Cellular Distribution of Ca\textsuperscript{2+} Pumps and Ca\textsuperscript{2+} Release Channels in Rat Cardiac Hypertrophy Induced by Aortic Stenosis

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Background—The response of ventricular myocytes to pressure overload is heterogeneous and not spatially coordinated. We investigated whether or not the alterations in SERCA and RyR gene expression are homogeneous within the myocardium.

Methods and Results—The cellular distribution of mRNAs and proteins encoding the 2 sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) isoforms (SERCA 2a and 2b) and 2 Ca\textsuperscript{2+} release channels (the ryanodine receptor, RyR, and the IP\textsubscript{3} receptor, IP\textsubscript{3} R) were analyzed by in situ hybridization and immunofluorescence, respectively. Analyses were performed during early (1 and 5 days) and late (1 month) stages of cardiac hypertrophy induced in rat by thoracic aortic stenosis (AS). The results indicated that 1 and 5 days after AS, the cellular distribution of SERCA 2a and RyR2 mRNAs in right ventricle and atrium was similar to controls but the mRNA levels appeared to decrease in some areas of the left ventricle (LV). One month after AS, the distribution of SERCA 2a mRNA and protein became heterogeneous throughout the LV, whereas RyR2 mRNA and protein levels were decreased in a homogeneous manner. SERCA 2b, poorly expressed in both cardiomyocytes and vessels of controls, was increased 4-fold 1 month after AS in coronary arteries only. In both sham (Sh) and AS, SERCA 3 and IP\textsubscript{3} R mRNAs were mainly found in the vessels.

Conclusions—In severe hypertrophy, decreased accumulation of SERCA 2a was heterogeneous and not compensated by an induction of SERCA 2b in the cardiomyocytes. Decrease in RyR2 expression was more homogeneous and not compensated by an increased IP\textsubscript{3} R expression. (Circulation. 1998;98:2477-2486.)

Key Words: sarcoplasmic reticulum ■ hypertrophy ■ calcium channels ■ remodeling

Cardiac expression of SERCA and RyR is regulated during physiological and pathological cardiac growth.\textsuperscript{8,9} In animal models, severe compensated hypertrophy secondary to pressure overload is accompanied by large decreases in SR Ca\textsuperscript{2+}-ATPase mRNA and protein levels.\textsuperscript{10-18} A low level of SERCA 2 mRNA is also observed in the ventricular myocardium of rats exhibiting signs of cardiac failure.\textsuperscript{19} Furthermore, decrease in SERCA gene expression is associated with decreased SR Ca\textsuperscript{2+} uptake.\textsuperscript{10,15,18} However, in rats with moderate (20\% to 30\%) cardiac hypertrophy, the level of SERCA 2 mRNA or protein was unaltered or upregulated.\textsuperscript{10,14-18,20} Data concerning SR protein gene expression in human myocardium are controversial. In failing versus nonfailing myocardium, SR Ca\textsuperscript{2+} pump activity is either unchanged or reduced, and SERCA 2 mRNA or protein levels are decreased or unchanged.\textsuperscript{21-25}

Changes in RyR expression have also been described during development of cardiac hypertrophy and failure. A decreased number of high-affinity Ryanodine binding sites...
are observed in compensatory cardiac hypertrophy in the rat, guinea-pig, and ferret, and in canine heart failure. In rat cardiac hypertrophy, the decrease in RyR2 mRNA and protein levels and the number of high-affinity binding sites are closely correlated to the length and severity of the pressure overload; however, no significant changes in RyR2 mRNA level or ryanodine binding were observed in other models. In failing human hearts, RyR2 mRNA level is decreased in various types of cardiopathies but unchanged in dilated cardiopathy. Decrease in ryanodine receptor mRNA level is correlated with decreased SERCA 2 and phospholamban mRNA levels, suggesting that these genes are coordinately regulated and that they are inversely correlated to the level of ANF and IP3 mRNAs. In contrast, RyR2 protein level and the number of high-affinity Ryanodine binding sites were similar in nonfailing and failing human hearts. Thus, as for SERCA 2, data concerning the expression of RyR2 in human cardiopathy are conflicting. In animal models, however, severe compensated hypertrophy is generally associated with a decrease in the expression of SERCA 2 and RyR2.

Because previous qualitative analysis has clearly demonstrated that the response of ventricular myocytes to pressure overload is heterogeneous and not spatially coordinated, we investigated the cellular distribution of Ca\textsuperscript{2+} pumps and Ca\textsuperscript{2+} release channels during development of rat cardiac hypertrophy secondary to coartation of the ascending aorta, a model where SERCA 2 and RyR2 gene expression were undoubtably reduced.

### Methods

#### Animals

Pressure overload of the left ventricle (LV) was produced in 3-week-old rats by coarctation of the ascending thoracic aorta (AS). Pressure overload of the left ventricle (LV) was produced in 3-week-old rats by coarctation of the ascending thoracic aorta (AS) as described. One day, 5 days, and 1 month later, operated and sham-operated (Sh) animals were killed by pentobarbital injection and the hearts were dissected and weighed. Animals did not have dyspnea, pulmonary edema, or ascites, and the weight of right ventricle was unchanged indicating that they did not develop cardiac failure. Part of the heart was frozen in liquid nitrogen and used for RNA preparation, part was used for in situ studies.

#### Complementary DNA Probes

SERCA 2a, 2b, and 3 probes were constructed as described previously. The common SERCA 2a (a+b) probe corresponds to nucleotides +2616 to 3120. IP3R and RyR probes were prepared by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification using 10 μg of total RNA from rat brain (IP3R) or rat heart RNA (RyR2). The sequence of the amplified fragments were confirmed using USB sequencing kit (Sequenase, Amersham). They correspond to nucleotide +4 to 336\textsuperscript{th} to 8604 to 9144, respectively, of the sequences previously published. The sequence of the RyR2 probe is presented below. It corresponds to the rabbit cardiac ryanodine receptor isolated by Otsu et al., except for few nucleotide changes (underlined), some of them leading to amino acid substitutions: 8603\textsuperscript{th} (ACCATCCTCTCTGGATGACACATCAGCAG-CAGAGGAGAAAGCAGGAGCGAGCGGAAGAGCCGAGCAC-ATCCCTGATTCCTACAGATCGGTTGATCCGGATACCGA-CAGGGGTTACCTCATTCCAGGAGCTGCTCGTCATTTTCAAACG-ATCGATATTTTCAAAACCCCATCGCCTTGTACCTCTTGTC-

\textsuperscript{cRNA Probes}

Complementary RNA probes were transcribed in vitro from HindIII (SERCA 2a and 2b) and BamHI (IP3R) linearized plasmids in the presence of T3 RNA polymerase or from XhoI (RyR2) and HindIII (SERCA 3) linearized plasmids in the presence of T7 RNA polymerase and (\textsuperscript{35}S)-UTP (1000 Ci/mmol; Amersham). All probes were diluted to a final specific activity of about 60 000 cpm/mL as described.

#### Immunolabeling

SERCA 2a polyclonal antibodies (gift from Dr F. Wuytack) were as described previously. The anti-chicken pectoralis RyR monoclonal antibody (MA3-925) was obtained from ABR, Inc (Golden, Colorado). Consecutive serial ventricular cryosections (5-μm thick, fixed as in next section) were incubated for 90 minutes at room temperature with a-SERCA 2a (1/150) and a-RyR (1/150). After 3 washings, the sections were incubated with a 30-fold dilution of Texas red conjugated anti-rabbit IgG and FITC conjugated anti-mouse IgG (Amersham). The sections were mounted in mounting medium for immunofluorescence (Fluoprep, BioMérieux) and analyzed using an epifluorescence microscope (Leica).

#### In Situ Hybridization

Rat hearts were divided transversally in 2 fragments, fixed in 2% paraformaldehyde (PFA) diluted in PBS for 2 hours at 4°C, washed in PBS plus 30% sucrose for 4 hours at 4°C, embedded in OCT (RUA), and frozen in liquid nitrogen– precooled isopentane. Serial cryosections (5-μm thick) were fixed in 4% PFA for 5 minutes, dehydrated in ethanol, and stored at −70°C with dessicant until use.

In situ hybridization conditions were as previously described. After prehybridization, 7 μL of hybridization mixture was applied to each section. Alternate serial sections were incubated with SERCA 2a, 2b, and 3; RyR2; and IP3R probes at 50°C overnight. After washings, RNase A treatment (20 μg/mL at 37°C for 30 minutes), and dehydration, the slides were immersed in Kodak NTB2 Nuclear track emulsion (Eastman Kodak) and autoradiographed. Sections were developed after 15 days in Kodak D19, mounted, and examined by light- and dark-field illumination.

#### Quantification of In Situ Hybridization Signals

Dark-field images magnified 100-fold were recorded as their light-field equivalent using a CCD videocamera (Hamamatsu C2400) and a computer (Power Macintosh) equipped with Optilab software (Graphitek). All slides were recorded under identical microscope lighting and camera settings, coded for unbiased blind analysis, and stored on zip disks. After recall, the hybridization signals appeared on the monitor as white grains. The 35 S-sensitized emulsion generated very homogeneous grains with surfaces of 2 to 5 pixels. The computer was instructed to count only perfectly white pixels (level 1 to 3 to 3 to 3 to 35 and 8604 to 9144, respectively, of the sequences previously published. The anti-chicken pectoralis RyR monoclonal antibody (MA3-925) was obtained from ABR, Inc (Golden, Colorado). Consecutive serial ventricular cryosections (5-μm thick, fixed as in next section) were incubated for 90 minutes at room temperature with a-SERCA 2a (1/150) and a-RyR (1/150). After 3 washings, the sections were incubated with a 30-fold dilution of Texas red conjugated anti-rabbit IgG and FITC conjugated anti-mouse IgG (Amersham). The sections were mounted in mounting medium for immunofluorescence (Fluoprep, BioMérieux) and analyzed using an epifluorescence microscope (Leica).
RNase Protection and RT-PCR Analysis
Total RNA from rat LV was extracted by the RNA-quick procedure (Bioprobe). Ten \( \mu g \) of total RNA was used for RNase protection analyses, which were performed according to the Ambion (Clini-sciences) protocol and as described previously.\(^1\) Each experiment included a control reaction in which total RNA was replaced by \( 10 \mu g \) of yeast tRNA.

Firststrand cDNA synthesis was performed on \( 5 \mu g \) of total RNA from LV of Sh and AS and \( 1 \mu g \) of RNA from brain using random hexamers at 42°C. Amplification was performed with an initial step of 120 seconds at 95°C and 40 cycles of 20 seconds each at 95°C, 30 seconds at 60°C and 30 seconds at 72°C, and a final step of 7 minutes at 72°C using oligonucleotides common to all IP3 R types as described.\(^3,7\) The PCR products were resolved in triplicates on 8% acrylamide gels, transferred to nylon membranes, and hybridized with \( ^{32}P \)-labeled oligonucleotides specific for each isoform, as in Perez et al.\(^3,7\)

Slot-Blot Analysis
Eight, 4, and \( 2 \mu g \) of total RNA were denatured and spotted onto the nylon membrane using a minifold apparatus (Schleicher & Schuell, Inc) After UV cross-linking, the membranes were prehybridized and hybridized with \( ^{32}P \)-labeled SERCA 2(a+b) and RyR2 probes as in De la Bastie et al.\(^10\) After dehybridization, a 24-mer oligonucleotide complementary to the rat 18S ribosomal RNA (\( ^{32}P \)-labeled using \( \gamma^{32}P \)-ATP and polynucleotide kinase) was hybridized in the absence of formamide to the same membrane.\(^10\) After hybridization and washing, the membrane was exposed to x-ray film for 1 to 4 days.

Statistical Analysis
For each sample, the densitometric values obtained for the SERCA 2a and 2b and RyR2 probes were divided by the densitometric values obtained with the 18S oligonucleotides. The ratio was determined for various amounts of RNA loaded and the values were expressed as mean±SE. Differences between independent samples were tested for significance by a nonparametric transformation of the unpaired \( t \) test: the Mann-Whitney \( U \) test.

Results
Specificity of the Probes
The specificity of SERCA 2a, 2b, and 3 cRNA probes has already been described.\(^1\) The specificity of RyR2 and IP3 R probes was determined by RNase protection and RT-PCR (Figure 1). The same 2 bands were observed in all samples by RNase protection analysis, the upper band corresponded to
type 1 IP,R and the second band to another isoform which could be type 2 or 3 (Figure 1A). RT-PCR analysis indicated that IP,R-1 and -2 (Figure 1B) were both expressed in the heart but type 3 was not (not shown). These data indicated that IP3 R-1 is the major isoform expressed in the heart, as recently described. No major change in the relative proportion of each isoform was observed in hypertrophy. The RyR2 probe hybridized to only 1 band of the expected size in cardiac mRNA. High levels of RyR2 mRNA were detected in the heart and only a faint signal was present in aorta (Figure 1C).

Quantitative Changes in SERCA 2 and RyR2 Expression in Rat Heart Subjected to a Sudden Pressure Overload

LV hypertrophy was determined as the ratio LV weight:body weight in operated versus Sh animals. As noted previously,33,38 coarctation of the ascending aorta in young rat induced a rapid (16.5±3.2% at day 1; 40.4±8.1% at day 5) and severe cardiac hypertrophy (116.3±8.1% after 1 month). One month after surgery, SERCA 2(a,b) and RyR2 mRNA levels were 60% and 40% lower in AS than in Sh animals (AS, n=5; Sh, n=4; P<0.01); this confirmed our previous data.10,29 Interestingly, the SERCA 2(a+b) and RyR2 mRNA levels were significantly higher than control values by day 1 (AS, n=4; Sh, n=6; P<0.05), but on day 5, the relative expression of both transcripts in the experimental group were close to the control values (AS, n=4; Sh, n=4; NS) (Figure 2).

Qualitative Changes in SERCA 2a and RyR2 mRNA Distribution During Development of Cardiac Hypertrophy

35S-labeled SERCA 2a and RyR2 probes gave strong hybridization signals in striated ventricular myocytes of controls (Figures 3A: a, b). One (Figure 3A: c, d) and 5 days (Figure 3A: e, f) after surgery, SERCA 2a and RyR2 mRNAs were found in most cardiomyocytes, although the distribution of SERCA 2a was heterogeneous in the LV but not in the right ventricle (not shown) or atrium. One month after surgery, substantial changes in the hybridization pattern were observed in the hypertrophied LV (Figure 3A: g, h; Figure 4). The hybridization signals with SERCA 2a and RyR2 probes were weaker in the hypertrophied LV than in the normal one (Figure 3A: g, h versus a, b). Labeled sense sequences synthesized in vitro from the same plasmids gave only background (Figure 3B). In AS (versus Sh), SERCA 2a mRNA level was markedly decreased in both left atrium and ventricle (Figure 4b versus 4a); it was higher in the right than in the left ventricle of AS (Figure 4).

At higher magnification, SERCA 2a mRNA signal in AS appeared to vary from cell to cell (Figure 5C and 5G), whereas the distribution of RyR2 mRNA appeared as small

![Figure 2. A, Slot-blot containing 3 different concentrations of total RNA from Sh and AS of 1 day and 1 month and RNA from liver (L) and tensor fascia latae (TFL) hybridized successively with SERCA 2, RyR2, and 18S probes. B, SERCA 2 and RyR2 mRNA levels (derived from the slot-blot analysis) at 1 day (n=4 for AS and n=6 for Sh), 5 days (n=4 for AS and n=4 for Sh), and 1 month (n=5 for AS and n=4 for Sh) after aortic banding. For each of the 3 groups of animals, the values represent the percentage of the mean value obtained for the age-matched Sh animals. Values are expressed as mean±SEM. *P<0.05; **P<0.01 versus Sh.](http://circ.ahajournals.org/)}
dots homogeneously distributed in the myocardium (Figure 5D). The RyR2 distribution was similar to that reported by Go et al.31

The amount of SERCA 2a and RyR2 mRNAs within the myocyte population was precisely analyzed at 1 month after surgery. The frequency histogram shows that in AS and Sh animals, the myocyte population follows a gaussian distribution as a function of mRNA concentration (Figure 6). The histogram of AS myocytes was significantly shifted toward lower values. As a result, myocytes containing high amount of SERCA 2a mRNA (140<wpm<210), which represented 45% of the population, decrease to <5% 1 month after AS. A similar leftward shift of frequency histogram was observed for RyR2 mRNA. Therefore, the mean hybridization signal per cell for SERCA 2a and RyR2 mRNAs decreased, respectively, from 123±22 wpm and 139±12 wpm in Sh animals to 80±8 wpm and 76±10 wpm in the AS myocytes (P<0.05, n=3 animals).

SERCA 2a and RyR protein distribution was assessed with specific antibodies (Figure 7): RyR2 (Figure 7a and 7d) and
SERCA 2 (Figure 7b and 7e) were found mainly in the cardiomyocytes. SERCA 2a was markedly heterogeneous in AS myocardium, with little signal being detected in some myocytes (Figure 7e). The amount of RyR2 was greatly decreased in AS hearts compared with controls but the distribution remained homogeneous (Figure 7a and d). Only background signal was observed when the first specific antibodies were omitted (Figure 7g and 7h).

Changes in SERCA 2a mRNA and RyR2 mRNA Levels Were Not Associated With Changes in SERCA 2b, SERCA 3, and IP3 R During Development of Cardiac Hypertrophy

Using in situ hybridization, we observed that SERCA 2b mRNA was equally present in cardiocytes from Sh and AS at 1 month after surgery, whereas a 4-fold increase was observed in the coronary arteries from AS ($P<0.01$ versus Sh) (Figures 8a, 8b, and 9a). IP3 R mRNA was more abundant in vessels (75 AU) than in myocytes (20 AU), as previously described. No change was observed at 1 month after AS in IP3 R mRNA abundance (Figures 8c, 8d, and 9b). In both Sh and AS, SERCA 3 mRNA was only detected in coronary endothelial cells (Figure 8e and 8f).

Discussion

The major finding of the present study is that the decrease in SERCA 2a mRNA and protein levels is heterogeneous throughout the hypertrophied LV myocardium. The decrease in SERCA 2a mRNA level was substantial in some myocytes (Figures 5 and 6) and was associated with a disappearance of the protein (Figure 7), whereas in other cells both protein and mRNA were abundant. Differences in SERCA 2a level between myocytes were observed throughout the entire LV, suggesting that not only an increase in transmural pressure but also humoral factors may be involved in the down-regulation of SERCA 2 gene expression. The disparity of neighboring cardiomyocytes either expressing or not expressing SERCA 2a may be deleterious for myocardial function because it could lead to different relaxation properties of myocytes. Indeed, recent data obtained on transgenic animals and isolated cardiocytes overexpressing SERCA 2 confirms the key role of this enzyme in cardiac function. SERCA 2 overexpression results in (1) increase in rate of the $Ca^{2+}$ transient decline and of cardiocyte relaxation indicating faster decrease in cytosolic $Ca^{2+}$ concentration, (2) increase in rate of myocyte shortening and of development of left ventricular pressure indicating faster $Ca^{2+}$ release, and (3) shortening of the time to half maximum postrest potentiation, indicating faster rate of SR $Ca^{2+}$ loading. In pressure overload–induced hypertrophy, prolonged myocardial relaxation has been demonstrated at the level of muscle fibers or isolated myocytes and was associated with reduced SERCA expression. On the other hand, SR function has been measured on total hearts and isolated cells in a similar model of hypertrophy. Surprisingly, the $Ca^{2+}$ balance in isolated hypertrophied cells was normal when normalized to cell volume, despite significant changes in myocardial relaxation and depressed SERCA 2 gene expression and $Ca^{2+}$ uptake rate measured on muscle. The authors pointed out that isolated cells might be the best survivors of the isolation procedure and represent a population of well-compensated cells with normal $Ca^{2+}$ balance that may not represent the total cell population. Reduced contractility, alteration of the $Ca^{2+}$ transient and of the ability of the $Ca^{2+}$ current to trigger $Ca^{2+}$ release, was also reported in strains of genetically hypertensive rats and in rats with heart failure. Because the density, or $Ca^{2+}$ sensitivity, of RyR was unaltered in these particular models, the authors proposed that alteration of E-C coupling was due to changes in the relation between the RyR and the plasma membrane $Ca^{2+}$ channel. In fact, all these alterations, including cell-to-cell heterogeneity, are likely to reflect different stages of the progression from moderate compensated hypertrophy to heart failure.

Figure 4. Expression of SERCA 2a mRNA in the atria (A), right (RV) and left ventricle (LV) of rat 1 month after AS. Compared with Sh (a, c), the SERCA 2 mRNA level is decreased in AS (b, d). However, hybridization signal is higher in the right than in the left ventricle (d). Note also the decreased SERCA 2a mRNA level in the left atrium (b). Magnification $\times 12$. 

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In our model of cardiac hypertrophy, we showed that the decrease in expression of SERCA 2a and RyR2 is a late event which is preceded by a transient increase (1 day) in the 2 specific mRNAs when the cardiac hypertrophy had not yet developed (Figure 2). This alteration in gene expression might be a nonspecific response due to global activation of the cardiac genome at the onset of adaptive response to overload as previously suggested for other gene products. However, such an increase in calcium-handling proteins has also been observed rapidly after an ischemic injury in the rat heart, suggesting that other regulatory processes might be involved. Five days after surgery, we observed that the level of SERCA 2 and RyR2 mRNAs return to basal level. Interestingly, a transient decrease in transcript level has also

Figure 5. Dark-field image of normal (A, B, E, F) and hypertrophied myocardium 1 month after AS (C, D, G, H) hybridized with SERCA 2a (A, C, E, G) and RyR2 (B, D) cRNA probes. F and H, Interferential contrast of E and G, respectively. Note that SERCA 2a hybridization signal varies between myocytes in both longitudinal (C) and transverse (G) sections. In contrast, RyR2 signal is equally decreased throughout the myocyte population. Some myocytes appeared almost free of SERCA 2 mRNA (arrowhead) compared with neighboring myocytes (small arrow) Magnification ×100.

Figure 6. Effect of pressure overload on SERCA 2a and RyR2 mRNA content in the cardiomyocyte population. Frequency histograms displaying the percentage of myocytes with various amounts of white pixels per myocyte (wpm) after in situ hybridization with either SERCA 2a or RyR2 35S-cRNA. Values are mean±SEM of 3 to 4 animals in each group. The percentage of myocytes expressing few or no (<80 AU) SERCA 2a and RyR2 mRNA is significantly higher in hypertrophied cardiomyocytes than in controls (P<0.001, χ² test).
been observed for genes such as ANF and β-MHC 5 to 7 days after surgery. Afterward, when the upregulation of these genes that are considered as markers of hypertrophy is sustained, the expression of SERCA 2 and RyR2 mRNAs is still repressed. The amplitude of the decrease in SERCA 2 and RyR2 mRNA levels is similar to that previously observed in other models of cardiac hypertrophy in the rat and Syrian hamsters. The reduction in SERCA 2 and RyR2 expression is

Figure 7. LV sections of Sh animals (a through c) and rats 1 month after AS (d through f). Double-immunolabelings with anti-RyR2 (a, d) and anti-SERCA 2a (b, e) antibodies and interferential phase contrast (c, f). Note the absence of label in the vessels and the marked decrease in the amount of SERCA 2a and RyR2 protein in AS. Arrows point to 2 consecutive cardiocytes from AS animals with various amounts of SERCA 2a and identical amounts of RyR. In absence of a-SERCA 2a (g) and a-RyR2 (h) antibodies, only low background and elastic lamina brightness was observed. Magnification ×400.

Figure 8. Nonconsecutive serial sections of left ventricles of Sh rats (a, c, e) and rats studied 1 month after AS (b, d, f), hybridized with SERCA 2b (a, b), IP₃R (c, d), and SERCA 3 (e, f) cRNA probes. In Sh animals, SERCA 2b and IP₃R mRNAs are ubiquitously distributed, whereas SERCA 3 mRNA is detected in the intimal layer of the coronary artery. Note that SERCA 2b level is increased only in the coronary artery. Magnification ×150.
smooth muscle cells as previously suggested. The change in can be related to a shift toward more immature phenotype of The enhanced expression of a ubiquitous SERCA 2 isoform mRNA level in the coronary arteries at 1 month after surgery. human heart failure. by IP3 R mRNA increase. Moreover, we did not find any directions with decrease in RyR mRNAs being accompanied of the other minor components (SERCA 2b, SERCA 3, IP, R). This is consistent with the concept that the decrease in SR

only relative and probably reflects a nonactivation of the genes which do not follow the global increase in gene expression leading to hypertrophy. In some studies, reduction in SERCA mRNA levels, observed only 4 to 5 months after surgery, was considered a molecular marker for impaired cardiac performance during the transition from compensated hypertrophy to failure. In our study, cardiac hypertrophy developed more rapidly as it reached 116.3% 1 month after surgery, (although the animals did not exhibit evident clinical signs of cardiac failure). The apparent discrepancy may be due to differences in the degree of coarctation and/or duration of the overload.

Another important finding of the present study is that the late downregulation of SERCA 2a mRNA within cardiomyocytes was not compensated by an increased expression of SERCA 2b or SERCA 3. In addition, the IP, R mRNA level was low and was unchanged in hypertrophy, indicating that the decrease in RyR2 mRNA level was also not compensated by upregulation of IP, R. These results are in contrast to those of Go et al., demonstrating that in failing human hearts the 2 intracellular Ca²⁺ release channels are regulated in opposite directions with decrease in RyR mRNAs being accompanied by IP, R mRNA increase. Moreover, we did not find any difference in the relative levels of the 2 IP, R isoforms between Sh and AS animals. Therefore, the increase in IP, R mRNA level previously observed may be a characteristic of human heart failure.

Finally, the data indicated an increase in SERCA 2b mRNA level in the coronary arteries at 1 month after surgery. The enhanced expression of a ubiquitous SERCA 2 isoform can be related to a shift toward more immature phenotype of smooth muscle cells as previously suggested. The change in SERCA 2b expression in vessels, however, was not associated with an alteration in IP, R mRNA level.

In summary, the present study indicates that the pressure overload–induced decrease of SERCA 2a and RyR2 mRNA levels is a late event and a heterogeneous process. The reduction in cardiocytes expression of the main Ca²⁺ pump and Ca²⁺ release channel is not compensated by the induction of the other minor components (SERCA 2b, SERCA 3, IP, R). This is consistent with the concept that the decrease in SR Ca²⁺ -ATPase and Ca²⁺ release channel levels are involved in the alteration of the excitation-contraction coupling.

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