Abstract  We developed protocols with intact cultured neonatal rat myocytes to directly evaluate the function of the sarcoplasmic reticulum (SR) Ca-ATPase (or SERCA2), Na-Ca exchange (Na-CaX), and slow Ca transport systems (mitochondria and sarcolemmal Ca-ATPase). Spontaneously beating control cells were compared with cells cultured for 2 days in the presence of verapamil (verapamil-arrested cells, VA). Intracellular calcium (Ca) transients were measured by use of indo-1 during (1) spontaneous twitches, (2) contractions induced by rapid application of caffeine (CaC, with and without NaC), and (3) twitches induced by brief depolarizations with high [K+] solution (K-twitches). We also measured mRNA levels for the SR Ca-ATPase and Na-CaX in the same experimental preparations. The t_{1/2} for [Ca], decline when both the SR Ca uptake and Na-CaX were prevented was the same for control and VA cells (=20 seconds), indicating unaltered slow Ca transport systems. Similarily, there was no significant difference in the t_{1/2} of CaC when Na-CaX was the main mechanism responsible for [Ca], decline (t_{1/2}=1.5 seconds), indicating unaltered Na-CaX. Conversely, we found nearly a twofold increase in the rate of [Ca], decline during K-twitches (control t_{1/2}, 0.84±0.05 seconds; VA t_{1/2}, 0.48±0.06 second; P<.001), indicating an increase in SR Ca-pumping activity in VA cells. This was also reflected by a 56% increase in the peak [Ca], reached during CaC used to assess maximal SR Ca content (427±49 nmol/L in control versus 665±75 nmol/L in VA cells). In agreement with these functional effects, we found no change in Na-CaX mRNA levels but a marked upregulation of both the SERCA2 mRNA and protein levels in VA cells (to 166±10% and 164±20%, respectively). Thus, verapamil arrest induced an increase in SR Ca uptake (and SERCA2 expression) without affecting the Na-CaX activity (or expression) or the slow Ca transport systems. (Circ Res. 1994;74:991-997.)

Key Words  • SR Ca-ATPase  • Na-Ca exchange  • caffeine  • hypothyroidity  • Ca, transients  • rat

Neonatal rat cardiac myocytes maintained in primary culture have been used extensively as a simple model to study the mechanisms involved in cellular growth and contractile protein gene expression.1-5 Neonatal rat myocytes in culture have also been used extensively to study cellular 45Ca fluxes.6 This type of cell culture model also allows relatively long-term study of the direct effects of contraction on myocyte metabolism without the confounding effects that alterations in contractile state may have on the viability of the intact organ or animal. It has been shown, for instance, that contractile arrest (produced by either membrane depolarization or L-type Ca channel blockers) inhibits not only the growth of neonatal cardiac myocytes in culture but also the expression of myosin heavy chain (β-MHC) mRNA and protein.7 This is the opposite of changes in β-MHC that accompany hypertrophic responses in both cultured cardiac myocytes and the intact heart.2,8,9 In addition, downregulation of the sarcoplasmic reticulum (SR) Ca-pump (SERCA2) mRNA and Ca transport has been reported during cardiac hypertrophy.10 Verapamil, by limiting intracellular calcium (Ca) transients, might also be expected to produce changes in expression of Ca transport proteins. Thus, we sought to determine whether chronic verapamil treatment altered the function and expression of the SR Ca pump and Na-Ca exchange (Na-CaX) in neonatal myocytes in culture.

A limitation with measurements of mRNA levels for Ca transport proteins is that changes in message levels do not necessarily correspond to changes in protein levels or Ca transport characteristics. Thus, it is important to measure changes in Ca transport in a system that is as intact as possible. This can help to overcome questions about protein synthesis/degradation rates, cellular regulation, and prevailing ionic conditions.

Since Ca interacts with many components in the cell simultaneously, it can be difficult to draw conclusions about individual Ca transport systems in the intact cell. Conversely, studying Ca transport in fractionated cell systems alters the environment and regulation of these systems. Recently, methods have been developed to analyze the individual function of several Ca transport systems during relaxation in intact cardiac myocytes.11-14 In intact ventricular myocytes, intracellular calcium concentration ([Ca]i) decline is mainly due to SR Ca uptake via SR Ca-ATPase and Ca extrusion via Na-CaX. However, the balance differs, because the SR Ca pump is stronger in rat than in rabbit and the Na-CaX is stronger in rabbit than in rat.13,15 These two mechanisms are responsible for 98% of [Ca], decline and cell relaxation, the remaining 2% being attributed to the combined action of mitochondrial Ca uptake and the Ca extrusion via the sarcoclemmal Ca ATPase (denoted below as the slow Ca transport systems).

In this work we developed protocols to evaluate the function of individual Ca transport systems responsible for 45Ca uptake and Na-CaX in culture.
for [Ca\textsubscript{i}], decline in individual newborn rat cardiac myocytes (SR Ca-ATPase, Na-CaX, and slow Ca transport systems). We also compared the levels of Ca transport in the intact cell with the mRNA levels for SERCA2 and Na-CaX in the same experimental model (with and without verapamil arrest).

**Materials and Methods**

**Ventricular Dissociation and Cardiac Myocyte Isolation**

Neonatal ventricular myocytes were isolated from the hearts of 2-day-old rats by collagenase digestion as previously described.\textsuperscript{2} RELEASED cells were plated onto collagen-coated six-well plates (for protein assay), 100-mm dishes (for RNA isolation), or homemade cell chambers (for Ca\textsubscript{i} transient measurement) at a density of about 400 cells/mm\textsuperscript{2} in PC-1 medium and left undisturbed in a 5% CO\textsubscript{2} incubator for 14 to 18 hours. Unattached cells were then removed by aspiration, and spontaneously contracting cells were maintained in DME/F12/PC-1 (1:1:1) medium for 48 to 72 hours (control). In some cultures, contractile activity (visible within 24 hours after plating) was prevented by the addition of verapamil (10 \textmu M/L) to the medium. Cells from these cultures will be referred to as verapamil-arrested (VA) cells. Media were changed daily.

**mRNA Analysis**

Total cellular RNA isolation and Northern blot analysis was performed as previously described.\textsuperscript{16,17} The following cDNA probes were used: rat cardiac SERCA2 (kindly provided by Dr Wolfgang Dillmann, University of California, San Diego); guinea pig cardiac Na-CaX (a gift from Dr Kenneth D. Philipson, University of California, Los Angeles); and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH, clone pHcGAP, obtained from American Type Culture Collection, Rockville, Md.). The inserts were isolated and labeled by random primer extension with (\alpha-\textsuperscript{32}P)dCTP. Oligonucleotides for rat a-MHC and \beta-MHC 3'-untranslated regions obtained from Oncogene Science (Uniondale, NY) were end-labeled with (\gamma-\textsuperscript{32}P)ATP by use of T4 kinase. The hybridization signals of specific mRNAs were normalized to that of GAPDH mRNA to correct for differences in loading and/or transfer.

**Western Blotting**

Equal amounts of total cell protein from control and VA myocytes (7.5 to 10 \mu g) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose sheets by electroblotting. Blots were blocked for nonspecific protein binding with 2% bovine serum albumin (BSA) and 0.075% Tween 20 and incubated with rabbit anti-rat SERCA2 antisemir (1:1000 dilution) kindly provided by Drs Ronald Hartong and Wolfgang Dillmann, University of California, San Diego. Blots were re-blocked with 2% goat serum, 2% BSA, and 0.075% Tween 20 and then incubated with a biotin-conjugated goat anti-rabbit IgG. Streptavidin-conjugated horseradish peroxidase and 4-chloro-1-naphthol were used for color development. Band intensities were quantified by laser densitometry.

**Intracellular Calcium Measurements**

The instrumentation and procedure for these measurements are described in detail elsewhere.\textsuperscript{12,18} The cell chamber was placed on an inverted microscope (Nikon Diaphot) adapted for epifluorescence measurements and superfused with normal Tyrode's solution (NT) for 30 minutes to wash out the culture medium. The cells were incubated with indo-1 AM (10 \mu M/L, Molecular Probes Inc, Eugene, Ore) for 15 minutes at room temperature and then perfused for at least 30 minutes to allow the washout of the extracellular dye and intracellular indo-1 deesterification.

During fluorescence experiments, excitation wavelength was 365 nm. The illumination field was a circular spot of approximately 30 \textmu m diameter. The emission field was restricted by a square window containing only the cell under study. After appropriate background subtraction (see below), the ratio of fluorescence emission at 405:485 was used to calculate [Ca\textsubscript{i}]. Calibration and conversion of the fluorescence to [Ca\textsubscript{i}] were performed as previously described,\textsuperscript{12} according to the equation [Ca\textsubscript{i}]=K_0[R-R_{\text{min}}]/(R_{\text{max}}-R), assuming a dissociation constant (K_0) of 250 nmol/L.\textsuperscript{19} The average in vivo values of R_{\text{max}} and R_{\text{min}} obtained in neonatal rat myocytes were 0.011±0.003 and 1.02±0.04 (n=6), respectively.

Background fluorescence was obtained from the same emission field as used to record the cell fluorescence after the cells were wiped off the chamber and the bath autofluorescence was measured for that particular microscope field. In some experiments we also estimated the [Ca\textsubscript{i}] values by using the cell autofluorescence (measuring before indo-1 loading for a particular cell under study) as background. The values obtained were not significantly different from those obtained by use of the bath autofluorescence.

**Solutions**

The NT solution had the following composition (mmol/L): NaCl 140, KCl 6, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, glucose 10, and HEPES 5. In the 0Ca solutions, CaCl\textsubscript{2} was omitted and 1 mmol/L EGTA was added. In the 0Na solutions, NaCl was replaced by choline chloride. In the 0Na,2Ca depolarization solution (HI-K+Ca), 24 mmol/L choline chloride was replaced by equimolar KCl (final KCl concentration of 30 mmol/L). Atropine (0.1 \mu M/L) was added to block muscarinic cholinergic receptors. Solutions containing no Ca were also prepared with 10 mmol/L caffeine. The pH of all solutions was adjusted to 7.4 at 22°C.

**Experimental Procedure**

Calcium transients were recorded during periods of 100 seconds from randomly chosen cells to evaluate Ca levels and spontaneous frequency. Fast and maintained caffeine application (using a fast solution switcher)\textsuperscript{19} was used to produce SR Ca release and to inhibit net accumulation of Ca in the SR. The relative contribution of the Na-CaX to the regulation of [Ca\textsubscript{i}] was estimated by comparison of the t\textsubscript{1/2} for [Ca\textsubscript{i}] decay of caffeine-induced Ca\textsubscript{i} transients in the presence and in the absence of Na (in 0Ca solutions). The SR contribution was evaluated by measuring the steady-state values of t\textsubscript{1/2} for [Ca\textsubscript{i}] decline during twitches induced by cell depolarization (K-twitch), which were obtained by brief (100-millisecond) application of the HI-K+Ca solution during perfusion with 0Na,0Ca. The reciprocals of these t\textsubscript{1/2} values from K-twitches and caffeine application with 140 mmol/L, Na\textsubscript{w} were used as indices of the rate of [Ca\textsubscript{i}] decline attributable to the SR (R\textsubscript{Na}) and the Na-CaX (R\textsubscript{NaCaX}), respectively. True rate constants were not used because in some cases (as in the K-twitches of VA cells), [Ca\textsubscript{i}] decline could not be well fit by a monoeponential function.

To evaluate the SR Ca reloading process, we first depleted the SR Ca content by fast caffeine application in the presence of Na. Then a variable number of K-twitches in the absence of Na\textsubscript{w} were evoked to reload the SR with Ca (with the number determined by pilot experiments). Thereafter, the SR Ca content was assessed by measurement of the peak of a Ca\textsubscript{i} transient induced by fast application of 0Na,0Ca caffeine solution.\textsuperscript{12} Values of the time to peak [Ca\textsubscript{i}] were also obtained and compared between the two groups of cells. All the procedures described above were applied to both control and VA cells. Further details are provided in the “Results” section.

**Statistical Analysis**

Data are presented as mean±SEM. Two-way analysis of variance was used for data comparison for Ca\textsubscript{i} transients. The Student-Newman-Keuls test was used for post hoc multiple
SERCA2 4.2 kb
NaCaX 7.0 kb
α-MHC 7.2 kb
β-MHC 7.2 kb
GAPDH 1.4 kb

Fig 1. Northern blots for α- and β-myosin heavy chain (MHC) isoforms, SERCA2, and Na-Ca exchange (Na-CaX). Data were obtained both from control (spontaneously contracting) and VA cells. Intensities were normalized to constitutively expressed GAPDH mRNA.

Comparisons. Paired Student's t test was used for analysis of Northern and Western blots. Values of P<.05 were considered statistically significant.

Results

Fig 1 shows examples of Northern blots for MHC isoforms, SERCA2, and Na-CaX (where intensities were subsequently normalized to constitutively expressed GAPDH mRNA). On the average, α-MHC message was increased to 200±24% of control, whereas β-MHC message was reduced to 9.9±2.9% of control (n=6) in VA versus control cells. These results are similar to those previously described and emphasize a switch from β- to α-MHC induced by VA. SERCA2 mRNA was also significantly increased to 166±10% of control. This increase in SERCA2 message was accompanied by a significant increase in Ca-pump protein estimated from densitometric scans of Western blots (164±20% of control, n=6 for both). Conversely, there was no change in Na-CaX expression (97±10% of control, n=6). To measure Ca transport, cells from these cultures were studied with indo-1 fluorescence microscopy.

The cells were firmly attached to the bath chamber in spiderlike clusters in which some cells were clearly accessible for fluorescence measurements. About 90% of the cells in a given microscope field exhibited spontaneous contractions in both the control and VA groups, the latter resuming spontaneous contractions in NT solution about 2 minutes after washout of the culture medium and verapamil. The average spontaneous frequency was not significantly different between the two groups (0.301±0.060 and 0.303±0.037 Hz for control and VA, respectively). When spontaneous activity was suppressed in 0Na,0Ca solution, resting [Ca], was not significantly different in control (143±9 nmol/L) versus VA cells (163±12 nmol/L).

Fig 2 illustrates the experimental protocol used for the fluorescence measurements. After some Ca, transients were acquired from a spontaneously active cell, the bath solution was switched to the 0Na,0Ca solution. This intervention stopped the spontaneous contractions within 3 to 5 seconds. After 10 to 15 seconds in 0Na,0Ca solution, caffeine in 140Na,0Ca solution was applied and maintained for about 35 seconds. Typically, the amplitude of the caffeine-induced Ca, transient was about 20% to 30% greater than that of the steady-state spontaneous twitches. The first caffeine application (in the presence of Na,) was sufficient to deplete the SR Ca content as tested in preliminary experiments by a second caffeine application (not shown). This result is in agreement with our previous observations in adult rabbit and rat myocytes.12,13 After the first caffeine application, 0Na,0Ca solution was reintroduced for 15 seconds (first interruption of trace in Fig 2), allowing complete washout of Na from the bath. With the SR depleted of Ca, a variable number (1, 2, 4, or 8) of K-twitches at 0.15 Hz were evoked in the same cell (see a sequence of 8 K-twitches in Fig 2). The amplitude of the K-twitches increased monotonically with the number of depolarizations, reaching a steady-state plateau value after about 4 to 8 contractions. For the cell in Fig 2, the eighth K-twitch was 98% of the maximal value. Further K-twitches in this Na-free Ca-containing solution typically caused an increase in the resting [Ca], and spontaneous [Ca], oscillations. We assumed that the plateau level corresponds to the maximum SR Ca content.

At the end of the 1-, 2-, 4-, and 8-K-twitch sequence, the cells were superfused for 15 seconds with 0Na,0Ca solution (second interruption of trace in Fig 2); then the 0Na,0Ca caffeine solution was applied to evaluate the SR Ca content in the absence of Na-CaX. The last K-twitch in a given sequence that produced the maximum caffeine-induced Ca transient amplitude was used to study the SR Ca uptake (see below).

As shown in Fig 2, there is a striking difference in the time for [Ca], decline between the two caffeine-induced Ca transients, the first in the presence and the second in the absence of Na. As observed in adult rat and rabbit cardiac myocytes,12 [Ca], still declines even in the absence of Na and Ca. This decay has been attributed to the combined effect of the mitochondrial Ca uptake and the Ca extrusion via sarcolemmal Ca pump.12 The same
protocol as in Fig 2 was applied to 6 to 10 cells from both control and VA groups. A quantitative analysis of the role of the Na-CaX and SR Ca uptake on the [Ca], regulation in cultured neonatal rat myocytes, as well as the correlation between these data and the molecular data, will be presented in the next sections.

**Role of the Na-CaX and Slow Systems**

Fig 3 illustrates caffeine-induced Ca, transients obtained in cultured neonatal rat ventricular myocytes from both control and VA groups in the absence and in the presence of extracellular Na. When the Na-CaX was inhibited in this manner (Fig 3A), the t$_{1/2}$ for [Ca] decline was 21.5±1.6 seconds (n=8) and 19.5±1.1 seconds (n=9) for control and VA groups, respectively. These values were not statistically different, which indicates that the combined contribution of the slow mechanisms of Ca, removal from the cytosol, namely the sarcolemmal Ca-ATPase and the mitochondrial Ca uptake, seems to be approximately the same in both groups of cells.

When Na was present (Fig 3B), the t$_{1/2}$ for [Ca] decline during contracture was markedly lower (control, 1.67±0.22 seconds; VA, 1.47±0.11 seconds; n=6) than that obtained in Na-free solution. This indicates that in our experimental conditions a very active Na-CaX is present, making the decline of Ca, transients during caffeine application about 13 times faster in the presence of Na than in the absence of Na. The t$_{1/2}$ of [Ca] decline attributable to the Na-CaX, however, was not significantly different between the two groups of cells (control and VA).

**Role of the SR Ca Uptake**

Fig 4 illustrates the time course of individual steady-state K-twitches (Fig 4A) and the K-twitch–dependent SR Ca accumulation (Fig 4B) for both control and VA cells. As in our previous observations in adult rabbit myocytes, about 4 to 8 K-twitches were sufficient to reload the SR to its maximum level of Ca (as tested by a 0Na,0Ca caffeine application; see Fig 2). During a K-twitch, the SR is expected to be the main mechanism responsible for [Ca] decline, since Ca extrusion via Na-CaX is inhibited. Cells pretreated with verapamil displayed a marked decrease in t$_{1/2}$ for [Ca] decline compared with nontreated cells (control, 0.843±0.046 second; VA, 0.483±0.056 second; n=6; P<.001; see Fig 4A). Additionally, compared with the control group, cells pretreated with verapamil had shorter time to peak (control, 0.788±0.06 second; VA, 0.392±0.04 second; n=6; P<.001). These results show that the SR Ca uptake was enhanced in VA cells, curtailing the peak and accelerating the decay of the [Ca], transients.

Neither the number of K-twitches necessary to reload the SR nor the fractional SR Ca reloading for a given number of twitches was different between control and VA cells (Fig 4B). However, the maximum levels of [Ca], reached during caffeine application in 0Na,0Ca solution were significantly higher in VA cells (control, 427±49 nmol/L; n=8; VA, 665±75 nmol/L; n=9; P<.05). The higher Ca accumulation in verapamil-treated cells could be due to a more powerful SR Ca uptake.

**Gene Expression and Functional Changes**

Fig 5 shows the effect of verapamil treatment on mRNA levels and Ca transport rates in cultured neonatal rat ventricular myocytes. To account for variation in gel loading and exposure conditions, the intensity of the mRNA band of the specific gene was first normalized to the intensity of the GAPDH band. The relative intensity was then calculated as a percentage of the normalized value in the contracting cells (control). Data are mean±SEM of six individual experiments (each experiment generated from a different cell isolation). VA markedly increased mRNA (and protein) levels of SERCA2 and the relative rate of [Ca] decline produced by the SR Ca pump (R$_{SR}$; control, 1.20±0.065 second$^{-1}$; VA, 2.64±0.278 second$^{-1}$; P<.05). However, it did not change the mRNA levels of Na-CaX exchange gene or the rate of [Ca] decline attributable to Na-CaX (R$_{Na-CaX}$; control, 0.642±0.072 second$^{-1}$; VA, 0.695±0.051 second$^{-1}$).

Fig 4. Time course of individual K-twitches (A) and K-twitch–dependent sarcoplasmic reticulum (SR) Ca accumulation (B) for both control and verapamil-arrested cells from experiments like that in Fig 2. The bar in A indicates the duration of exposure to 30 mmol/L K, 2 mmol/L Ca solution. The pooled data (n=6) in panel B show the number of K-twitches required to refill the SR to the steady-state level in 0Na,0Ca solution.
Changes in mRNA and Ca-Transport Induced by Verapamil Arrest in Neonatal Rat Myocytes

![Graph showing changes in mRNA and Ca-Transport](image)

**Fig 5.** Effect of verapamil arrest (Verap) on mRNA and Ca transport rates in cultured neonatal rat ventricular myocytes. Message levels are normalized to GAPDH mRNA and plotted as a percent of the value in control cells. The indices of [Ca], decline rate via sarcoplasmic reticulum Ca pump and Na-Ca exchange action (RNaCaX and RNaCaX respectively) are the inverse of the t1/2 values for the last K-twitch and a CaFIC in 140Na, respectively.

**Discussion**

In the present work we showed that VA induces changes in Ca transport during cell relaxation in cultured neonatal rat cardiac myocytes. The SR Ca uptake was markedly increased by VA, but the [Ca], extrusion by Na-CaX was not affected. These data were paralleled by an upregulation of the SERCA2 gene expression at both mRNA and protein levels and no change in the Na-CaX.

**Assumptions and Limitations**

Since the neonatal rat myocytes in culture exhibit spontaneous contractile activity, we had to design new experimental protocols to control contractile frequency and measure Ca transients during contractions when different Ca transport systems were specifically inhibited. Twitches induced by high [K], depolarizations in the absence of Na, (choline-substituted, Ca-containing solution) were used to activate contraction without Ca extrusion via Na-CaX. Caffeine, in the presence or absence of Na, was used to activate Ca release, whereas SR Ca accumulation was prevented.

In previous work we used electrical stimulation to activate a single twitch in 0Na (Li-substituted) solutions. This method could not be applied here, because the neonatal rat cells undergo spontaneous activity in the presence of Li. Conversely, because the neonatal rat myocytes were firmly attached to the perfusion chamber, we were able to use high flow velocity in our superfusion system, allowing solution change in the vicinity of a given cell within 50 to 100 milliseconds and consequently fast application of the depolarizing (high-K+Ca) solution.

One of the potential problems in comparing twitch-induced by cell depolarization with caffeine-induced Ca, transients resides in the voltage-dependence of the Na-CaX activity. However, it has been shown previously in adult rat and rat myocytes that relaxation and Ca decline during a twitch is not significantly affected by voltage, probably because the relaxation (and most of the [Ca], decline) occurs late during cell activation, when the action potential is already repolarized.

It is interesting that it took more K-twitches to reach a steady state (often 8 to 10) than the number required to refill the SR (=4, see Fig 2 versus Fig 4B). This is similar to results in adult rabbit and guinea pig ventricle. This may be due to a small increase in baseline [Ca], an increase in the fraction of SR Ca released, an increase of Ca influx, or even saturation of some slow intracellular Ca buffer or compartment.

It should also be noted that the effects of verapamil arrest on these cultured myocytes do not necessarily provide information about developmental changes or effects of culturing per se. Rather, our results only provide information about how Ca handling is altered by verapamil arrest in this cultured myocyte model.

**Slow Transport Systems, Na-CaX, and SR Ca Uptake in Control Neonatal Rat Ventricular Myocytes**

In the present study [Ca], still declined, albeit slowly (t1/2 =20 seconds), to the resting levels during caffeine-induced Ca, transients in 0Na,0Ca solutions. A combined participation of mitochondrial Ca uptake and Ca extrusion by the sarcolemmal Ca pump could explain the slow Ca, decay in the absence of both SR Ca uptake and Ca extrusion via Na-CaX (as in adult rabbit and rat myocytes).12,13,23

We have already compared t1/2 for Ca, decline during caffeine-induced Ca, transients in the presence and in the absence of Na, in adult rat and rabbit myocytes.12,13 This kind of comparison allows evaluation of the role of the Na-CaX and other mechanisms (eg, mitochondrial Ca uptake and Ca extrusion via sarcolemmal Ca-ATPase) in conditions in which the net accumulation of Ca by the SR is inhibited.12 The Na-CaX had a marked impact on the decay of [Ca], during caffeine-induced Ca, transients in the presence of Na,. We found a 13-fold reduction (from 21.1±2.0 to 1.67±0.22 seconds) in t1/2 for Ca, decline when Na was present in the external medium. This is higher than the change found in adult rat ventricular myocytes (about sevenfold), in agreement with the finding that the Na-Ca exchanger is more abundant during late fetal and early newborn development both in rabbit and rat myocytes.24-26 This also confirms that the Na-CaX is much stronger than the slow systems, such that the t1/2 for [Ca], decline during caffeine application (and RNaCaX) is a reasonable reflection of the Ca transport rate attributable to Na-CaX alone.

During a K-twitch the main mechanism responsible for cell relaxation and [Ca], decline is the SR Ca uptake. In this case the Na-CaX is inhibited and the other mechanisms (mitochondrial uniporter and sarcolemmal Ca pump) are much too slow to compete with the SR Ca ATPase in removing Ca from the cytosol.13 In the present experiments the SR contribution to [Ca], decline was even greater than the contribution of the Na-CaX (see Fig 1, time course of K-twitch versus time course of the caffeine-induced contracture in 140Na solution). In control cells the SR Ca transport was ~25-fold faster than the slow systems. That is a smaller factor than in adult cells, in which the SR is ~64-fold faster than the slow systems.13 This would be consistent with increased SR Ca uptake in adult rat cells.

The ratio of RNaCaX to RNaCaX in control cells was 1.9. This suggests that in these neonatal rat myocytes the SR transports Ca only about twice as fast as the Na-CaX. That is more like adult rabbit than adult rat myocytes, in
which similar ratio values are 1.7 and 6.8, respectively. Thus, the relative contribution of the Na-CaX is higher in neonatal compared with adult rat myocytes, such that about one third of the Ca transport is via Na-CaX and two thirds via the SR Ca pump. The increase in importance of the SR Ca pump and decrease of the Na-CaX during development in rat is consistent with other results.

Functional and Molecular Changes and Ca Transport Systems Induced by VA in Neonatal Rat Myocytes

In our experimental conditions VA did not significantly change the rate of cellular Ca removal via Na-CaX as evaluated by comparing the rates of Ca, decline during caffeine-induced contractures in 140Na,0Ca solutions (Fig 3B). These data paralleled the mRNA levels for the Na-CaX measured in the same biological preparation (Fig 5). The simplest interpretation of this result is that there is no change in Na-CaX gene expression or regulation in VA cells (rather than a change in expression or number of molecules along with an offsetting regulatory change). Since Na-CaX is crucial in setting resting [Ca], for the cell, this result is also consistent with there being no significant change in resting [Ca] levels.

Verapamil arrest, on the other hand, induced a marked increase (see Fig 5) in SR Ca uptake rate evaluated by measuring the \([\text{Ca}^2+]_p\) for Ca, decline during K-twitches. Simultaneously, in the same experimental model, we found an upregulation of SERCA2 expression at both mRNA and protein levels. Again, the simplest interpretation of this result is that there is an increase in the number of SR Ca pumps, with concomitant increase in Ca transport by the SR Ca pump.

An increased SR Ca pump rate might also explain the decreased time to peak [Ca], since the faster SR Ca pump would balance Ca entering the cytoplasm sooner and curtail the peak of the Ca, transient sooner. Simple exponential modeling of Ca uptake and release suggests that slowing of the Ca pump alone (or with a higher amount of Ca released) may not be sufficient to explain the decreased time to peak [Ca] in VA cells. It is possible that SR Ca release is also “faster” in VA cells (although the time to peak [Ca] during caffeine application is not changed). In this scenario the Ca current trigger could be larger in VA cells. Although we have no direct support for this possibility, it might also explain why the larger SR Ca store in VA cells is refilled by the same number of K-twitches (although caffeine-induced Ca, transients have the same time to peak). A possible explanation is a larger Ca influx signal triggering faster SR Ca release. This could also explain why the SR refills with Ca in the same number of K-twitches in VA cells despite a higher Ca load than control cells. This possibility remains to be tested.

The maximum SR Ca content was also increased by ~50% in VA cells. This could also result from an increase in the SR Ca pump. Presumably the SR Ca content is established by a pump-leak balance. If the SR Ca pump is increased without a change in Ca leak, then an increase in Ca content is expected. If this interpretation is correct, it emphasizes that the SR Ca content is determined by the pump-leak balance rather than reaching the thermodynamic limiting [Ca] gradient of the SR Ca-ATPase. The latter could, however, be the case if there were a concomitant increase in SR volume or calsequerin (the intra-SR Ca buffer).

In the rat, myocardial hypertrophy is associated with a decrease in SERCA2 expression and the ratio of \(\alpha\)-MHC/\(\beta\)-MHC. There is also much interest in the possibility of a simple program of coordinate expression of proteins involved in contraction and Ca regulation. Although we find that VA increases both SERCA2 and \(\alpha\)-MHC/\(\beta\)-MHC, we do not see a significant change in Na-CaX message or function. Thus, if there is a “program” of coordinate gene expression in these cells and our experimental conditions in response to VA, the Na-Ca exchanger does not seem to be involved.

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Contractile arrest increases sarcoplasmic reticulum calcium uptake and SERCA2 gene expression in cultured neonatal rat heart cells.

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