Age-Related Changes in Sarcoplasmic Reticulum
Ca$^{2+}$-ATPase and $\alpha$-Smooth Muscle Actin Gene
Expression in Aortas of Normotensive and
Spontaneously Hypertensive Rats

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The expression of the sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase gene and the SR Ca$^{2+}$ pump function were investigated in thoracic aortas of 5- and 17-week-old normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). The relative level of the two isoforms of SR Ca$^{2+}$-ATPase mRNA expressed in the aorta (i.e., SERCA 2a and SERCA 2b) was determined by quantitative S1 nuclease protection analysis and normalized to the level of $\alpha$-smooth muscle ($\alpha$-Sm) actin mRNA. The level of $\alpha$-Sm actin mRNA itself was normalized to the level of 18S ribosomal RNA using slot-blot hybridization assays. Total SR Ca$^{2+}$ pump activity was estimated by measuring the rate of oxalate-supported Ca$^{2+}$ uptake in homogenates. At 5 weeks, the amount of SERCA 2a and SERCA 2b mRNA, normalized to 18S ribosomal RNA, and the ratio of $\alpha$-Sm actin mRNA to 18S RNA were identical in SHR and WKY rats. The Ca$^{2+}$ pump activity was similar in the two strains of rats at 5 weeks. From 5 to 17 weeks, the amount of SERCA 2a mRNA increased in both strains while the level of SERCA 2b mRNA remained constant. The Ca$^{2+}$ pump activity was unchanged in SHRs and tended to decrease in WKY rats. Accordingly, the change in the ratio of the SR Ca$^{2+}$-ATPase mRNA isoforms does not appear to influence SR function. The level of $\alpha$-Sm actin mRNA and SERCA 2a mRNA increased in parallel from 5 to 17 weeks in both strains. The regulation of the expression of SERCA 2a and $\alpha$-Sm actin mRNA seems to be coordinated, whereas that of SERCA 2b and $\alpha$-Sm actin mRNA appears to be unrelated. When related to $\alpha$-Sm actin mRNA, the level of total SR Ca$^{2+}$-ATPase mRNA at 17 weeks in thoracic aortas was lower in SHR than in WKY rats. (Circulation Research 1993;72:341–348)

KEY WORDS • sarcoplasmic reticulum Ca$^{2+}$-ATPase • $\alpha$-smooth muscle actin • rat aortas • hypertension • aging

When compared with Wistar-Kyoto (WKY) normotensive rat arteries, large arteries of spontaneously hypertensive rats (SHRs) develop a thicker media during growth, with hypertrophy and polyplody of vascular smooth muscle cells.1-2 Of note, in other models of hypertension, hyperplasia may occur.3-4 Vascular smooth muscle cell growth is accompanied by quantitative and qualitative changes in gene expression that involve cytoskeletal proteins and contractile proteins.5-6 Specifically, an increased expression of $\alpha$-smooth muscle ($\alpha$-Sm) actin as opposed to non-muscle $\beta$-actin appears to be responsible for the increase in actin content observed during development6-8 and the hypertrophy induced by angiotensin II or arginine vasopressin.5

Vascular relaxation is impaired in aortic strips from hypertensive rats, and intracellular Ca$^{2+}$ concentration is increased.9-12 In large arteries, relaxation and regulation of cytosolic Ca$^{2+}$ concentration are predominantly mediated by sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase.13 Alterations in gene expression could be responsible for the reduced SR Ca$^{2+}$ transport in preparations from aortas of hypertensive compared with normotensive rats.14-16 Two SR Ca$^{2+}$-ATPase isoforms, produced by alternative splicing of a single gene, are expressed in the aorta: SERCA 2a, which is also expressed in heart, slow skeletal muscle, and some smooth muscles, and SERCA 2b, which is expressed in smooth muscles and in non-muscle tissues.17-23 The functional significance of the presence of the two isoforms in vascular smooth muscle is unclear. Conversion from the 2b to the 2a Ca$^{2+}$-ATPase phenotype occurs during differentiation of the BC3H1 cell line toward the muscle phenotype24 but is not observed during differentiation of the C2C12 muscle cell line.25

The expression of the SR Ca$^{2+}$-ATPase gene in relation to SR Ca$^{2+}$ pump activity was studied in rat aortas during normal development in normotensive
WKY rats and under the influence of age-related increases in arterial blood pressure in SHR. The study was performed on young SHR (5 weeks, before the increase in blood pressure) and on adult SHR (17 weeks, when blood pressure is markedly elevated). The level of expression of SERCA 2a and SERCA 2b mRNA isoforms was compared with that of the α-Sm actin gene, which encodes the major contractile protein of the aorta.

Material and Methods

Animals

SHR and normotensive WKY rats of 5 and 17 weeks were obtained from Ifa Credo, France. Arterial blood pressure was measured by the tail-cuff method (blood pressure recorder 8005, W+W Electronics, Apelab, France). Mean blood pressures of 5-week-old SHR and WKY rats were 124±4 mm Hg (n=27) and 122±4 mm Hg (n=27), respectively (p=NS). At 17 weeks, the mean blood pressure was 148±2 mm Hg (n=41) in WKY rats and 234±4 mm Hg (n=41) in SHR (p<0.001). Animals from each strain and each age were divided into two groups. One was used to investigate gene expression, and the other to study SR function. After intraperitoneal injection of pentobarbital or cervical dislocation, the descending portion of thoracic aortas (aortic root excluded) was dissected and prepared in a cold saline solution. The adventitia was removed, and the medial layer was frozen in liquid nitrogen for the samples used for RNA preparation. The samples used to measure the Ca2+ pump activity were processed immediately.

RNA Preparations

Aortas from 3 rats were pooled and stored at −80°C. Heart, liver, intestine, and fast skeletal muscle (tissue of fascia lata) from normal Wistar rats as well as aortic smooth muscle cells in subculture (more than four passages) were used as controls. Total RNA was prepared using the guanidinium isothiocyanate–phenol–chloroform method.26

Probes

Three probes were used. The first was a 24-nucleotide oligomer complementary to nucleotides 1,046–1,070 of the rat 18S ribosomal RNA,27 which was labeled at its 5’ end using [γ-32P]ATP and polyoxynucleotide kinase. It was diluted to a specific activity of 5×10⁶ dpm/μg without unlabeled oligomer. The second was a 130-nucleotide Dde I–HindIII DNA fragment complementary to the 3’ untranslated region of the α-Sm actin mRNA that was subcloned in the pSP64 vector.28 A radiolabeled fragment was synthesized by primer extension from the SP6 promoter primer after denaturation and linearization by EcoRI of the double-stranded DNA. The third probe was the most 3’ Pst I fragment from the rat cardiac Ca2+-ATPase cDNA subcloned in the phage M13.21

Filter Hybridization Assays

Two and 4 μg total RNA were size-fractionated on 3% formaldehyde agarose gels and transferred to a nylon membrane (Hybond N, Amersham) by capillarity. After transfer, RNA was cross-linked by ultraviolet irradiation. Sequential hybridizations of the irradiated membranes were carried out with the α-Sm actin probe and thereafter with the 18S probe. Prehybridization and hybridization were performed at 42°C, as already described, in the absence of formamide for the ribosomal probe and in the presence of 50% formamide for the α-Sm actin probe. The final washing conditions were as follows: 1× standard saline citrate (0.15 M sodium chloride and 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 45°C for 30 minutes for the α-Sm actin probe and 2× standard saline citrate at room temperature for 30 minutes for the 18S probe. After the last washing, the membranes were exposed to x-ray films.

Quantitation of α-Sm actin RNA was performed by slot-blot analysis. Total RNA (1, 2, and 4 μg) was denatured for 15 minutes at 65°C in 15× standard saline citrate and 3% formaldehyde and directly spotted onto the membrane. The membranes were treated as described above for Northern analysis.

S1 Nuclease Protection Assays

Total RNA was hybridized simultaneously to specific SR Ca2+-ATPase and α-Sm actin cDNA probes. The antisense Ca2+-ATPase probe was transcribed from the single-stranded template using a primer located in the 3’ untranslated region, a mixture of the four deoxynucleotides including [α-32P]dATP (3,000 Ci/mmol) and DNA polymerase I (Boehringer Mannheim). The single-stranded 507-nucleotide fragment released by Pst I digestion, which included the last 373 nucleotides of the coding sequence and 134 nucleotides of the 3’ untranslated region, was purified on 6% acrylamide sequencing gels.

The actin probe was labeled by primer extension as described previously. After linearization by EcoRI, the 200-nucleotide single-stranded DNA fragment, containing the α-Sm actin sequence and 70 nucleotides of vector sequence, was separated from the large DNA fragment on denaturing gels.

Both probes were simultaneously hybridized to total RNA at 39°C in 80% formamide for 16 hours. Nuclease S1 digestion was performed at 26°C for 1 hour in the presence of 20 or 100 units nuclease S1 per microgram RNA, depending of the nuclease batch. Each experiment included a control reaction in which total RNA was replaced by 5 μg yeast tRNA. The probes had been prehybridized separately to total RNA to rule out potential interaction and inhibition of specific hybridization. To validate the approach, simultaneous hybridization of various amounts of total RNA from uterine muscle to the two probes and subsequent digestion with a fixed amount of S1 nuclease were carried out. After digestion, the protected probes were separated by electrophoresis on 6% denaturing polyacrylamide gels. The gels were dried and exposed to x-ray films for 2 days.

Evaluation of the Amount of Specific RNA

The autoradiograms corresponding to S1 nuclease protection assays or to slot blots were analyzed by densitometry.

Densitometric analysis was performed on unsaturated autoradiograms. α-Sm actin levels were corrected for the amount of total RNA applied on the membrane by calculating the ratio of the signals obtained with the
α-Sm actin and the 18S probes for each RNA sample at 1, 2, and 4 μg RNA and averaging the three values. The intensity ratios of SERCA 2a to SERCA 2b mRNAs and SERCA mRNA isoforms to α-Sm actin mRNA were calculated for each sample from the S1 nuclease protection assays and multiplied by 100. Because the probe was uniformly labeled and completely protected by SERCA 2a mRNA but only partially protected by SERCA 2b mRNA, the 2a/2b ratio is only indicative of changes that may occur during aging but not of the actual relative amount of each SERCA mRNA present in the samples. Each RNA sample was tested at least twice. The n value represents the number of independent RNA samples tested in each group.

Preparation of Crude Homogenates

The aortic tissue was weighed, finely chopped, and homogenized for 1–3 minutes at 4°C using a PolyLab P21 glass homogenizer in 0.7 ml isolation medium containing 0.1 M KCl, 5 mM sodium azide, 30 mM Tris-HCl buffer (pH 7.4), and 1 mM dithiothreitol. The sample was centrifuged in an Eppendorf centrifuge (Eppendorf Inc., Fremont, Calif.) at 1,250g for 2 minutes. The supernatant was collected, and the pellet was resuspended in 0.5 ml isolation medium. The extraction procedure was performed three times. The pooled postnuclear supernatants contained more than 80% of the total calcium pump activity of the thoracic aortas.

Measurements of Calcium Uptake

The calcium pump activity of the postnuclear fractions was measured 1–2 hours after the isolation. A standard filtration technique (Millipore Corp., Bedford, Mass.) was used.29,30 The incubation medium contained 0.1 M KCl, 5 mM sodium azide, 5 mM ATP, 6 mM MgCl₂, 10 mM potassium oxalate, 30 mM Tris-HCl (pH 7.0), 0.15 mM EGTA, and 0.12 mM 45CaCl₂ (concentration of ionized calcium, 0.9 μM). The reaction was initiated by adding 5–20 μg protein to 0.5 ml medium (37°C) and terminated at 10 minutes by filtration through 0.45-μm Millipore filters. The oxalate-supported Ca²⁺ uptake, which represents the activity of the SR in various types of muscle,30,31 was linear for at least 15 minutes. Nonspecific binding of calcium was estimated in the presence of 5 μM calcium ionophore A23187 or in the absence of either ATP or oxalate and did not exceed 7% of the value obtained under our standard conditions.

Concentration of protein was measured by a protein assay (Bio-Rad Laboratories, Richmond, Calif.). Bovine serum albumin was used as a standard.

Statistical Analysis

Each RNA sample was analyzed twice, and values were averaged. Three measurements were performed for Ca²⁺ uptake and averaged. Values represent mean±SEM. Differences between independent samples were tested for significance by a nonparametric transformant of the unpaired t test: the Wilcoxon–Mann-Whitney U test. A value of p≤0.05 was considered significant.

Results

Relative Level of Expression of the Two SR Ca²⁺-ATPase mRNA Isoforms

Hybridization of total RNA from aortas of 5- and 17-week-old SHR and WKY rats to the Ca²⁺-ATPase probe yielded two fragments (Figure 1, left panel). The fully protected fragment (507 bp) corresponds to the 2a isoform, which is also present in heart (Figure 1, right panel). The partially protected fragment (360 bp) corresponds to the 2b isoform, which is expressed in smooth muscle and nonmuscle tissues (Figure 1). Simultaneous hybridization to the α-Sm actin probe yielded additional fragments. The fully protected fragment was shorter than the probe because of the presence of vector sequences in the cDNA. The other fragments probably originated from partial nilling in a homologous but AT-rich region. All the fragments were specific for α-Sm actin since they were not observed after hybridization to total RNA from heart (α-cardiac), intestine, or aortic cells in subculture (β and γ) (Figure 1, right panel). Quantification of α-Sm actin mRNA included all the fragments. None of the probes hybridized to yeast tRNA.

The relative levels of 2a and 2b isoforms were similar at 5 weeks in aortas of SHR and WKY rats (Figures 1, left panel, and 2). From 5 to 17 weeks, the ratio of SERCA 2a to SERCA 2b mRNA increased by 94% in WKY rats (p<0.05) and by 84% in SHRs (p=0.01). At 17 weeks, the ratio of 2a to 2b isoforms was slightly greater in thoracic aortas of WKY rats when compared with that of SHRs (60.7±4.8 versus 47.1±3.1, p<0.05) (Figure 2).

Quantification of the α-Sm Actin mRNA

Northern blot analysis of total RNA from thoracic aortas of 17-week-old SHR and WKY rats indicates that under our hybridization conditions the α-Sm actin probe reacted with a single band migrating at 1.7 kb that was not present with heart (Figure 3), skeletal muscle, and liver (not shown) RNAs. These data confirmed the specificity of the probe previously demonstrated by Kocher and Gabbiani.28 The same blot was hybridized to an 18S ribosomal RNA probe to normalize the amount of α-Sm actin mRNA to the amount of total RNA present on the blot. The level of α-Sm actin mRNA was then quantified by slot-blot hybridization in the same conditions, and the α-Sm actin mRNA/18S RNA ratios are illustrated in Figure 4.

The amount of α-Sm actin mRNA was similar at 5 weeks in SHR and WKY rats. From 5 to 17 weeks, the level of α-Sm actin mRNA increased by 36% in thoracic aortas of WKY rats (p<0.05) and by 83% in thoracic aortas of SHRs (p<0.01).

Level of Expression of the Two Ca²⁺-ATPase mRNA Isoforms Normalized to 18S RNA and α-Sm Actin mRNA

The amounts of SERCA 2a and SERCA 2b, normalized to 18S RNA, in aortas of 5- and 17-week-old SHR and WKY rats are illustrated in Figure 5. At 5 weeks, the level of SERCA 2a was similar in thoracic aortas of SHR and WKY rats. From 5 to 17 weeks, the amount of SERCA 2a increased by 53% in thoracic aortas of WKY rats (p<0.05) and by 131% in thoracic aortas of SHRs (p<0.01), whereas the amount of SERCA 2b mRNA did not vary between 5 and 17 weeks in the aortas of both SHR and WKY rats.

The ratio of SERCA 2a to α-Sm actin (×100) did not change significantly from 5 to 17 weeks in WKY rats and SHRs (8.5±1.5 versus 13.7±3.5 and 4.1±0.5 versus
4.8±0.5, respectively). The ratio of SERCA 2b to α-Sm actin (×100) decreased from 5 to 17 weeks in SHRs (21.8±2.2 versus 10.1±1.4, p<0.01), whereas it remained constant in WKY rats (21.2±3.5 versus 21.7±1.8, p=NS). Consequently, the ratio of total SR Ca\(^{2+}\)-ATPase mRNA to α-Sm actin mRNA (×100) was lower at 17 weeks in SHR aortas than in WKY aortas (15.2±1.7 versus 34.9±6.4, p<0.05).

**Calcium Uptake Measurements in Homogenates**

The oxalate-supported calcium uptake, which reflects the activity of the SR Ca\(^{2+}\)-ATPase,\(^{30,31}\) was measured in postnuclear fractions (Table 1). Age did not significantly affect the velocity of calcium transport, which was normalized to the weight of the tissue in SHRs. However, in WKY rats, there was a nonsignificant tendency to decrease (p=0.06). At 17 weeks, the activity of the postnuclear fraction from SHR aortas was significantly greater than that from WKY aortas (p=0.02), whereas at 5 weeks, there was no significant difference in activity in preparations from SHR and WKY rats.

**Discussion**

The present data clearly indicate that from 5 to 17 weeks the level of SERCA 2a mRNA increases in parallel to that of α-Sm actin mRNA, whereas the level of SERCA 2b remains unchanged. This change in phenotype does not appear to influence SR function since with age the Ca\(^{2+}\) pump activity was unchanged in SHRs and tended to decrease in WKY rats. In addition, our data demonstrate that the SR Ca\(^{2+}\) pump activity is not depressed, as previously reported, but rather slightly increased in aortas from adult SHRs when compared with WKY rats.

The levels of SERCA 2a and 2b and α-Sm actin mRNAs were normalized to 18S ribosomal RNA, since the level of 18S ribosomal RNA closely reflects both muscle mass and total protein. When normalized to 18S ribosomal RNA, the SERCA mRNA levels can then be compared with SR Ca\(^{2+}\) pump activity, determined on postnuclear fractions of crude homogenates, and expressed per gram tissue.\(^{29,30}\)

To our knowledge, a shift in the SERCA phenotype is for the first time demonstrated in vascular smooth muscle cells. Increase in SERCA 2a mRNA without alteration in SERCA 2b was also documented during the differentiation of the BC3H1 cell line (obtained from a brain tumor) toward the striated muscle phenotype.\(^{24}\) In contrast, in another cell line with a striated muscle phenotype, C2C12, which expresses the SERCA 2 gene, the 2b isoform is present in differentiated and undifferentiated cells. The SERCA 2 gene appears to be transcriptionally activated in C2C12 but is not differentially spliced.\(^{25}\) The SERCA 2 gene is also expressed in

**Figure 1.** Analysis of total RNA from thoracic aortas of 5- and 17-week-old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats (left panel) and RNA from rat heart, intestine, and aortic cells in subculture (Ao Subcult) (right panel) by S1 nuclease protection assay. Yeast tRNA was used as a control of unspecific hybridization and of complete digestion of the probe. When RNAs were hybridized to the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase probe, two protected fragments were observed: a fully protected fragment, 2a, and a partially protected one, 2b. Simultaneous hybridization of the RNAs to the α-smooth muscle (α-Sm) actin probe resulted in the bands migrating below the undigested probe. RNA from heart, intestine, and subcultured aortic cells did not hybridize to the α-Sm actin probe. The fragment detected with RNA from intestine was of the same size than the probe and thus represents undigested DNA. The size of the undigested probes is indicated in the lane probe in the left panel and in the lane labeled no RNA in the right panel.
heart, where the total amount of SERCA 2a mRNA increases during development and decreases during aging. In contrast, SERCA 2b mRNA is present at a constant and extremely low level at all ages. The mechanisms responsible for the change in phenotype observed in vascular smooth muscle cells could involve alteration in the splicing pattern of the SERCA 2 gene or preferential stabilization and destabilization of one of the two mRNA populations.

The amount of Ca\textsuperscript{2+}-ATPase in homogenates was too low to be estimated by the immunological technique, and high basal phosphorylation prevented us from accurately measuring the Ca\textsuperscript{2+}-dependent phosphoenzyme, the latter finding confirming results obtained by Eggermont et al. With either approach, the Ca\textsuperscript{2+} pump protein could only be detected in microsomal fractions. Since comparison of the amount of SERCA mRNA within total RNA with the level of SERCA protein within microsomal protein may be misleading, we compared the mRNA level with the Ca\textsuperscript{2+} pump activity in homogenates.

Change in the relative amount of SERCA 2a and SERCA 2b mRNA isoforms did not appear to influence SR function. The level of SERCA 2a mRNA increased from 5 to 17 weeks in both SHR and WKY rats, but the activity of the SR Ca\textsuperscript{2+} pump did not significantly change in SHRs, and it rather tended to decrease in WKY rats. However, the relative amount of SERCA 2a increased from 15–20% of total Ca\textsuperscript{2+}-ATPase mRNA at 5 weeks to only 30–40% at 17 weeks. Such an increase may not be sufficient to detect a change in pump activity. The specific activity of SERCA 2a and SERCA 2b could not be measured in our conditions. SERCA 1, SERCA 2a, and SERCA 2b isoforms were recently shown to possess similar activity in a wide range of free Ca\textsuperscript{2+} concentration by transfection of cDNA corresponding to the different SERCA isoforms into Cos cells. Nevertheless, one cannot definitively rule out that, when expressed in the proper environment, the specific activity of SERCA 2a is lower than that of SERCA 2b.

The higher intracellular Ca\textsuperscript{2+} concentration observed in aortas of adult SHRs has been attributed to a lower SR Ca\textsuperscript{2+} pump activity (see review in Reference 16). The intracellular Ca\textsuperscript{2+} concentration has been reported to be similar in rat aortic cells from 4-week-old SHR and WKY rats and markedly increased in aortic cells...


TABLE I. Calcium Uptake in Postnuclear Fractions Isolated From Aortas of Spontaneously Hypertensive and Wistar-Kyoto Rats of Two Different Ages

<table>
<thead>
<tr>
<th>Calcium uptake</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nmol Ca²⁺·g tissue⁻¹·min⁻¹)</td>
<td></td>
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</tr>
<tr>
<td>5 Weeks</td>
<td>82.5±6.0</td>
<td>93.0±8.9</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(n=12)</td>
<td></td>
</tr>
<tr>
<td>17 Weeks</td>
<td>67.2±5.1*</td>
<td>82.7±4.4</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(n=11)</td>
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</tr>
</tbody>
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WKY, normotensive Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Values are mean±SEM.

The preparations were isolated and tested under conditions described in "Material and Methods." Since the calcium uptake was linear during the first 10 minutes, the activities are expressed as nanomoles per gram tissue per minute. Calcium uptake tended to decrease from 5 to 17 weeks in WKY rats \( p=0.06 \) but did not change in SHRs \( p=0.6 \).

\* \( p=0.02 \) vs. SHR at 17 weeks.

from 8- and 12-week-old SHR but not WKY rats. Since we did not find the function of SR to be depressed in adult SHRs compared with young normotensive SHRs but, to the contrary, to be slightly increased when compared with adult control rats, our data do not support the hypothesis that higher intracellular Ca²⁺ concentration results from depressed SR function.

Nevertheless, the change in SERCA phenotype may have some functional significance in view of recent data on Ca²⁺ compartmentalization in vascular smooth muscle cells. Two subpopulations of aortic smooth muscle cells, differing by their intracellular Ca²⁺ release mechanisms, have been described. Several Ca²⁺ compartments have also been found within a single cell. The various cell types or Ca²⁺ compartments may possess different ATPases and regulation of the expression of the SERCA isoforms may potentially control the Ca²⁺ pools. Analysis of the Ca²⁺ pools is important for understanding the mechanisms by which vasoactive substances exert their effects under both physiological and pathological conditions.

Our data confirm the important change in \( \alpha \)-Sm actin gene expression during development of the thoracic aorta in the rats. In addition, the development of hypertension appears to regulate the expression of the actin genes; at 17 weeks, when the rats had become hypertensive, the level of \( \alpha \)-Sm actin mRNA was greater than that in normotensive rats. However, when hypertension is induced by aortic constriction, the level of expression of the different actin genes does not appear to be altered. Interestingly, a significant decrease in total actin gene expression with a downregulation of the \( \alpha \)-Sm actin gene occurs in the aorta distal to the constriction. These variable results can be explained by different mechanisms underlying the increase in muscle mass: hypertrophy and polyplody of preexisting cells in SHRs and hyperplasia in the model of abrupt aortic constriction. Our findings indicate that both age and development of hypertension play an important role in the quantitative change in \( \alpha \)-Sm actin gene expression in the vascular smooth muscle.

The age-related increase in the level of SERCA 2a mRNA is associated with a similar increase in \( \alpha \)-Sm actin mRNA in thoracic aortas of both SHR and WKY rats. In contrast, the level of SERCA 2b mRNA, which represents the most abundant SR Ca²⁺-ATPase mRNA, does not increase during development in SHR and WKY rats. Consequently, in thoracic aortas of 17-week-old SHRs, which are characterized by an extremely high level of \( \alpha \)-Sm actin mRNA, the levels of total SR Ca²⁺-ATPase and \( \alpha \)-Sm actin mRNA are discordant. This disparity in expression of the \( \alpha \)-Sm actin and SR Ca²⁺-ATPase genes during the development of hypertension may result in an uncoupling of the proteins involved in the vascular contractile machinery and in the intracellular transport of Ca²⁺ and thereby in abnormal vascular relaxation in SHRs.

A shift in the relative ratio of two SR Ca²⁺-ATPase mRNAs without significant alteration in SR function or
total SERCA mRNA level occurs during growth of the vascular smooth muscle in SHRs. In contrast, such a shift does not occur in cardiac hypertrophy induced by hemodynamic overload. The SR Ca\(^{2+}\)-ATPase gene does not follow the general increase in gene expression, which leads to cardiac hypertrophy in chronic pressure overload in rats\(^2,3\) and rabbits\(^4\) and in chronic heart failure in humans.\(^1,4,2\) Consequently, a decrease in the density of Ca\(^{2+}\) pumps and a lower rate of Ca\(^{2+}\) uptake\(^2,3\) are observed in the hypertrophied heart compared with the control heart.

In summary, both age and the development of hypertension appear to be important factors for the induction of the α-Sm actin gene in the media of thoracic aortas of SHR and normotensive rats. The expression of the SR Ca\(^{2+}\)-ATPase gene is also developmentally regulated: the level of SERCA 2a mRNA increases with age in parallel to the level of α-Sm actin mRNA, whereas the level of SERCA 2b mRNA remains constant. Change in SERCA phenotype does not appear to alter total SR function but may be involved in the control of intracellular Ca\(^{2+}\) pools.

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