Expression of Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase and Calsequestrin Genes in Rat Heart During Ontogenic Development and Aging

A.M. Lompré, F. Lambert, E.G. Lakatta, and K. Schwartz

Little is known concerning the molecular mechanisms responsible for changes in sarcoplasmic reticulum (SR) function during ontogenic development and aging except that the amount of SR Ca\(^{2+}\)-ATPase mRNA varies in these conditions. The aim of the present work was to determine whether SR maturation requires expression of specific isoforms and synchronous accumulation of mRNAs encoding proteins located in SR. Thus, we have studied expression of SR Ca\(^{2+}\)-ATPase and calsequestrin genes in the rat at different developmental stages from 14 fetal days to 24 months of age. Analysis of alternative splicing of the major Ca\(^{2+}\)-ATPase gene expressed in heart by nuclease S\(_{1}\) mapping led us to conclude that the Ca\(^{2+}\)-ATPase gene expressed in heart was not differentially spliced during ontogenic development and senescence. A single calsequestrin mRNA isoform was also detected in rat heart whatever the developmental stage. The amount of specific mRNA was then measured by dot blot and normalized to 18S ribosomal RNA or to myosin heavy chain mRNA. The amount of Ca\(^{2+}\)-ATPase mRNA relative to 18S RNA increases substantially at the end of fetal life and in the early postnatal period (9.5±0.5% in the 14–15-day fetus versus 99±7% in the 4-day-old rat). A stable high level is observed during adulthood. In aged rats (24 months), Ca\(^{2+}\)-ATPase mRNA represents only 44.6% the amount observed in young adults (1–2 months). In the fetal–neonatal period the increase in Ca\(^{2+}\)-ATPase mRNA concentration is parallel to the increase in myosin heavy chain mRNA concentration, but during senescence the two mRNAs do not evolve in parallel. The concentration of calsequestrin mRNA also peaks at day 4 but is equally abundant in fetal, adult, and senescent rats, which supports and extends previous observations showing that, during maturation of SR, calsequestrin is synthesized earlier than ATPase. Our results indicate that maturation of SR, which is in part responsible for changes in relaxation rate, does not involve a Ca\(^{2+}\)-ATPase and calsequestrin isoform switch but, rather, changes in the concentration of single isoforms. (Circulation Research 1991;69:1380–1388)

Myocardial function changes during ontogenic development\(^1\)–\(^3\) and aging,\(^4\)–\(^7\) and some of the developmental and age-associated changes in the mechanical properties of the heart have been hypothesized to result from differences in sarcoplasmic reticulum (SR) properties. The SR is intimately related to the control of free intracellular Ca\(^{2+}\) and, thus, to the contractile process, because release of Ca\(^{2+}\) from the SR is probably largely responsible for muscle contraction and Ca\(^{2+}\) uptake by the SR results in relaxation.\(^8\) Several studies that have compared SR properties in aged and adult rats have reported a decreased rate of Ca\(^{2+}\) uptake and a decreased Ca\(^{2+}\)-ATPase activity with age.\(^5\)–\(^9\) In addition, the sensitivity to Ca\(^{2+}\) of the uptake does not vary with age, whereas Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity are lower in fetal than in adult SR.\(^10\)–\(^14\) Moreover, the Ca\(^{2+}\)-induced release of Ca\(^{2+}\) from the SR becomes functional only after birth.\(^15\) Stereological measurements show low abundance of SR network\(^2,16\) and low surface density of the dyads\(^17\) before birth. Thus, the changes in cardiac contractility occurring during development and aging appear to result from maturation of SR. However, the molecular mechanism for SR maturation remains...
unresolved. The relative abundance of SR proteins has been measured in fetal and adult hearts, and both decreased or unchanged levels of Ca\(^{2+}\)-ATPase and increased or unchanged levels of calsequestrin in fetuses compared with adults have been reported.\textsuperscript{12–14} These conflicting results could be due to differences between species, since studies have been conducted in sheep\textsuperscript{12,13} and rats.\textsuperscript{14} They may also reflect the preferential isolation of a particular SR fraction from fetal and adult hearts by the various investigators. Indeed, the SR is a heterogeneous intracellular compartment that includes the network (free) SR, the peripheral junctional SR, and specialized nonjunctional SR referred to as corbular SR. The major SR proteins involved in Ca\(^{2+}\) transport are not evenly distributed through these vesicles. Thus, Ca\(^{2+}\)-ATPase and phospholamban are located mainly in the free SR; calsequestrin and ryanodine receptors are found in the corbular and peripheral junctional vesicles.\textsuperscript{18,19} Alternatively, differences in abundance of SR proteins may result from the modulation of expression of specific genes during development. It has been recently shown that the amount of Ca\(^{2+}\)-ATPase mRNA increases in the rodent heart during ontogenic development\textsuperscript{14} and decreases during senescence.\textsuperscript{20} However, several isoforms of Ca\(^{2+}\)-ATPase and of each SR protein have been described, and for other proteins, the developmental expression of various isoforms is well documented (for a review see Reference 21). Sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) is encoded by three different genes.\textsuperscript{22–24} Gene 1 is expressed in fast skeletal muscle, and alternative splicing of the 3' end of the primary transcript gives rise to two mRNAs that are differentially expressed during development.\textsuperscript{25,26} The gene 2 transcript is also alternatively spliced at its 3' end. One of the mRNAs (SERCA 2a) has been detected in adult cardiac and slow skeletal muscles, and the other (SERCA 2b) has been detected in adult smooth and nonmuscle tissues.\textsuperscript{27–33} The possibility that the gene 2 transcript is alternatively spliced during cardiac development, as it was demonstrated for gene 1 during skeletal muscle development, has not been ruled out. Gene 3 is expressed in muscle and nonmuscle tissues.\textsuperscript{24} Similarly, calsequestrin is encoded by two genes differentially expressed in tissues.\textsuperscript{34–37} At present, there is evidence for alternate polyadenylation\textsuperscript{37} but not for alternative splicing\textsuperscript{16} of calsequestrin gene primary transcripts.

The purpose of the present study was to investigate the qualitative expression of SERCA 2 and calsequestrin gene products during ontogenic development and aging in the rat heart. We also wanted to determine whether SR maturation requires synchronous accumulation of mRNAs encoding proteins located in the SR. Myosin heavy chain (MHC) mRNA was used as a reference for myocyte-specific RNA and 18S ribosomal RNA as a reference of total RNA. Our results indicate that maturation of the SR during cardiac development does not involve the transition of Ca\(^{2+}\)-ATPase and calsequestrin isoforms to adult isoforms but requires changes in the concentration of single mRNA isoforms.

**Materials and Methods**

**Animals and RNA Preparation**

Wistar rats including 14–15- and 19-day fetuses, 4- and 8–10-day-old neonates, and adults of increasing age (1–2, 8, and 24 months old) were used in the study. Hearts were collected from all age groups and dissected free of atrial tissue and large blood vessels before freezing. Liver, soleus, tensor fasciae latae, and aorta were used as controls of nonmuscle, slow skeletal muscle, fast skeletal muscle, and smooth muscle, respectively, and were collected from young adults only. The tissues were blotted dry, frozen in liquid nitrogen, and kept at \(-80^\circ\text{C}\) until RNA preparation. Either individual left ventricles (including septa) of adult rats or both ventricles pooled from eight to 24 fetal or neonatal rats (one to three litters) were used for each RNA preparation. Total RNA was isolated according to Chirgwin et al\textsuperscript{38} or, for fetuses, according to Chomzinsky and Sacchi\textsuperscript{39} and kept in 70% ethanol, 0.3 M sodium acetate at \(-20^\circ\text{C}\). Specific mRNA species were analyzed either by filter hybridization or nuclease S\(_1\) protection assay.

**Nuclease S\(_1\) Mapping Analysis**

Nuclease S\(_1\) protection assays were performed with a rat Ca\(^{2+}\)-ATPase single-stranded cDNA probe prepared as follows: The PstI fragment covering nucleotide 2618 to the poly(A) tail of the rat heart cDNA\textsuperscript{21} was inserted into M13 mp19 vector. Phage DNA containing the sense strand was purified and used as a template for the synthesis of the single-stranded probe. Synthesis was initiated by the addition of a specific primer located in the untranslated region, a mixture of the four deoxynucleotides, including \[^{32}\text{P}\]dCTP (3,000 Ci/mmol) and the DNA polymerase I (Boehringer Mannheim Corp., Indianapolis, Ind.). After PstI digestion, a 507-bp fragment comprising the last 374 coding nucleotides and 133 bp of untranslated sequences was released and purified on 6% sequencing gel. The probe was hybridized at 42°C to 1 \(\mu\)g total RNA, and nuclease S\(_1\) (Bio-Lab Corp., St. Paul, Minn.) digestion was performed in the conditions described previously,\textsuperscript{40} using 10 units enzyme/\(\mu\)g RNA. In each experiment, we included a control containing 5 \(\mu\)g *Escherichia coli* tRNA processed in the same hybridization and digestion conditions as the samples.

**Probes**

Several probes were used in filter hybridization assays. The Ca\(^{2+}\)-ATPase probe corresponds to a coding portion of the rat heart Ca\(^{2+}\)-ATPase cDNA (nucleotide 265–1,446), which we have previously described.\textsuperscript{31} The calsequestrin probe (a gift of Dr. B. Nadal-Ginard, Harvard Medical School, Boston) was isolated from a canine cardiac library by Scott et al\textsuperscript{37}; it is an *EcoRI* fragment that includes the entire 5'
untranslated and coding sequences and part of the 3′ untranslated region (probe 1 in Figure 1A of Reference 37). Both probes were random-prime labeled (multiprime labeling system, Amersham Corp., Arlington Heights, Ill.) to a specific activity of 1-3×10^6 dpm/μg.

The MHC probe used was pMHC_{26,41}. Since we wanted to measure the total amount of MHC mRNA present in the sample, we chose this probe, which corresponds to a coding portion of the mRNA and reacts with equal intensity with α- and β-MHCs but does not hybridize to other MHC mRNAs (see Figure 1). The MHC cDNA was nick-translated to a specific activity of 5×10^6 dpm/μg plasmid DNA.

The last probe was a 24 oligomer complementary to nucleotide 1,046-1,070 of the rat 18S ribosomal RNA.42 It was used to determine the actual amount of RNA present on the blot. The oligonucleotide was 5′ end-labeled using [γ-32P]ATP and polynucleotide kinase (Boehringer Mannheim) and diluted to a specific activity of 5×10^6 dpm/μg with unlabeled oligomer.

Filter Hybridization

Total RNA was analyzed by dot blot or Northern blot hybridization assays. For dot blot analysis, total RNA was denatured in 15x standard saline citrate (SSC) (1x SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate) at 65°C for 15 minutes and rapidly cooled on ice. Aliquots containing 1.25, 2.5, and 5 μg RNA were spotted directly onto nylon membranes (Hybond N, Amersham), using a mini-fold apparatus (Schleicher & Schuell, Inc., Keene, N.H.). For Northern blot analysis, 8 μg total RNA was denatured in 6% formaldehyde/2.2 M formamide and size-fractionated on 1% agarose gel containing 3% formaldehyde and 0.5 μg/ml ethidium bromide. In some experiments, RNA markers (Bethesda Research Laboratories 0.16-1.77-kb and 2.4-9.5-kb standards) were run in parallel to the samples. RNA was visualized with ultraviolet light and blotted onto a nylon membrane. RNA was cross-linked to the membranes by ultraviolet irradiation, and the irradiated membranes were prehybridized at 42°C for at least 4 hours in the presence of 50% formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.05 M sodium phosphate (pH 6.5), 5x SSC, 0.1% sodium dodecyl sulfate (SDS), and 250 μg/ml salmon sperm DNA. Sequential hybridizations were performed under the same conditions after the addition of 32P-labeled probes in excess. When the 18S ribosomal probe was used, formamide was omitted. The final washing conditions were as follows: Ca^{2+}-ATPase at 50°C in 0.5x SSC, 0.1% SDS; calsequestrin at 45°C in 0.5x SSC, 0.1% SDS; MHC at 63°C in 0.2x SSC, 0.1% SDS; and 18S at room temperature in 2x SSC. Each wash was performed for 45 minutes. The blots were exposed to x-ray films using Quanta III intensifying screens at -70°C. Three exposure times were chosen to obtain densitometric readings within the linear response range of the film. The membranes were washed at least twice in boiling 0.1% SDS and exposed to x-ray films for 24-48 hours to assess the efficacy of probe-RNA dissociation after each round of hybridization.

Evaluation of the Amount of Specific mRNA

For each RNA preparation, the ratio of specific mRNA to 18S RNA was determined for the three RNA concentrations, and the mean value was calculated. The number of RNA preparations is indicated by n, which for adults and aged rats is equal to the number of rats but is less for fetuses and newborns, since several rats were pooled. The ratios Ca^{2+}-ATPase/calsequestrin and Ca^{2+}-ATPase/MHC were deduced from the corresponding mRNA/18S ratios. Results were expressed as mean±SEM. The statistical significance of differences between the various groups was determined by one-way analysis of variance, and group-to-group comparisons were determined by Scheffe’s F test.

Results

Qualitative Analysis of Gene Expression

Figure 1 shows the results obtained when the same Northern blot containing total RNA from hearts of rats at different developmental stages was sequentially hybridized with the SR Ca^{2+}-ATPase, the calsequestrin, the MHC, and the ribosomal 18S probes. Fast (tensor fasciae latae) and slow (soleus) skeletal muscles and liver total RNA are included as controls.

Hybridization with the 18S probe indicates the actual amount of RNA loaded for each sample, so that the relative abundance of MHC, SR Ca^{2+}-ATPase, and calsequestrin mRNAs can be assessed in each tissue. Under our conditions of hybridization, the MHC cDNA probe hybridizes with either of the two cardiac MHC mRNAs but not with other striated MHC mRNAs present in tensor fasciae latae or with liver RNA. Thus, the amount of hybridizable MHC mRNA is a measure of cardiac mRNA contributed by the myocytes, whereas 18S RNA represents total cardiac RNA (from myocytes and nonmyocytes).

A single hybridization band of 4.4 kb was observed when the blot was probed with the SR Ca^{2+}-ATPase. The washing stringency used was such that the Ca^{2+}-ATPase probe did not cross-react with the fast Ca^{2+}-ATPase mRNA (SERCA 1) isof orm present in tensor fasciae latae. Thus, the signal obtained with tensor fasciae latae was considered as the background in our study. The absence of signal with liver RNA is due to the low abundance of SERCA 2 mRNA in this tissue.

The SERCA 2 primary transcript is known to be differentially spliced to give rise to two mRNAs of identical size.27-33 The fast SERCA 1 gene transcript has been shown to be alternatively spliced during ontogenic development of fast skeletal muscle.25,26 The possibility that the 4.4-kb species observed in heart represents two mRNA species was tested by nuclease S_{1} digestion of the cDNA-mRNA hybrids. SERCA 2a and 2b exhibit divergent sequences in
their 3’ untranslated region that give rise to protected cDNA fragments of different size after S1 digestion.27,30,31 As can be seen in Figure 2, left panel, the major fragment observed after S1 digestion of cardiac hybrids corresponds to the fully protected probe whose abundance varies with the age of the animals. The minor fragment of 360 nucleotides originating from protection by mRNA 2b is abundant in aorta (Figure 2, right panel), but in heart, regardless of the animal’s age, the proportion of this isoform is low and remains constant. This indicates that RNA splicing of the SERCA 2 primary transcript is not regulated during cardiac ontogenic development and aging.

For calsequestrin, whatever the developmental stage, a single hybridization band that corresponds to an mRNA of 2.5 kb was observed. This result contrasts with the data reported by Scott et al.,37 who described two mRNAs of different sizes (2.9 and 2.2 kb) in dog heart. In fast skeletal muscle, the calsequestrin probe hybridizes with an mRNA of smaller size (1.8 kb), in agreement with the size reported for the fast skeletal muscle calsequestrin mRNA.34 Surprisingly, slow skeletal muscle (soleus), which expresses the slow isoform of MHC and SR Ca2+-ATPase, contains mostly the fast calsequestrin isoform. The fast isoform could not be detected in any of the cardiac RNA samples, indicating that if it is present at all during cardiac development, it is much less abundant than the slow isoform. No apparent hybridization was observed with liver RNA. Since we were unable to detect the fast calsequestrin mRNA isoform in heart and since there is no evidence to date for alternative splicing of the calsequestrin genes,36,37 we conclude that a single calsequestrin mRNA isoform is expressed in heart throughout development.

**Quantitative Analysis of Gene Expression**

To quantitate more precisely the variations in the level of expression of the genes, we performed dot blot analysis. The relative amounts of Ca2+-ATPase, calsequestrin, and MHC mRNAs present in the different groups of rats, normalized to 18S ribosomal RNA, are presented in Figure 3. It is interesting to note that the concentration of all three mRNAs is highest in the heart of 4-day-old rats; therefore, the data are expressed as a percentage of the value observed at day 4.

Although it is significantly more abundant at day 4 (p=0.01 versus individual age group and p=0.001 versus all groups), calsequestrin mRNA is equally abundant in the fetus and in the aged rat. Moreover, at all ages it is expressed at a high level (50% of the maximum). In contrast, SERCA 2 mRNA concentration is very low in the fetus, increases rapidly at the end of the fetal life (9.5±0.5% versus 36±0.6%), and peaks at neonatal day 4. It remains relatively constant during adulthood but is much lower in 24-month-old rats. When compared with the maximal amount of Ca2+-ATPase mRNA accumulated (neo-
natal day 4), the decrease in the aging rats was 59.2% 
\((p=0.01)\), and when compared with the group of 
adults (1–2 months), it was 44.6% \((p=0.01)\). Thus, 
the ratio of SR Ca\(^{2+}\)-ATPase mRNA to calsequestrin 
mRNA is not constant throughout life.

During fetal and juvenile life (until day 10), the 
level of Ca\(^{2+}\)-ATPase mRNA increases linearly with 
age \((r=0.86, p<0.001)\), whereas no significant 
regression was observed for calsequestrin mRNA \((r=0.37, 
p=0.2)\). Moreover, Ca\(^{2+}\)-ATPase mRNA and MHC 
mRNA accumulate in parallel. Indeed, the slopes of 
the regression representing Ca\(^{2+}\)-ATPase mRNA ac-
cumulation \((slope=66.6)\) and MHC mRNA accumu-
lation \((slope=66.6)\) are not significantly different.

The concentration of MHC mRNA is similar to that 
of SERCA 2 mRNA during fetal and juvenile life 
(Figure 3). However, unlike SERCA 2 mRNA and in 
agreement with our previous observation,\(^4\) there is a 
significant decrease in the young adult, with no signif-
ificant change during senescence. Because the MHC 
cDNA probe does not cross-react with smooth muscle 
and nonmuscle mRNA, MHC mRNA levels are a 
measure of cardiomyocyte-specific RNA. Our results 
indicate that this age-related decrease is specific to 
SERCA mRNA and, thus, cannot be accounted for by 
loss of myocyte specific RNA in senescent hearts.

**Discussion**

The present study was undertaken to determine 
whether qualitative changes in the accumulation of 
mRNA species encoding SR proteins occur during 
cardiac development and senescence. Our data 
indicate that, for both Ca\(^{2+}\)-ATPase and calsequestrin, 
the same mRNA species is expressed throughout the 
life, indicating that, in heart, developmental pro-
gramming of the SR does not involve alternate 
transcription or splicing of these two SR-coding 
protein genes. We found also that the SERCA 2 gene 
is upregulated around birth and downregulated dur-
ingsenescence, whereas calsequestrin mRNA is also 
 maximal soon after birth, but in contrast to Ca\(^{2+}\)- 
ATPase mRNA, it is equally abundant in fetal, adult, 
or aged rats. Thus, our observations indicate that 
maturation of SR involves quantitative modulation of 
the concentration of single mRNA isoforms. More-
over, the increase in the Ca\(^{2+}\)-ATPase mRNA level 
during development paralleled the increase in MHC 
mRNA, whereas the decrease of the Ca\(^{2+}\)-ATPase 
mRNA level in senescence was independent of changes in the total MHC mRNA level.

Our results provide evidence for age-related regu-
lation of the SERCA 2 mRNA and are in agreement 
with recently reported evidence of an upregulation of
served in C2C12 myoblasts as well as in myotubes. In BC3H1 cells, however, SERCA 2b is associated with the undifferentiated state, and SERCA 2a is associated with differentiation.43 Thus, differentiation of the cells in which the SERCA 2 gene is expressed is associated either with Ca2+-ATPase pre-mRNA splicing or with an upregulation of the gene. Another mRNA corresponding to the SERCA 3 gene has been detected in muscle and nonmuscle tissues.24 In heart, however, it represents a very minor proportion of the total Ca2+-ATPase mRNA.

The increase in the Ca2+-ATPase mRNA level around birth is parallel to the increase in the MHC mRNA level, suggesting a common mechanism for induction of the two genes. The increase in the MHC level at birth is mainly due to induction of the α-MHC gene,40 which is thought to be triggered by thyroid hormone.4445 The SERCA 2 gene is positively regulated by thyroid hormone,4647 and putative thyroid hormone receptor DNA binding sequences have been defined in the 5' flanking region of the SERCA 2 gene,23 but their functional importance has to be defined. Thus, the accumulation of Ca2+-ATPase mRNA that we observed around birth may be induced by thyroid hormone. During senescence, only a slight decrease in thyroxine level and no change in the biologically active thyroid hormone have been reported,2048 suggesting that thyroid hormone is not involved in the age-dependent decrease in Ca2+-ATPase mRNA. Changes that occur with senescence in the rodent ventricular myocytes are in many aspects similar to those in the rodent hypertensive hearts. In aged rats, the ventricular myocytes enlarge49 and change their phenotype in the same way as the hypertensive heart.2150 The Ca2+-ATPase mRNA level and the density of Ca2+ pumps in heart also decrease in both hypertension145152 and senescence (our present study and Reference 20). Altered loading53 or age-related neuroendocrine regulation affecting genetic expression directly or indirectly could be involved.

Species specificity of expression may explain why in rat heart of all ages we detected a single calsequestrin mRNA species, whereas in adult dog heart two mRNAs of different size have been described.37 We also found that the calsequestrin isoform expressed in fast-twitch skeletal muscle is a major component of the calsequestrin mRNA expressed in slow-twitch skeletal muscle (soleus), which is in agreement with previous studies.35 However, we could not detect the presence of this RNA in heart whatever the developmental stage. Both heart and soleus are slow-twitch muscles, and the phenotype of the proteins expressed in the two muscle types are often similar. This is the case for Ca2+-ATPase,2531 phospholamban,54 and most of the contractile proteins.21 The high level of expression of the fast-twitch calsequestrin gene in soleus and its absence from cardiac muscle is thus surprising. Careful analysis of the fast calsequestrin gene failed to reveal evidence for splicing.36 No evidence for splicing of the cardiac gene

the Ca2+-ATPase gene in the perinatal period14 and of a downregulation during senescence20 in the rat. Moreover, we show that there are no changes in the splicing of the SERCA 2 gene. Similar results have been obtained during differentiation of the C2C12 muscle cell line,23 where SERCA 2b mRNA is ob-

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Bar graphs showing relative amounts of Ca2+-ATPase (panel A), calsequestrin (panel B), and myosin heavy chain (panel C) mRNAs, normalized to ribosomal RNA, in the different groups of rats. NS, not significant; d, days; mo, months. Quantitation of the different mRNAs was performed by dot blot analysis as described in "Materials and Methods." The results are expressed as the percentage of the maximal mean value (observed at postnatal day 4). The data are expressed as mean±SEM. The values represent the mean of n different RNA preparations, which corresponds to n rats for adults or n pools of hearts for fetuses and neonates (one to three litters were included in each group): for 14−15d fetal rats, n=2; for 19d fetal rats, n=3; for 4d newborns, n=3; for 10d newborns, n=5; for 1−2mo rats, n=6; for 8mo rats, n=3; for 24mo rats, n=5. In panels A and C, the asterisks indicate the significance of Scheffe's F test for the comparison of the various groups with the group of 4-day-old newborns. In panel B, the asterisks indicate the significance of the comparison of the group of 4-day-old newborns with all other groups taken together, since they were not significantly different from each other. *p=0.05; **p=0.01; ***p=0.001.
transcript has been provided either. Altogether, the data lead us to conclude that a single calsequestrin isoform is expressed in hearts of all ages.

The amount of calsequestrin mRNA was found to be as high in fetal as in adult or aged rats. This contrasts with what we observed for Ca\(^{2+}\)-ATPase and clearly indicates that the two genes are under different regulatory mechanisms. In rabbit, it has been recently shown that both Ca\(^{2+}\)-ATPase and calsequestrin mRNAs increase in parallel during cardiac development.\(^5\) Our results, however, are in agreement with previous data showing that, in skeletal muscle cells in culture, calsequestrin is synthesized earlier than Ca\(^{2+}\)-ATPase.\(^6\)–\(^9\) Moreover, it has been shown recently that, in human skeletal muscle, the amount of Ca\(^{2+}\)-ATPase decreases with age, whereas there is no concomitant decrease in the amount of calsequestrin or ryanodine receptors.\(^10\) In sheep, the level of calsequestrin is two times higher in the late fetus than in the adult.\(^11\) In rats, we found that the level of calsequestrin mRNA is the same in fetal rats and in adult or aged rats but is two times higher in neonates. Species differences may explain the two sets of data. Mahony and Jones\(^12\) reported for heart and Klitgaard et al\(^13\) reported for skeletal muscle that ontogenic development and aging affect extrajunctional (phospholamban and Ca\(^{2+}\)-ATPase) and junctional (ryanodine receptors and calsequestrin) proteins differently. Our data support the idea that the differences observed in SR protein composition between fetuses, adults and aged animals is due to different expression of specific genes at the various stages of development and that the regulation occurs at the level of mRNA accumulation.

The alteration in Ca\(^{2+}\)-ATPase gene expression that we and others\(^14\)–\(^20\) observed is consistent with the modifications in SR Ca\(^{2+}\) uptake reported during ontogenic development and senescence. Indeed, a lower level of Ca\(^{2+}\)-ATPase will account for the slower rate of SR Ca\(^{2+}\) transport observed in immature and senescent hearts.\(^5\)–\(^7\),\(^9\)–\(^14\) Among the numerous age-dependent alterations in contractile properties is the decrease in maximal velocity of isotonic relengthening,\(^2\)–\(^6\) which has been attributed to a defective SR and may thus be explained in part by decreased density of Ca\(^{2+}\) pumps. However, other mechanisms can compensate for the relative immaturity of SR, and it has been shown that force development and relaxation are more dependent on transsarcolemmal Ca\(^{2+}\) fluxes in the fetus and newborn than in the adult.\(^2\),\(^10\) The prolonged relaxation time observed in senescent myocardium may be regulated, at least in part, by the substantial reduction in the density of SR pump sites, which is a likely consequence of the decrease in the SR Ca\(^{2+}\)-ATPase mRNA that we observed.

In conclusion, our results indicate that maturation of cardiac SR does not require expression of specific isoforms of Ca\(^{2+}\)-ATPase or calsequestrin but rather quantitative modulation of the expression of single isoforms. The qualitative modifications observed during development and senescence can account for some of the impairments of Ca\(^{2+}\) movements and relaxation properties observed during that period.

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