Ca flux, contractility, and excitation-contraction coupling in hypertrophic rat ventricular myocytes

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McCall, Eileen, Kenneth S. Ginsburg, Rosana A. Bassani, Thomas R. Shannon, Ming Qi, Allen M. Samarel, and Donald M. Bers. Ca flux, contractility, and excitation-contraction coupling in hypertrophic rat ventricular myocytes. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1348–H1360, 1998.—Left ventricular hypertrophy (~40%) was induced in rats by banding of the abdominal aorta. After 16 wk, ventricular homogenates were prepared for biochemical measurements and ventricular myocytes were isolated for functional studies. In myocytes, the effects of banding on intracellular Ca handling, contraction, and excitation-contraction (E-C) coupling were determined using indo 1 fluorescence and whole cell voltage clamp. After steady-state field or voltage-clamp stimulation to load the sarcoplasmic reticulum (SR), SR Ca content assessed by caffeine-induced Ca transients was the same in sham and banded groups. Despite this, cell shortening amplitudes were significantly depressed in the banded group, suggesting altered contractile properties. In banded rats, the SR Ca-adenosinetriphosphatase (Ca-ATPase) mRNA level was reduced, as was homogenate thapsigargin-sensitive SR Ca-ATPase, but cytosolic Ca-ATPase (Ca-ATPase) mRNA level was reduced, as was homogenate Ca-ATPase. In banded rats, the SR Ca-adenosinetriphosphatase (Ca-ATPase) mRNA level was reduced, as was homogenate thapsigargin-sensitive SR Ca-ATPase, but cytosolic free Ca concentration ([Ca]i) decline attributed to SR Ca-ATPase activity in intact cells was not slowed. Banding also reduced Na/Ca exchange mRNA level but did not affect either Na-dependent sarcoplasmal 45Ca transport in homogenate or the rate of [Ca]i decline in intact cells attributed to Na/Ca exchange (during caffeine-induced contractures). Banding also did not change the rate of [Ca]i decline mediated by the combined function of the mitochondrial Ca uptake and sarcoplasmal Ca-ATPase in intact cells. Ca current (Ica) density and voltage dependence were the same in sham and banded groups. Ryanodine receptor mRNA, protein content, and ryanodine affinity were also unchanged in the banded group. At 1 mM extracellular Ca concentration ([Ca]o), banding did not affect E-C coupling efficacy in intact cells under voltage clamp (i.e., same contraction for given Ica and SR Ca load). However, when [Ca]o was reduced to 0.5 mM, the efficacy of E-C coupling was greatly depressed in the banded group (even though Ica and SR Ca content were matched). In summary, unloaded myocyte contraction was depressed in these hypertrophic hearts, but Ca transport was little altered, at 1 mM [Ca]o. However, reduction of [Ca]o, to 0.5 mM appears to unmask a depressed fractional SR Ca release in response to a given Ica trigger and SR Ca load.

cardiac muscle; sarcoplasmic reticulum; calcium current

CARDIAC HYPERTROPHY is an initial adaptive response to several types of cardiovascular stress and can precede the decompensatory phase of heart failure (42). Many different animal models have been developed and studied to determine the responses of cardiomyocytes to chronic insults (see Refs. 3 and 25 for reviews). Cardiac hypertrophy induced by pressure overload in response to aortic constriction in rats for various periods has yielded much information about Ca and myofilament changes (e.g., Refs. 2, 4, 15, 17, 20, 29, 30, 34, 40, 41, 46, 51). Nevertheless, hypertrophic responses of individual cells are still far from clearly understood.

Studies of pressure-overload hypertrophy, the model used here, have concentrated on how and whether Ca homeostasis and cellular function are altered. In the rat pressure-overload model, there is a switch from the fast isoform of myosin heavy chain (α-MHC) to the slow isoform (β-MHC) (19), but in skinned fibers, no alteration in myofilament Ca sensitivity has been shown (30, 37). However, reports of other changes in Ca homeostasis during the progression of hypertrophy are controversial and conflicting. Most studies have reported that individual contractions in hypertrophy are both reduced in amplitude and prolonged in time (e.g., 35, 52). A slower relaxation in hypertrophy may be due to prolongation of the Ca transient by reduced sarcoplasmic reticulum (SR) Ca uptake (3, 17, 20, 30, 32, 34, 51). There has also been speculation that downregulation of the SR Ca-adenosinetriphosphatase (Ca-ATPase) is responsible for the transition from compensated cardiac hypertrophy to decompensated heart failure. Reduced Na/Ca exchange and sarcoplasmal Ca-ATPase activities have been measured in hypertrophy (1, 24), but in hypertrophic rat and failing human heart tissue, expression of the Na/Ca exchanger has been reported to increase (21, 40, 49).

Although there is general agreement on some findings, a wide array of models have been used to study various different aspects of hypertrophy and heart failure. In some cases, authors have examined changes in a rather limited number of specific biochemical markers, without much data from intact cells. Using a well-developed rat abdominal aortic banding model (18, 19, 43), we here combine expression studies in homogenates with functional studies in isolated ventricular myocytes, in an attempt to characterize cellular Ca regulation in cardiac hypertrophy more comprehensively.

To examine how chronic pressure overload-induced hypertrophy alters contractility, Ca fluxes during relaxation, and excitation-contraction (E-C) coupling in isolated cardiac myocytes, we recorded contractions as well as cellular Ca transients using indo 1 fluorescence. In field-stimulated myocytes, we assessed the contributions of the four mechanisms responsible for removing Ca from the cytosol during cytosolic free Ca concentration ([Ca]i) decline and relaxation (i.e., the SR Ca-ATPase, Na/Ca exchange, mitochondrial Ca uniporter, and sarcoplasmal Ca-ATPase; Refs. 5 and 8). In myocytes under voltage clamp, we examined the relation-
ship between Ca current (I_{Ca}) and contraction while SR Ca content (assessed by caffeine-induced contractures) was controlled. In cells from banded rats we found little change in the competition among Ca transport systems during relaxation (despite reduced levels of SR Ca-ATPase activity in homogenates). We also found reduced cell shortening for a comparable Ca transient, no change in SR Ca content, and a reduced efficacy of E-C coupling, which was only apparent at 0.5 mM extracellular Ca concentration ([Ca]_o). Some of this work has appeared in abstract form (39).

**MATERIALS AND METHODS**

Induction of pressure-overload hypertrophy. The banding procedure was as described previously (19). Sprague-Dawley rats (175- to 200-g male; Harlan Sprague Dawley, Indianapolis, IN) were anesthetized by ketamine (60–90 mg/kg im) and xylazine (1–2 mg/kg im). The aorta was dissected and a suprarenal abdominal constriction was applied (closure equivalent to 25-gauge needle) using a hemodip. Sham-operated animals underwent the same surgical procedure without hemodip placement.

Fifteen to eighteen weeks later, using the same anesthetic conditions, systemic blood pressure was recorded using a micromanometer-tipped catheter (3-Fr, Teflon, Gaeltec) at the aortic arch. The catheter was pushed through the aortic valve into the left ventricle to measure end-diastolic pressure, when possible. The thorax was then opened, and the heart was removed, weighed, and used to obtain isolated cardiac myocytes.

Myocyte isolation. After the hemodynamic measurements, myocytes were isolated from the left ventricular free wall as previously described (e.g., Refs. 8 and 18). The heart was excised, mounted on a Langendorff perfusion apparatus, and perfused with nominally Ca-free, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered Tyrode solution containing 1.5–2 mg/ml collagenase (type I, Worthington) until it started to become flaccid (~10 min), after which the left ventricular free wall tissue was separated, transferred to a flask containing fresh enzyme, and incubated for a further 10–20 min. Free cells were separated, and the remaining undissociated tissue was reincubated (2 or 3 times if necessary) until most of the tissue had been dissociated. The resultant cell suspension was rinsed several times, with [Ca]_o gradually increased to 1 mM. Myocytes were then plated onto laminin-pretreated glass-bottomed Plexiglas superfusion chambers.

Measurement of Ca transients and cell shortening. In field stimulation experiments, myocytes were loaded with membrane-permeant indo 1-acetoxymethyl ester by a 20-min incubation at 22°C (concentration of indo 1 = 10 µM) followed by washing for 30 min to allow deesterification. Cells from both banded and sham animals were loaded similarly using the same technique, and this degree of loading does not affect contraction parameters significantly (5). Ca-dependent fluorescence was recorded using a microscope-based fluorescence system (Photon Technology International, Monmouth Junction, NJ). Fluorescence emitted at 405 (F_{405}) and 485 nm (F_{485}) was recorded from a field restricted to one cell (excitation at 365 nm). Autofluorescent backgrounds, measured on comparably sized cells from the same heart, were subtracted from each signal before the ratio F_{405}/F_{485} was obtained. This ratio (R) was converted to [Ca], using minimum and maximum R values and the apparent dissociation constant (K_d) which was determined in separate calibration runs (6). System background fluorescence was negligible.

In all intact myocyte studies, shortening was measured as previously described (8) using a video-edge detection system (Crescent Electronics, Sandy, UT) and stored using pCLAMP software (Axon Instruments, Burlingame, CA) as well as on videotape for off-line analysis. In most cells in which [Ca] was measured, shortening was recorded simultaneously.

Field stimulation solutions and protocols. Myocytes were continuously superfused with Tyrode solution at 22°C and a flow rate of 2–5 ml/min. The basic (normal) Tyrode solution (NT) contained (in mM) 140 NaCl, 10 glucose, 5 HEPES, 6 KCl, 1 MgCl_2, and 2 CaCl_2, adjusted to pH 7.4 at 22°C with NaOH. Steady-state twitch Ca transients were evoked by field stimulation at 0.5 Hz with platinum electrodes.

Caffeine-induced contractures and Ca transients were activated by rapid application of 10 mM caffeine in either NT solution or Na- and Ca-free solution (0 Na-0 Ca solution; NT with Li replacing Na and 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N’-tetraacetic acid (EGTA) replacing Ca). The amplitude of these contractures and Ca transients is an index of SR Ca content (8, 33, 48). Because continuous application of caffeine prevents net SR Ca reuptake, the rate of [Ca] decline and relaxation during caffeine-induced contracture provides information about Ca extrusion by Na/Ca exchange (when in NT) or the combined action of the mitochondrial Ca uniport and sarcoplasmic Ca-ATPase (when in 0 Na-0 Ca solution; Refs. 5 and 8). In measuring caffeine-induced contractures, we stopped steady-state stimulation for 5 s before caffeine solutions were introduced via a quick-switching device (8). For the caffeine-induced contracture in 0 Na-0 Ca solution, the solution was first switched to 0 Na-0 Ca for 10 s (to remove residual Ca) before caffeine application.

In some experiments single twitches were evoked and [Ca] decline was followed in the absence of Na/Ca exchange. In this case 0.5-Hz stimulation was stopped, cells were predepleted of Na for 8–10 min in 0 Na-0 Ca solution, and then Ca was reintroduced in a Na-free NT (Li substituted) for the test twitch with Na/Ca exchange blocked (5). In other experiments twitches were recorded after maximal SR Ca loading. Here, stimulation frequency was increased from 0.5 to 5.0 Hz for 10 s (in NT) before a single twitch was evoked.

Analysis of contributions of Ca transport systems. During twitch relaxation, all of the transport systems removing Ca from the cytosol operate simultaneously and are interdependent because of their common influence and dependence on [Ca]. We analyzed separately the [Ca] dependence of Ca transport by these systems to further evaluate their relative contributions to Ca removal during twitch relaxation as described by Bassani et al. (5). This analysis uses Ca transient amplitudes (∆[Ca]), diastolic [Ca], and time constants (τ) of [Ca] decline during twitches, as well as during caffeine-induced contractures in both NT and 0 Na-0 Ca. The analysis was performed for each group (sham or banded) using the mean values of these parameters for that group (see Table 3).

The [Ca] decline during the caffeine-induced contracture in 0 Na-0 Ca was first converted to total Ca using known passive cytosolic and indo 1 binding constants from rabbit ventricular myocytes (26) and then differentiated with respect to time. The dependence of this d[Ca]_total/dt on [Ca] was fit to a Hill equation, $V_{max}/(1 + K_d[Ca])^n_H$, where d[Ca]_total/dt is the first derivative of total Ca concentration with respect to time, $V_{max}$ is the maximum Ca transport velocity, $K_d$ is apparent affinity, and $n_H$ is the Hill coefficient. This fit describes empirically how the lumped slow Ca removal systems (mitochondrial Ca uniport and sarcoplasmic Ca-ATPase) depend on [Ca]. To determine how Na/Ca exchange flux depends on [Ca], we similarly fit the caffeine-NT tran-
sient data to a two-term Hill equation (as this transient is governed by both the slow removal processes and Na/Ca exchange), with one term constrained to use the parameters just estimated from the caffeine-induced contracture in 0 Na-0 Ca. Next, the twitch d[Ca]i/dt was fit to a three-term Hill equation, with the first two terms being constrained by the slow process and Na/Ca exchange removal models as determined above. This fit predicts how SR reuptake flux depends on [Ca]. Finally, using the mean twitch [Ca], parameters (diastolic [Ca], δ[Ca], and τ of [Ca] decline) as the driving function, we calculated the flux due to each removal process with all operating simultaneously. The resulting fluxes were then integrated numerically to get cumulative Ca transport. This provides information about how these systems interact independently but simultaneously with [Ca].

Determination of Ica-contraction relationship. In these experiments cell shortening and Ica were measured simultaneously during test pulses to different membrane potentials (E_m). To ensure comparable SR Ca loading, the test pulses were preceded by a conditioning train (38). Ica was measured in whole cell voltage clamp, using an Axopatch 1B patch-clamp amplifier (Axon Instruments, Burlingame, CA) and patch electrodes with 1- to 3-MΩ resistances (glass type 1B150F-6, World Precision Instruments, Sarasota, FL) containing (in mM) 140 CsCl, 5 MgATP, 5 HEPES, and 50 µM oxalate, 1.87 mM MgCl2, 5 mM EGTA, and 5.14 mM CaCl2, which contained 140 mM KCl and 10 mM HEPES (pH 7.2). The solution was put into 5 ml cold KCl buffer (see below) with protease inhibitors (75 mM aprotonin and 1 µM leupeptin) and homogenized by three 30-s bursts at 67% maximum setting with a Polytron (Brinkmann Instruments, Westbury, NY). Aliquots were used as homogenate for analysis of Na/Ca exchange activity.

Na/Ca exchange activity was assessed in ventricular homogenates using a modification of the method of Reeves and Sutko (45). Homogenate aliquots were diluted 1:2 into a buffer solution (140 mM NaCl, 10 mM HEPES, pH 7.2) to load the vesicles passively with Na (final concentrations 46.7 mM KCl, 93.3 mM NaCl) and then treated with either 20 µM digitonin (to selectively permeabilize the sarcolemma and prevent Na/Ca exchange) or 0.2% ethanol (vehicle). The Na-loaded homogenate (3 µl) was added to 97 µl of a solution containing 25 µM 45CaCl2, 10 mM HEPES (pH 7.2), and either 140 mM KCl (Na free) or 46.7 mM KCl-93.3 mM NaCl (control incubate without Na gradient). Na-dependent Ca uptake via Na/Ca exchange should only occur in Na-loaded vesicles diluted into Na-free solution without digitonin. After 10 s, Ca uptake was stopped by addition of 3 ml of an ice-cold solution containing 1 mM EGTA, 200 mM KCl and 3-(N-morpholino)propanesulfonic acid-Tris (pH 7.4). Membranes were collected by vacuum filtration onto glass-fiber filters (Whatman, GF/C), with the 45Ca content determined by liquid-scintillation spectroscopy.

Na/Ca exchange activity is notoriously difficult to measure in homogenates. Part of the problem with homogenates is the possible interference of mitochondrial Na/Ca exchange. However, using sarcotemmalar-enriched preparations requires assumptions about relative purification factors. We measured digitonin-sensitive Na/Ca exchange activity in homogenates, which should reflect only sarcotemmalar Na/Ca exchange. We also incubated samples overnight on ice, which resulted in lower digitonin-insensitive 45Ca uptake (background), making homogenate measurements more practical. The sarcotemmolar Na/Ca exchange is not inhibited by this procedure, but it is possible that this results in mitochondria with less ability to accumulate Ca (and thus lower backgrounds).

Ryanodine binding assay. Ryanodine binding was measured as described by Bers and Stiffel (12). Homogenate (1–2 mg/ml) was incubated in 1 M NaCl, 20 mM HEPES, 25 mM Tris, 5 mM AMP, 0.5 mM CaCl2, and 1–100 nM [3H]ryanodine (New England Nuclear) at pH 7.4 for 90 min at 37°C. Unlabeled ryanodine (17 µM) was used to displace [3H]ryanodine, allowing measurement of specific binding. The reaction was terminated by vacuum filtration through Whatman GF/B filters using a Brandel cell harvester. Filters were washed three times with 3 ml distilled water to eliminate excess [3H]ryanodine. Membrane-bound [3H]ryanodine on the filter was estimated by β-scintillation spectroscopy. The data on Scatchard plots were fit by linear regression.

RNA isolation and Northern blotting. Total RNA was extracted from left ventricular tissue using the guanidinium thiocyanate method (16). Fifteen micrograms of total RNA were used for Northern blot analysis as described by Qi et al. (43). The following cDNA probes were used for Northern blot analysis: 1) SR Ca-ATPase (2.3-kilobase (kb) cDNA fragment of the rat cardiac SERCA2, kindly provided by Dr. Wolfgang Dillmann, University of California, San Diego); 2) Na/Ca exchanger (1.5-kb cDNA of guinea pig cardiac Na/Ca exchanger from Dr. Kenneth Philipson, University of California, Los Angeles); 3) phospholamban (710-bp cDNA fragment of the rat cardiac phospholamban provided by Dr. Huapin He, University of California, San Diego); 4) ryanodine receptor (580-bp cDNA of rabbit cardiac ryanodine receptor from Dr. Andrew Marks, Mount Sinai Medical Center, New York, NY); 5) calsequestrin (1.4-kb cDNA of canine cardiac calsequestrin from Dr. Larry J. ones, Indiana University, Indianaapolis, IN); 6) atrial natriuretic factor (ANF; 0.8-kb cDNA of rat ANF from Dr. Tadashi Inagami, Vanderbilt University Medical Center, Nashville, TN); and 7) glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 750-bp cDNA from human
fetal liver obtained from American Type Culture Collection). The cDNA probes were radiolabeled with [32P]dCTP and hybridized as previously described (19). The amounts of Ca transporter mRNAs and ANF mRNA were quantified by autoradiography at ~80°C and laser densitometry and are expressed relative to the amounts of GAPDH mRNA. Unless otherwise stated experimental reagents used were of analytical grade and were supplied by Sigma (St. Louis, MO).

RESULTS

Hemodynamic parameters. Table 1 shows the hemodynamic data obtained from the animals used in this study. Aortic banding for 16 wk resulted in significant cardiac hypertrophy, increased systemic blood pressures, and elevated left ventricular end-diastolic pressure (LVEDP), as we have reported previously with this hypertrophic model (18, 43). Heart weight or heart weight-to-body weight ratio increased by ~25%. This probably underestimates the degree of left ventricular hypertrophy. The left ventricle could not be weighed separately in most hearts because the cell isolation procedure precluded removal of atria or the right ventricle before weighing. In parallel experiments where we measured both heart weight and left ventricular weight, this 25% cardiac hypertrophy corresponds to ~43% left ventricular hypertrophy (43). The elevated arterial pressures indicate that this is a hypertensive hypertrophy model.

SR Ca-ATPase, Na/Ca exchange, ryanodine binding, and mRNA in homogenates. Previous studies in this same 16-wk banding model have shown that the SR Ca-ATPase is downregulated by 76% at the mRNA level and 34% at the protein level; further, thapsigargin-sensitive, oxalate- and ATP-supported 45Ca uptake is reduced by 27–50% (41, 43). Consistent with these results, we find here that the thapsigargin-sensitive Ca-stimulated ATPase activity in ventricular homogenates was decreased by 34%, comparable to reductions in mRNA (see Table 2). However, despite a depression of Na/Ca exchange mRNA in the banded group, we could not detect a difference in sarcolemmal Na-dependent 45Ca uptake activity in homogenate (Table 2). Because a major focus in the present study is on E-C coupling, we also measured the number of ryanodine receptors using radioligand binding. As shown in Fig. 1, there was no significant difference in ryanodine binding between sham and banded animals. The Scatchard plot (inset) shows that maximal binding (Bmax) was ~400 fmol/mg in both and there was no apparent difference in affinity under these assay conditions (Kd = 3.3 nM). Figure 2 shows sample Northern blots for expression levels of mRNAs for several proteins. Quantitative analysis showed no significant change in ryanodine receptor or calsequestrin message but a reduction in phospholamban mRNA that roughly paralleled the decrease in SR Ca-ATPase activity and mRNA levels (Table 2). ANF mRNA was greatly increased in the

Table 1. Hemodynamic parameters of animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Banded</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>473 ± 4 (68)</td>
<td>475 ± 5 (64)</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>1.77 ± 0.05 (48)</td>
<td>2.21 ± 0.06* (59)</td>
</tr>
<tr>
<td>Heart wt:body wt, %</td>
<td>3.79 ± 0.09 (48)</td>
<td>4.70 ± 0.12* (59)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117 ± 2 (45)</td>
<td>180 ± 4* (46)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81 ± 2 (45)</td>
<td>113 ± 2* (56)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>93 ± 2 (45)</td>
<td>135 ± 3* (56)</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>2.6 ± 0.8 (21)</td>
<td>11.8 ± 1.4* (38)</td>
</tr>
</tbody>
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Table 2. Biochemical data on Ca transport proteins

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Banded</th>
<th>Banded/Sham, % of sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR Ca-ATPase mRNA</td>
<td>2.17 ± 0.18 (5)</td>
<td>1.59 ± 0.10* (6)</td>
<td>73</td>
</tr>
<tr>
<td>Ca-ATPase, mmol·mg⁻¹·min⁻¹</td>
<td>44.7 ± 4.9 (5)</td>
<td>29.7 ± 3.4* (6)</td>
<td>66</td>
</tr>
<tr>
<td>Na/Ca exchange mRNA</td>
<td>3.99 ± 0.44 (5)</td>
<td>2.58 ± 0.29* (6)</td>
<td>65</td>
</tr>
<tr>
<td>Na-dep 45Ca uptake, mmol·mg⁻¹·min⁻¹</td>
<td>3.97 ± 1.78 (4)</td>
<td>3.55 ± 1.54 (4)</td>
<td>90</td>
</tr>
<tr>
<td>Ryanodine receptor mRNA</td>
<td>0.094 ± 0.018 (5)</td>
<td>0.078 ± 0.019 (6)</td>
<td>83</td>
</tr>
<tr>
<td>Ryanodine binding at 100 nM, fmol/mg</td>
<td>395 ± 51 (5)</td>
<td>412 ± 43 (6)</td>
<td>104</td>
</tr>
<tr>
<td>Calsequestrin mRNA</td>
<td>1.42 ± 0.13 (5)</td>
<td>1.13 ± 0.19 (6)</td>
<td>80</td>
</tr>
<tr>
<td>Phospholamban mRNA</td>
<td>634 ± 68 (5)</td>
<td>402 ± 48* (6)</td>
<td>63</td>
</tr>
<tr>
<td>ANF mRNA</td>
<td>2.52 ± 0.21 (5)</td>
<td>22.4 ± 3.5* (6)</td>
<td>888</td>
</tr>
</tbody>
</table>

Data are means ± SE; nos. in parentheses are no. of animals. ANF mRNA data are expressed in arbitrary densitometric units relative to glyceraldehyde 3-phosphate dehydrogenase mRNA, which was unchanged with respect to total RNA (see Fig. 2). SR, sarcoplasmic reticulum; Na-dep, Na-dependent; ANF, atrial natriuretic factor. *Significant difference from sham group (P < 0.05, unpaired t-test).
banded group, excluding the possibility that there was simply a generalized reduction in mRNA levels compared with GAPDH.

Thus in ventricular homogenates from banded animals there is reduced SR Ca-ATPase activity and mRNA, but no evidence indicating altered Na/Ca exchange activity or the number of SR Ca release channels (per mg protein). We also previously showed in this model that the density of L-type Ca current was unchanged (18). In addition to assessing the number or behavior of these key Ca transport proteins in a controlled in vitro environment, it is essential to evaluate the function of these systems in the ventricular myocyte during activity. Therefore we next evaluated the function of the key Ca transport proteins in intact ventricular myocytes.

Contraction and Ca transients in field-stimulated myocytes. Isolated ventricular myocytes in this aortic banding model are hypertrophied compared with the sham myocytes (in length by ~10%, width by 5%, and volume by ~28%; Ref. 18). Figure 3 shows, for a typical myocyte from a banded animal, Ca transients and contractions associated with steady-state twitches followed by caffeine-induced contractures in either NT or 0 Na-0 Ca solution. The three types of contractions shown provide sufficient data to evaluate Ca transport via the SR Ca-ATPase, Na/Ca exchange, and slow systems (mitochondrial Ca uniport and sarcolemmal Ca-ATPase) in ventricular myocytes (5). The amplitudes and time constants (τ) of decline of the corresponding types of contractions and Ca transients were pooled across cells, and statistical comparisons appear in Table 3.

Diastolic [Ca]i was not different between sham and banded groups. Steady-state twitch Ca transient amplitude was similar in both groups although the contraction amplitude was significantly depressed (by 35%). Caffeine-induced Ca transient amplitudes (ΔCa) were not different between groups, but the corresponding contractions were again significantly decreased under banding (by 19–21%). This disparity between contraction and ΔCa indicates that mechanical contraction is depressed in banded myocytes despite unaltered Ca transients.

The amplitude of the caffeine-induced Ca transient is an index of the SR Ca content, especially in 0 Na-0 Ca solution where Na/Ca exchange is inhibited (5, 8, 45).
As there was no difference in the caffeine-induced Ca transients in Table 3, we infer that there was no difference in SR Ca content in the two groups under steady-state conditions. This means we can reasonably base our interpretations of twitch- and caffeine-induced contractions and Ca transients on a constant SR Ca load.

Twitch Ca transient amplitudes are much smaller than caffeine-induced transients for two reasons. First, only 37–55% of SR Ca is released during E-C coupling during the normal twitch, whereas all is released during sustained caffeine application (7, 18). Second, rapid SR Ca uptake during the twitch Ca transient can curtail the peak of the measured Ca transient, whereas net Ca uptake by the SR is prevented during sustained caffeine application (5, 8). It is possible that a reduced SR Ca-ATPase activity could cause the larger twitch Ca transients (for a given SR Ca load), but this is not consistent with effects on the time course of [Ca], decline or relaxation (see below).

Time constants of [Ca], decline and relaxation provide further information about the processes that remove Ca from the cytosol during relaxation. During caffeine-induced Ca transients in 0 Na-0 Ca the slow relaxation and [Ca], decline are due to the slow transport of Ca by the mitochondrial Ca uniporter and the sarcosomnal Ca-ATPase (5). These Ca transients declined with \( \tau = 15–16 \text{ s} \) and were not significantly different between the groups. Relaxations of caffeine-induced contractures in 0 Na-0 Ca were seldom fit well by exponentials and so were not included in Table 3. From the constant \( \tau \) we infer that banding did not affect Ca removal by the combined sarcosomnal Ca-ATPase and mitochondrial Ca uniporter. We found similar \( \tau \) for the slow processes previously in rat ventricular myocytes (5). These systems are very slow in removing Ca compared with the SR Ca-ATPase and Na/Ca exchange.

Relaxation and [Ca], decline during a caffeine-induced contracture in NT solution are due primarily to Ca extrusion via Na/Ca exchange (8). The \( \tau \) for [Ca], decline was \( \approx 3.1 \text{ s} \) for both sham and banded groups. Similarly, the relaxation \( \tau \) was \( \approx 3.8 \text{ s} \) for both groups. This is more than five times faster than the [Ca], decline with Na/Ca exchange blocked, indicating that most of the Ca is removed via Na/Ca exchange. We conclude that there is no demonstrable difference in Na/Ca exchange activity between the sham and banded groups.

The \( \tau \) of [Ca], decline during the twitch in rat ventricular myocytes is strongly dominated by the SR Ca-ATPase (see below and Ref. 5). This is also supported by the fact that the \( \tau \) for [Ca], decline is an order of magnitude faster when the SR can take up Ca during relaxation (i.e., twitch- vs. caffeine-induced contracture-NT in Table 3). In view of the reduced expression of SR Ca-ATPase in homogenates, a slowing of [Ca], decline and relaxation was expected in the banded group. We were therefore surprised that the \( \tau \) of [Ca], decline was \( \approx 20\% \) faster in cells from banded rats (214 vs. 263 ms) without any change in the \( \tau \) of relaxation between the groups.

Single twitches were also recorded in Na-free, Ca-containing solution from some cells (see MATERIALS AND METHODS). By eliminating the contribution of Na/Ca exchange to twitch [Ca], decline and relaxation, we may better isolate any changes in SR uptake with hypertrophy (5). With SR Ca uptake isolated in this way, \( \tau \) of [Ca], decline was 405 \( \pm \) 41 ms in banded vs. 339 \( \pm \) 47 ms in sham cells \( (n = 21 \text{ and } 16) \). This 19\% longer mean \( \tau \) in the banded group was not significantly different (nor was the 15\% smaller \( \Delta \text{[Ca]} \), during these twitches). Thus we do not find any compelling cellular evidence of reduced SR Ca-ATPase function.

Ca flux analysis. As we noted above, parameters of twitches and caffeine contractures in NT reflect the operation of multiple Ca removal processes, which simultaneously influence [Ca], but the contributions of the processes can be measured separately (5). Measuring the relative strengths of Ca removal processes within a group (banded or sham) overcomes the limitation of comparing \( \tau \) values for Ca transients of different amplitudes between the groups. To make these comparisons we have to take into account the buffering capacities of sham and banded cells. The assumption we have made is that there are no major differences in passive cytosolic Ca buffering between these two groups. Evidence from a previous study using this model (18) and our results regarding E-C coupling at 1 mM [Ca], (see below) suggest that this is a reasonable assumption.

Figure 4 shows integrated Ca removal fluxes for an average twitch Ca transient due to SR Ca uptake, Na/Ca exchange extrusion, and the combined slow processes for all of the cells from the sham group. Ca fluxes were derived using mean values for diastolic [Ca], \( \Delta \text{[Ca]} \), and \( \tau \) of [Ca], decline as in Table 3. As expected for rat cells (5) Ca removal was heavily dependent on SR function. Total Ca removal flux during
Hypertrophy and Cardiac Myocyte Ca

The SR Ca-ATPase removed ~96% of the activating Ca from the cytosol. Na/Ca exchange accounted for only ~3% of the Ca removal from the cytosol and the slow systems for <1% (see legend to Fig. 4 for corresponding values for banded group). There was almost no difference in the relative SR contribution in cells from sham and banded animals.

Maximal SR Ca load. Stimulating at high frequency could increase SR Ca loading toward a maximum and thereby potentiate twitch amplitudes (7). This potentiation could be greater in cells from sham rats, if functional expression of SR Ca-ATPase was reduced in hypertrophy. To test this, we paced cells at 0.5 Hz for 10 s, followed by 5 Hz for 10 s. As soon as diastolic [Ca] fell to a stable value, a single twitch was evoked. Figure 5 shows records of Ca transients and contractions elicited in response to this protocol in a representative cell from a banded animal. Cells used in this protocol are a subpopulation of those used in the steady-state twitch and caffeine-induced contracture protocols described above.

At 5-Hz stimulation frequency, [Ca], and contraction reached a steady tetanic level. The average [Ca], determined by a straight line fit to the last 3 s of the 5-Hz period in the record, was similar in banded and sham cells (means ± 5E: sham 291 ± 30 nM, n = 22; banded 354 ± 48 nM, n = 8). Posttetanic twitch Δ[Ca], values were significantly higher in banded cells (sham Δ[Ca], 631 ± 87 nM, n = 22; banded Δ[Ca], 1,038 ± 226 nM, n = 8; P = 0.048). However, the relative enhancement (ratio of maximum to steady-state twitch Δ[Ca],) was unchanged, as was the τ of [Ca], decline in steady state under either SR loading condition.

E-C coupling in voltage-damped cells. To study E-C coupling in a controlled manner, we voltage-damped cells from each group so that contractile amplitude could be expressed as a function of ICa. The mean membrane capacitance values were significantly higher in banded (354 ± 78 pF, n = 17) than in sham cells (209 ± 23 pF, n = 16; P < 0.05), consistent with cellular hypertrophy in the banded group. On the other hand, the membrane charging time constants (2.2 ± 0.3 ms in sham vs. 2.4 ± 0.2 ms in banded) were not significantly different. Although the speed of voltage clamp is less than ideal in these large myocytes, this constitutes only a systematic limitation that was not different between the groups of cells. Thus comparing peak ICa values here is reasonable and the results also agree with our prior study (18) that focused more on ICa characteristics.

Figure 6, top, shows the protocols used in these experiments. Figure 6A shows conditioning and test contractions and test ICa (elicited at 0 mV) with 1 mM [Ca], in representative myocytes of comparable size. The conditioning pulses (from −90 to 0 mV for 400 ms, 0.5 Hz) to load the SR to a steady-state level were followed by test pulses to different ECa (after holding the voltage for 400 ms at −40 mV to inactivate Na channels). The amplitudes of the conditioning contractions were fairly constant, and the responses to test pulses to 0 mV are similar in cells from banded and sham animals. Figure 6, right, shows that trigger ICa levels were also similar (2.06 pA/pF in sham vs. 1.97 pA/pF in banded). In this example, insofar as the SR Ca load is the same in sham and banded cells (refer to caffeine-evoked [Ca] transients described above), similar ICa produced similar contractions, so we infer that E-C coupling is unaltered by banding. These data showing a lack of effect of hypertrophy on ICa are in agreement with the results previously reported by us in this model (18).
Because 1–2 mM [Ca\textsubscript{o}] is sufficient to produce nearly a maximal twitch contraction in rat ventricular muscle (11), E-C coupling could be maximally activated with respect to [Ca\textsubscript{o}]. In this case a modest change might not be detectable. Therefore, we also measured E-C coupling at lower [Ca\textsubscript{o}] where subtle differences might be more apparent.

Figure 6B shows that at 0.5 mM [Ca\textsubscript{o}], the contractions were smaller than at 1 mM [Ca\textsubscript{o}]. In this example, the test contraction was smaller in the banded cell, but the test I\textsubscript{Ca} was comparable when normalized to cell capacitance (1.6 vs. 1.4 pA/pF). Indeed, the conditioning contractions at 0 mV were also smaller in the banded cell than in the sham (even in cells of comparable size). Thus, in contrast to the case at 1 mM [Ca\textsubscript{o}], there seems to be less contraction for a given I\textsubscript{Ca} in the banded cells. If SR Ca content were indeed the same, this would indicate weaker E-C coupling in the banded group.

Figure 7 shows complete mean current-voltage and contraction-voltage curves for banded and sham groups with 0.5 and 1.0 mM [Ca\textsubscript{o}]. Contraction and I\textsubscript{Ca} exhibited bell-shaped voltage dependences peaking around 0 mV, in both sham and banded cells. With 1 mM [Ca\textsubscript{o}], both I\textsubscript{Ca} and contraction were superimposable in both groups. However, the I\textsubscript{Ca}-contraction relationship was different with 0.5 mM [Ca\textsubscript{o}]. The test I\textsubscript{Ca} was comparable between sham and banded groups, but the contractions were significantly smaller in the banded group at each voltage tested (at most points by ~50% or more). Therefore, banding seems to depress E-C coupling in a way that is only apparent at [Ca\textsubscript{o}] < 1 mM.

Ca is known to be a major factor in the inactivation of cardiac L-type Ca channels (23, 47). In both sham and banded cells, reduction of [Ca\textsubscript{o}] from 1 to 0.5 mM resulted in significant slowing of I\textsubscript{Ca} decline during the pulse. For example, I\textsubscript{Ca} decay time constants on stepping to +10 mV increased from 41.5 ± 3.3 to 55.6 ± 3.8 ms in sham cells and from 36.2 ± 2.8 to 47.1 ± 3.0 ms in banded cells (on the basis of single exponential fits; n = 14–16, P < 0.05). The slower I\textsubscript{Ca} inactivation with 0.5 mM [Ca\textsubscript{o}] is sufficient to produce nearly a maximal twitch contraction in rat ventricular muscle (11), E-C coupling could be maximally activated with respect to [Ca\textsubscript{o}]. In this case a modest change might not be detectable. Therefore, we also measured E-C coupling at lower [Ca\textsubscript{o}] where subtle differences might be more apparent.

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Fig. 6. Contraction (left) and calcium current (I\textsubscript{Ca}; right) at extracellular Ca concentration ([Ca\textsubscript{o}]) of 1.0 (A) or 0.5 mM (B). Top: voltage-clamp protocol. Conditioning depolarizations from membrane potential (E\textsubscript{m}) of −90 to 0 mV were followed by depolarization to −40 mV to inactivate sodium current (I\textsubscript{Na}) and then stepped to test potential (in case shown at 0 mV). Left: conditioning and test contractions in a sham and a banded cell. Right: expanded view of test step and I\textsubscript{Ca} traces. Contraction and I\textsubscript{Ca} were recorded simultaneously during test pulses (indicated by * on contraction traces). Data were obtained from sham and banded cells of comparable size (RCLs: sham, 192 µm and 152 µm for 1 mM and 0.5 mM [Ca\textsubscript{o}], respectively; banded, 170 µm and 182 µm for 1 mM and 0.5 mM [Ca\textsubscript{o}], respectively).

Fig. 7. Voltage dependencies of test contraction and I\textsubscript{Ca} amplitudes at 1(left) and 0.5 mM [Ca\textsubscript{o}] (right) in cells from sham and banded animals. I\textsubscript{Ca} of each cell was normalized to cell capacitance (in pA/pF; plotted on downward ordinate), and contraction was normalized to individual RCL (plotted on upward ordinate). Voltage-clamp protocol was as shown in Fig. 6. Data are means ± SE; n = 19 cells (1 mM [Ca\textsubscript{o}]) and 15 cells (0.5 mM [Ca\textsubscript{o}]): RCLs were 204 ± 8 µm (banded) and 146 ± 7 µm (sham). Absolute amplitude of last conditioning contraction was smaller at 0.5 mM [Ca\textsubscript{o}] than at 1 mM [Ca\textsubscript{o}], respectively, for both sham (23.1 ± 1.2 µm vs. 27.6 ± 0.5 µm) and banded groups (15.1 ± 0.8 µm vs. 24.4 ± 1.0 µm).
mM [Ca]o compared with 1 mM is expected and likely reflects less Ca-dependent inactivation due to both the smaller Ca influx into the cell (23) and the smaller amount of Ca released from the SR (47). At 1 mM [Ca]o there was no difference in Ica decline between banded and sham groups at any test potential. This suggests that when Ica and SR Ca release are comparable between sham and banded groups, Ica inactivation is also comparable.

SR Ca content and E-C coupling. An important aspect of the interpretation of the E-C coupling results in Figs. 6 and 7 is the state of SR Ca loading when Ica and contraction are measured. Caffeine-induced contractions and Ca transients in Fig. 3 and Table 3 suggested that the SR Ca content of cells from sham and banded groups in steady-state field stimulation was the same, as we have also reported previously for similar voltage-clamp pulses in this model (18). In the same cells undergoing E-C coupling protocols summarized in Fig. 7, caffeine was also applied after the same conditioning trains to assess SR Ca content available for release during the test twitches. Figure 8, left, shows conditioning trains and subsequent caffeine-induced contractions in example cells from sham and banded groups. Figure 8, right, shows pooled data for caffeine-induced contracture in both groups and at both [Ca]o levels.

After the steady-state conditioning train of voltage-clamp pulses at 1 mM [Ca]o, the caffeine-induced contracture amplitudes in the banded group were 77% of those in the sham group (not significantly different). This mean value is close to the 79 and 82% during caffeine-induced contracture in Table 3 (in NT and 0 Na-0 Ca) where we know that Δ[Ca] values were almost identical. At 0.5 mM [Ca]o the caffeine-induced contracture amplitudes in the banded group were similarly 86.1% of those in the sham group (see Fig. 8, not significantly different). We infer that the SR Ca load is really the same between the sham and banded groups at any given [Ca]o during the voltage-clamp experiments of Figs. 6 and 7. We also conclude that the much smaller twitch contractions (with comparable Ica) in the banded group at 0.5 mM [Ca]o indicate a depression of E-C coupling and cannot be explained by a reduced SR Ca content.

On the basis of the foregoing discussion, we could expect that a comparable twitch SR Ca release in the banded group would produce 79–82% of the twitch contraction observed in the sham group (Table 3). Indeed, at 1 mM [Ca]o the last steady-state conditioning pulse to 0 mV was moderately smaller in cells from the banded group as a percentage of that in sham (85.8%, n = 19, P < 0.01). This would be consistent with unaltered E-C coupling at 1 mM in the banded group. However, when [Ca]o was 0.5 mM the last steady-state conditioning twitch amplitude was only 43.3% of the sham value (n = 19, P < 0.001). This dramatic depression of contraction is consistent with depressed E-C coupling in the banded rats at 0.5 mM [Ca]o (despite comparable Ica and SR Ca load).

The absolute amplitudes of twitch contractions were higher in the voltage-clamped vs. field-stimulated cells. This might be due to higher SR Ca load and relatively long voltage-clamp pulses (300–400 ms) vs. the short action potential duration characteristic of field-stimulated rat cells. The effect of [Ca]o reduction from 1 to 0.5 mM on steady-state twitch amplitude was much more profound in the banded group (52% decrease) than in the sham group (5% decrease; see Fig. 7 legend). The minor effect of [Ca]o reduction on steady-state twitch amplit-
tude in the sham group supports the notion that in adult rats E-C coupling efficacy is nearly maximal at 0.5–1 mM [Ca\textsubscript{o}]. Obviously this situation is strikingly different in the banded rats, and this may be another reflection of depressed E-C coupling in the banded rat when [Ca\textsubscript{o}] is 0.5 mM.

Relationship between I\textsubscript{Ca} and contraction. The way contraction depends on I\textsubscript{Ca} (at comparable SR Ca load) is at the core of E-C coupling. In Fig. 9 the data of Fig. 7 are replotted using I\textsubscript{Ca} rather than E\textsubscript{m} as the independent variable. The direction of the arrows indicates increasingly positive test voltage steps. The steepness of the relationship indicates the gain or efficacy of E-C coupling, i.e., the relationship between a given trigger I\textsubscript{Ca} and SR Ca release (as measured by contraction in this study) for a given SR Ca load. At 1 mM [Ca\textsubscript{o}] the sham and banded groups follow similar trajectories, with the banded falling just lower than the sham. This small reduction is consistent with the modest reduction of contraction for a comparable [Ca\textsubscript{i}] in the banded rats.

In contrast, with lower [Ca\textsubscript{o}] there is a striking difference in the I\textsubscript{Ca}-contraction relationships between the two groups. In the sham cells the relationship has a similar steepness to that seen at 1 mM [Ca\textsubscript{o}], although the peak levels of both contractility and current are smaller, as would be expected. In the banded group the I\textsubscript{Ca}-contraction relationship is much flatter such that the contraction for a given I\textsubscript{Ca} is 2–5 times smaller in the hypertrophied cells. Indeed, a linear regression for the 0.5 mM [Ca\textsubscript{o}] data in Fig. 9 has a much lower slope for the banded compared with the sham rats (3.23 vs. 7.07) and also a lower apparent y-intercept (1.8 vs. 4.2, not shown).

**DISCUSSION**

Hypertrophic model. Many animal models have been employed to study hypertrophy, hypertension, and heart failure (3, 25). We do not intend to review the merits of different models here. The rat suprarenal aortic constriction model of left ventricular hypertrophy has been well characterized by us (18, 19, 43) and others (e.g., 2, 4, 15, 17, 29, 30, 34, 40, 41, 46). Although models using ascending aortic constriction have also produced valuable results (e.g., 20, 51), these models differ functionally (e.g., with respect to systemic hypertension and cardiac load). The duration and severity of constriction varies among the above 10 studies of abdominal aortic constriction, but the degree of left ventricular hypertrophy (23–62%, mean 42%) is similar to what we find in this model (43%). Here we study relatively long-term (~16 wk) effects and changes in cellular Ca transport and E-C coupling.

In this 16-wk hypertensive hypertrophic model we have previously documented that there is a significant reduction in SR Ca-ATPase (at the mRNA, protein, and thapsigargin-sensitive SR Ca uptake levels) and an increase in arterial pressures, LVEDP, time constant of isovolumic pressure decline, collagen, and the fraction of β-MHC (43). We have also recently determined that despite cellular hypertrophy in the left ventricular myocytes (in banded vs. sham animals), there was no significant difference with respect to cellular surface area-to-volume ratio, I\textsubscript{Ca} characteristics (including current density, activation, inactivation, and isoproterenol stimulation) or in the steady-state SR Ca content in dialyzing voltage-clamp experiments (18). Although we measured a slight reduction in the mean fraction of SR Ca released at a twitch (from 55 to 37% in sham vs. banded, respectively), this difference was not statistically significant.

In the present study we sought to further determine if hypertrophy caused differences in E-C coupling (how SR Ca release depends on I\textsubscript{Ca}) or on the processes that remove Ca from the cytosol during relaxation (SR Ca-ATPase, Na/Ca exchange, mitochondrial uniporter, and sarcolemmal Ca-ATPase), with the latter two lumped together as the slow systems (5).

![Fig. 9. Efficacy of excitation-contraction (E-C) coupling in myocytes from sham (○) and banded rats (●) at 1 (left) and 0.5 mM [Ca\textsubscript{o}] (right). Data from Fig. 7 are plotted to show dependence of peak contraction on I\textsubscript{Ca} amplitude at test pulses. Arrows indicate direction of increasingly positive E\textsubscript{m} during test pulses. Maximal I\textsubscript{Ca} and contraction occurs at the 0- or +10-mV test pulse. E-C coupling efficacy was reduced in cells from banded animals at 0.5 mM [Ca\textsubscript{o}].](http://ajpheart.physiology.org/
Decreased mechanical response. During caffeine-induced contracture in isolated ventricular myocytes, the extent of cell shortening was reduced in cells from the banded group despite unchanged Ca transient amplitudes. This suggests a reduced contractile response to Ca in these cells. Although there is clearly a shift in MHC isoform (β-MHC increases from 25 to 59%; Ref. 43), skinned fibers from rats made hypertrophic by aortic banding pressure overload have not shown any difference in myofilament Ca sensitivity (30, 37). Other factors, such as increased stiffness, restoring force, or changes in microtubules, could depress unloaded shortening in isolated myocytes (50). Notably, these effects could have much less effect on isometric twitch force or steady-state myofilament Ca sensitivity. Although there is increased collagen in the banded rat hearts (19, 43), this seems like an unlikely explanation for the cellular results here because the myocytes are isolated with extensive collagenase treatment. The caffeine-induced contracture produces a relatively long Ca transient duration, removing most kinetic concerns that complicate myofilament Ca sensitivity conclusions drawn from twitches. If the caffeine-induced increase in myofilament Ca sensitivity was smaller in the banded group, that might explain the reduced caffeine-induced contracture amplitudes. However, the opposite result was reported in ferret pressure overload (9). In addition, even without caffeine, the twitch contractions here were significantly reduced, despite comparable Δ[Ca]. Thus there was a significant reduction in cell contraction for a given Δ[Ca]. Although our focus here is mainly on Ca transport, this is a potentially important mechanical alteration exhibited at the cellular level.

Ca removal fluxes. Increased Na/Ca exchange has recently been reported in human heart failure and was suggested to be compensatory for the reduced levels of SR Ca-ATPase (21, 49). However, in banded vs. sham rats we did not find any difference in Na/Ca exchange activity either in homogenates or functionally in intact cells (on the basis of the r of [Ca], decline or relaxation of caffeine-induced contracture). There was also no difference in the rate of [Ca], decline attributed to the combined action of the mitochondrial Ca uniporter and the sarcolemmal Ca-ATPase. These three systems only make a very minor contribution to Ca removal during twitch relaxation in these experiments in adult rats, whereas the SR Ca-ATPase is responsible for 96–98% of Ca removal flux.

Given the significant decrease in SR Ca-ATPase mRNA, protein, SR Ca-ATPase, and SR 45Ca uptake measured in homogenate from banded rats (Ref. 43 and data of current study), we anticipated some slowing of [Ca], decline and relaxation during the twitch. We have previously estimated that SR Ca-ATPase transport rate in rabbit ventricle is 40% of that in rat ventricle (5, 27). Bassani et al. (5) found a comparable significant reduction in the rates of twitch relaxation and [Ca], decline in rabbit vs. rat (to 40–48%) especially when Na/Ca exchange was prevented in both species. In the present study we found that homogenates from the banded rats had 68% as much SR Ca-ATPase activity as the sham rats. Although this decrease is only one-half as large as the interspecies difference between rat and rabbit, we were surprised not to detect any slowing of [Ca], decline or relaxation in the cells from the banded group. On the contrary [Ca], decline was slightly faster in the banded group (although relaxation was not different).

We do not have a compelling explanation for this dichotomous result with respect to SR Ca-ATPase but can offer some speculations. On the basis of our experience comparing rat with rabbit, our methods should be adequate to detect a 30–40% decrease in SR Ca-ATPase function. When Na/Ca exchange was prevented the twitch [Ca], decline was 19% slower in the banded group, but this result was not significant. However, a higher degree of variation within the banded group (cells or animals) could have obscured a statistical difference. In any case, this would lead us to expect no significant difference, rather than a change in the opposite direction from that expected. The 25% higher twitch Δ[Ca], measured in the banded group could also bias the r value to be smaller in Table 3, even with unaltered SR Ca-pump function (10). Another possible explanation is that the cell isolation procedure introduces a selection bias (18). That is, the cells that survived the isolation might have a smaller decrease of SR Ca-ATPase than average and exhibit relatively normal Ca transients. Although this possibility might be dismissed on grounds that the cell viability was not different (18), recent experiments in multicellular preparations in this same banded rat model have shown slower [Ca], decline and relaxation, which was only significant at higher frequencies or work loads (36). This raises an additional point, because all of the experiments presented here were carried out at 23°C and at low work levels (unloaded shortening at 0.5 Hz). This could well mask differences between the groups. It is also conceivable that the SR Ca pump is in a different regulatory state in the banded group, making it more effective. A final speculative possibility is the following. The banded cells that contract less strongly will remain at longer sarcomere length on average and may consequently have higher Ca affinity (28). This could result in stronger effective cytosolic Ca buffering during contraction in the banded cells causing free [Ca], to decline more rapidly for a given SR Ca-ATPase activity. Presently we cannot distinguish how much each of these factors might contribute to why a functional slowing of [Ca], decline or relaxation was not detected here.

E-C coupling. We found no difference in I Ca characteristics between sham and banded groups in the present study. This is consistent with previous, more detailed studies of I Ca in this rat hypertrophy model (18, 46) and with the lack of alteration in dihydropyridine receptor density (44). In addition, we found no difference in the number of ryanodine receptors (Fig. 1). Whereas Kim et al. (29) found a reduction in ryanodine binding in a comparable rat model of hypertrophy, our data agree with those of Rannou et al. (44), who found no difference in ryanodine binding in moderate hypertrophy in rat (~40%, comparable to the present study). However,
in rat when hypertrophy was more severe (60%). Ran-
nou et al. (44) found a decreased number of ryanodine
receptors. Furthermore, they found that reduced ryan-
odine binding occurred at more moderate levels of
hypertrophy in guinea pig or ferret heart, consistent
with heart failure results in dogs and humans (13, 53).
Thus the amount of ryanodine receptor downregulation
may depend on the species and severity of hypertrophy
or failure.

Here we studied the relationship between $\Delta F_{\text{Ca}}$ and
contraction in intact cells, where banding did not affect
$\Delta F_{\text{Ca}}$, the number of ryanodine receptors, or the SR Ca
content. At 1 mM $[\text{Ca}]_o$, there was no apparent differ-
ence in E-C coupling. However, when $[\text{Ca}]_o$ was reduced
to 0.5 mM, there was a dramatic reduction in E-C
(coupling (Figs. 7 and 9). Why this difference in E-C
coupling is manifest only with 0.5 mM $[\text{Ca}]_o$ is not clear,
but it could be related to E-C coupling being nearly
maximal at higher $[\text{Ca}]_o$ in the rat (due to both maximal
SR Ca load and sufficiently high $I_{\text{Ca}0}$ trigger). That is,
twitch contractions in adult rat ventricle are nearly
maximal at 1–2 mM $[\text{Ca}]_o$, whereas contraction amplitu-
din most mammalian species continues to increase
with $[\text{Ca}]_o$, up to $\sim$0.5 mM (11, 14). Thus moderate
changes in E-C coupling that reduce the efficacy of a
given $I_{\text{Ca}0}$ trigger may be less apparent at $[\text{Ca}]_o$ of 1 mM
or higher in rat. A maximized E-C coupling at the
higher Ca level might mask subtle differences in E-C
coupling between cells in the two groups. This might
also explain why the fractional release of Ca from the
SR during twitches was reduced only from 55 to 37% in
this same model, which was not significant (18). Given
the undiminished levels of $I_{\text{Ca}0}$, ryanodine receptor,
and SR Ca load, our results suggest a decreased effi-
cacy of E-C coupling in banded, hypertrophic rat hearts
that is due to a regulatory modulation of the process.
Such depression of E-C coupling could progress in more
severe hypertrophy or failure and greatly compromise
systolic function, even with relatively normal $I_{\text{Ca}0}$ and
SR Ca content.

In this regard Gómez et al. (22) very recently re-
ported a very similar depression of E-C coupling in
genetic rat strains that develop hypertrophy and heart
failure in comparison to those that we report here at 0.5
mM $[\text{Ca}]_o$. Indeed they found that boosting $I_{\text{Ca}0}$ by
adding isoproterenol could bring E-C coupling back in
salt-sensitive hypertensive rats but not in a failing strain (SH-HF).

Gómez et al. (22) also raised the possibility that the
E-C coupling defect might reflect a geometric distortion
of the space between the L-type Ca channel and ryan-
odine receptor. They took slowing of $I_{\text{Ca}0}$ inactivation in
the hypertensive rats (i.e., less Ca-dependent inactiva-
tion) as evidence for this type of effect. However,
because the Ca transients were also smaller, less
Ca-dependent inactivation is expected on that ground
alone (making this intriguing geometric argument not
compelling). Our experiments at 1 mM $[\text{Ca}]_o$ provide an
excellent test of this hypothesis in our model. Because
$I_{\text{Ca}0}$ decline at 1 mM $[\text{Ca}]_o$ was the same in sham and
banded rats (as were $I_{\text{Ca}0}$ amplitude and SR Ca load), the
released Ca must have had a comparable effect on
inactivation of the L-type Ca channels. This does not
support the geometric model, but further tests of this
hypothesis would be valuable. Thus, although the
influence of Ca influx (via $I_{\text{Ca}0}$) on the SR Ca release
channel is clearly diminished in the banded rats, the
feedback of Ca released from the SR on the sarcolem-
mal Ca channel appears normal.

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