ extrusion can decrease the amplitude of the caffeine-induced $Ca^{2+}$ transient, this might suggest that the SR $Ca^{2+}$ content...
was lower in detubulated cells, consistent with a loss of Ca\(^{2+}\) influx pathways; such a decreased load may also contribute to the smaller rapid upstroke of the Ca\(^{2+}\) transient observed in detubulated cells\(^6\) (as seen in this study). In addition, however, the small fraction of \(I_{Ca}\) remaining in the surface membrane,\(^6\) and the loss of the main Ca\(^{2+}\) efflux pathway \(I_{Na,Ca}\) in detubulated cells would result in the SR retaining more Ca\(^{2+}\), thus helping to maintain its content;\(^2\) the longer diffusion distances for Ca\(^{2+}\) from the center of the cell to extrusion at the cell surface and the relatively slow Ca\(^{2+}\) extrusion rate of the remaining extrusion pathways may also help maintain SR Ca\(^{2+}\) uptake and content.

The prolonged decline of the caffeine-induced increase of Ca\(^{2+}\) in detubulated cells is expected, because Na\(^+-\)Ca\(^{2+}\) exchange is normally the main pathway removing Ca\(^{2+}\) from the cytoplasm in the presence of caffeine.\(^7,8\) However, previous work showed a more modest slowing of the rate of decline.\(^6\) It is likely that the more marked effect observed in the present study is a better assessment of the response to caffeine, because (1) in the present study we ensured detubulation of each cell before application of caffeine, by checking that electrical stimulation caused the pattern of Ca\(^{2+}\) release shown in Figure 1B. Because not all cells are detubulated by formamide,\(^6\) it is likely that the mean data in the previous study included data from normal cells. (2) A higher concentration of caffeine was used in the present study, to ensure more complete Ca\(^{2+}\) release from the SR and to minimize the contribution of Ca\(^{2+}\) reuptake by the SR to the change of Ca\(^{2+}\).\(^2\) (3) In the present study, caffeine was applied directly and rapidly to the cell being studied, whereas in the previous study the rapidity of application depended on the position of the cell in the bath, which is likely to have influenced the observed response.

In previous studies, the rate constant of decline of the caffeine-induced rise of Ca\(^{2+}\) has been used to determine the relative contributions of different Ca\(^{2+}\) extrusion pathways.\(^7,8\) Although detubulation decreased the rate constant of decline (from 0.54 s\(^{-1}\) to 0.25 s\(^{-1}\)), it is difficult to compare the relative contribution of different efflux pathways in control and detubulated cells using this technique, because other factors may influence the rate of decline in detubulated cells. For example, diffusion distances to the cell membrane will be different and the t-tubules, which appear to reseal within the cell although uncoupled from the surface membrane, may play some role in intracellular Ca\(^{2+}\) cycling. However, Figure 2 shows that in detubulated cells, all of the Ca\(^{2+}\) efflux that occurs on application of caffeine is inhibited by barboxyosin, so that in the absence of t-tubules there appears to be no efflux via Na\(^+-\)Ca\(^{2+}\) exchange: all the efflux appears to be via the Ca\(^{2+}\) ATPase. This is in contrast to control cells, in which Na\(^+-\)Ca\(^{2+}\) exchange is the most important efflux pathway.\(^7,8\)

The Ca\(^{2+}\) extrusion pathways responsible for the decline of the caffeine-induced increase of Ca\(^{2+}\) in detubulated cells are not clear. In the absence of barboxyosin, the sarcoplasmal Ca\(^{2+}\) ATPase will extrude Ca\(^{2+}\) (and a continuing low rate of Ca\(^{2+}\) efflux may be difficult to detect). It is also possible that in both the absence and presence of barboxyosin, intracellular organelles such as the mitochondria may sequester Ca\(^{2+}\).\(^7,8\)

The location of the exchanger is important for the normal function of the cell because of its role as (1) a route for Ca\(^{2+}\) influx, which may act as a trigger for Ca\(^{2+}\) release from the SR, and load the SR with Ca\(^{2+}\); and (2) the main Ca\(^{2+}\) efflux pathway from ventricular myocytes.\(^7,8\) Concentration of Na\(^+-\)Ca\(^{2+}\) exchange activity in the t-tubule membrane of rat myocytes is germane to its trigger function, because Ca\(^{2+}\) influx via the exchanger will occur adjacent to the RyRs, which are concentrated at the t-tubules. However, it is not clear that the exchanger is located sufficiently close to the ryanodine receptors,\(^3\) or that the flux rate of Ca\(^{2+}\) through the exchanger is sufficient to make Ca\(^{2+}\) entry via this pathway an effective trigger under normal conditions.\(^2,2\) This observation could, however, explain why it has proved so difficult to measure an exchange current accompanying putative Na\(^+-\)Ca\(^{2+}\) exchange--generated Ca\(^{2+}\) release: a small \(I_{Na,Ca}\) flowing in the t-tubules might, under some conditions, be sufficient to cause release, because Ca\(^{2+}\) will be delivered close to the ryanodine receptor, but might be difficult to detect. The location of the exchanger may also enable Ca\(^{2+}\) influx via the exchanger to load the SR with Ca\(^{2+}\), because SR Ca\(^{2+}\)-ATPase is found adjacent to the t-tubules,\(^2\) so that Ca\(^{2+}\) entering the cell via the exchanger may be sequestered by these pumps.

The location of Na\(^+-\)Ca\(^{2+}\) exchange activity may also be important for its role as the main route for Ca\(^{2+}\) efflux. First, because it will enable a relatively rapid and synchronous removal of Ca\(^{2+}\) throughout the cell. Second, because the exchanger is located close to the site of SR Ca\(^{2+}\) release, Ca\(^{2+}\) efflux via the exchanger may be sensitive to modulation by Ca\(^{2+}\) released from the SR. Because Na\(^+-\)Ca\(^{2+}\) exchange is electrogenic, current carried by the exchanger, which influences action potential configuration, will also be concentrated in the t-tubules and modulated by the local environment.

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References


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