Age-Induced Decreases in the Messenger RNA Coding for the Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase of the Rat Heart


Age-associated slowing of cardiac relaxation related to the decline in the Ca\(^{2+}\) pump function of cardiac sarcoplasmic reticulum (SR) has been previously described. It is unclear if the decreased Ca\(^{2+}\) pump function results from a lower amount of Ca\(^{2+}\)-ATPase protein or a decreased pumping activity of the enzyme. To determine if these alterations could be mediated by changes in the amount of the protein itself, the level of the messenger RNA (mRNA) coding for the Ca\(^{2+}\)-ATPase of the SR of Fischer rat hearts (4- and 30-month-old rats) were quantitated with a Northern blotting technique. We observed that the levels of SR Ca\(^{2+}\)-ATPase mRNA were 60% lower in old rats as compared with young rats, suggesting that a quantitative reduction in the levels of the corresponding protein could occur during aging to explain the delayed diastolic relaxation documented in old animals as opposed to a change in the specific activity of this enzyme. The thyroid hormone responsiveness of SR Ca\(^{2+}\)-ATPase mRNA has been previously established. We have found in this study that the thyroxine levels were consistently lower in old rats; however, this difference was relatively small (4.3±0.7 and 3.1±0.8 μg/dl [mean±SD], respectively, in young and old rats). In addition, no age-induced decrease in 3,5,3′-triiodothyronine levels was observed, suggesting that the aging process itself may be responsible for the changes in SR Ca\(^{2+}\)-ATPase mRNA levels. (Circulation Research 1990;67:230–234)

Prolonged duration of contraction is one of the most prominent and well-documented effects of aging on the mechanics of cardiac muscle in humans\(^1\) as well as in animals.\(^2\)\(^-\)\(^5\) Previous studies have suggested that this prolonged contraction is due to an age-associated slowing of cardiac relaxation, because age does not seem to change the speed of tension development by heart muscle during isometric contraction.\(^2\)\(^,\)\(^6\)

In the heart, contraction and relaxation are dependent on the Ca\(^{2+}\) concentration in the cytoplasm. Ca\(^{2+}\) concentrations are changed by translocation across the sarcoplasmic reticulum (SR) and sarcolemmal membrane systems, and any modification in this process related to aging could affect changes in myocardial contraction and relaxation. This possibility is supported by reports\(^7\)\(^,\)\(^8\) showing that the rate of Ca\(^{2+}\) uptake is reduced in isolated SR vesicles prepared from senescent rat hearts. These in vitro results reflect a decreased Ca\(^{2+}\) pump function of the SR, that is, a decreased activity of the SR Ca\(^{2+}\)-dependent ATPase enzyme. It is currently unclear whether the decreased Ca\(^{2+}\) pump function results from a lower amount of Ca\(^{2+}\)-ATPase protein or a decreased pumping activity of an unchanged number of pump units. Because the complementary DNA (cDNA) coding for Ca\(^{2+}\)-dependent ATPase of the SR has recently been cloned,\(^9\) we used Northern blot analysis to investigate the influence of aging on the level of SR Ca\(^{2+}\)-ATPase messenger RNA (mRNA).

Materials and Methods

Animals

Two groups of male Fischer 344 rats purchased from the National Institute of Aging, Bethesda, Md., were used in this study. One group was composed of nine animals aged 4 months and the other of nine rats aged 30 months.

Studies of the same rat strain have shown that in these animals adult physiological competence as measured by maturity of the nervous and endocrine functions is reached around 6 months of age in males. Aging as measured by coat deterioration, incidence of tumors, and increased mortality begins at 22 months of age. The average life span is approximately
28 months, with the maximum being 33 months.10 The two ages chosen in this study are, therefore, representative of young adult and old rats. All animals were free of grossly visible disease and received a standard block diet and water ad libitum.

Animals were anesthetized with sodium pentobarbital, blood was collected from the tail, and after clotting and centrifugation the sera were stored at −20°C for further analysis. After blood collection, the chest was opened, and the heart was removed, rinsed in ice-cold 0.9% sodium chloride, and immediately used for RNA preparation.

RNA Preparation

Total RNA was prepared by the proteinase K–phenol procedure previously described.11 Briefly, each ventricle was homogenized in a Waring blender (New Hartford, Conn.) in the presence of proteinase K. The homogenate was then extracted once with equal volumes of phenol and chloroform. After this, the RNA was precipitated with ethanol and subsequently with 3 M sodium acetate; a final ethanol precipitate yielded total RNA with a ratio of absorbance at 260 nm to absorbance at 280 nm of 1.8:2. The RNA was translationally active. Equal RNA recovery was obtained from the heart of old and young rats yielding 1 mg RNA/g heart wt.

Northern-Blot Analysis

Each animal's RNA was processed separately. A total of 15 μg RNA was denatured in the presence of 2.2 M formaldehyde and 50% deionized formamide at 65°C for 10 minutes and was separated through electrophoresis in 0.8% agarose gels made of 20 mM MOPS (pH 7.4), 5 mM sodium acetate, 1 mM EDTA, and 2.2 mM formaldehyde. The positions of 28S and 18S RNAs were marked by staining with acridine orange in 10 mM sodium phosphate buffer (pH 6.5) and 1.1 M formaldehyde. The ratio of 28S to 18S RNA was about 2:1. The RNA was transferred to a Nytran membrane (Schleicher & Schuell, Inc., Keene, N.H.) in the presence of 10× standard saline citrate (SSC) (1× SSC is 300 mM sodium chloride, 30 mM sodium citrate, pH 7.0).

The membrane was air dried for 20 minutes, baked at 80°C in a vacuum oven, and prehybridized at 42°C for 10–12 hours in a solution containing 5× SSPE (1× SSPE is 150 mM sodium chloride, 0.01 M sodium dihydrogen phosphate, 0.001 M EDTA), 20× Denhardt’s solution (1× Denhardt’s solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and deionized bovine serum albumin), 25 μg/ml sheared herring sperm DNA, 0.1% sodium dodecyl sulfate (SDS), and deionized formamide at a final concentration of 50%.

Labeling Probes

Ca2+-ATPase cDNA was labeled by using a nick translation kit (Bethesda Research Laboratories, Gaithersburg, Md.) according to the manufacturer’s instructions. The specific activities obtained were about 1×10⁸ cpm/μg DNA. [32P]dNTPs (3,000 Ci/mmol) were purchased from Amersham, Arlington Heights, Ill. The Ca2+-ATPase cDNA is a 2.3-kilobase pair rat cDNA clone corresponding to the 3' end of slow SR Ca2+-ATPase. The rat Ca2+-ATPase cDNA clone was obtained by screening a rat heart cDNA library cloned into the λZAP vector (Stratagene Inc., La Jolla, Calif.) with a cDNA clone of the 3' end of the rabbit skeletal muscle SR Ca2+-ATPase kindly provided by D. MacLennan, Toronto, Canada.9 The rat heart SR Ca2+-ATPase cDNA clone was verified by nucleotide sequencing.12

An unidentified cDNA clone (C5) of 1,000 base pairs cloned into the vector pT5/T7-18 (Bethesda Research) was isolated in this laboratory by D. Rohrer and used as a reference for equivalent RNA loading. This plasmid was also labeled by nick translation to a specific activity of 1×10⁸ cpm/μg. All probes were purified on a Bio-Gel A-5m column (Bio-Rad, Richmond, Calif.).

After hybridization, the filter was washed twice with 2× SSC and 0.1% SDS at room temperature for 20 minutes, twice with 1× SSC and 0.1% SDS at 55°C for 30 minutes, once with 0.2× SSC and 0.1% SDS at 55°C for 30 minutes, or 0.1× SSC and 0.1% SDS at 50°C for 30 minutes for Ca2+-ATPase or C5.

A myosin light chain (MLC2) cDNA probe, kindly provided by Dr. K. Chien,13 was simultaneously hybridized with the Ca2+-ATPase probe on an additional Northern blot made with RNA from five individual young and five individual old rat hearts.

Autoradiograms were obtained after exposure to Kodak XAR films (Rochester, N.Y.) at −70°C baked by intensifying screens.

Relative mRNA ratios were determined by scanning densitometry of the autoradiograms and corrected for loading differences by using C5 and MLC2, respectively, when probed simultaneously as a standard. It was assured that a linear relation existed between dpm film darkening and scanning signal.

Radioimmunoassay Measurement of Serum Total Thyroxine and 3,5,3'-Triiodothyronine

Total thyroxine (T₄) and 3,5,3'-triiodothyronine (T₃) levels in unextracted serum were measured by using a commercial radioimmunoassay kit (Monobind, Costa Mesa, Calif.). Hormone measurements for a given experiment were all performed in the same assay.

Statistics

The results are expressed as mean±SEM and were compared between the two groups by Student’s t test to unpaired data. The level of significance was set at p<0.05.

Results

The steady-state levels of total cardiac Ca2+-ATPase mRNA of 30- and 4-month-old Fischer 344 rats are compared in Figure 1A. An equal amount of total RNA obtained from young and old rat hearts was loaded per lane and checked by acridine orange
staining. The autoradiogram shows that the mRNA level of SR Ca\textsuperscript{2+}-ATPase mRNA is significantly lower in the aged as compared with the young rat heart (Figure 1A).

The same Northern blot was probed with C5 cDNA (Figure 1B). No difference was observed in mRNA levels between the two groups by the C5 cDNA probe. The level of C5 mRNA was used to correct for slight differences in RNA loading.

The quantification of total Ca\textsuperscript{2+}-ATPase mRNA levels by densitometric scanning of autoradiograms shows a 60% decrease in the mRNA coding for SR Ca\textsuperscript{2+}-ATPase from old rats (Figure 2). These data indicate that the level of the mRNA coding for slow SR Ca\textsuperscript{2+}-ATPase significantly decreases with age (*p*=0.0002). Furthermore, a second Northern blot containing RNA from five individual young and five individual old rats was probed simultaneously with SR Ca\textsuperscript{2+}-ATPase and MLC\textsubscript{2} cDNA probes. The ratio of SR Ca\textsuperscript{2+}-ATPase mRNA over MLC\textsubscript{2} mRNA in young versus old rats revealed a 4.7-fold age-induced decrease. These data are shown in Table 1.

Serum T\textsubscript{3} concentrations in aged male rats were significantly lower than those observed in young rats (Table 2). However, no appreciable changes in serum levels of T\textsubscript{3} were documented between the two groups. In addition, a significantly positive correlation between the levels of mRNA for Ca\textsuperscript{2+}-ATPase evaluated by the relative density and the serum levels of T\textsubscript{3} was observed for all animals grouped together (*r*=0.69, *p*<0.001). However, the decrease of T\textsubscript{3} levels in old animals is relatively small (4.3±0.7 versus 3.1±0.8 µg/dl). No correlation of SR Ca\textsuperscript{2+}-ATPase mRNA levels to T\textsubscript{3} levels was observed.

**Discussion**

Aging could be considered as a process of gradual disorganization of complex physiological systems responsible for maintaining homeostasis and it may result, at least in part, from the impairment of key cellular functions. The results of this study demonstrate that the mRNA coding for the cardiac SR Ca\textsuperscript{2+}-ATPase decreases markedly and selectively with age when compared with other mRNAs, including the mRNA coding for MLC\textsubscript{2}. Our findings provide additional support for previous observations that showed a decrease in the activity of Ca\textsuperscript{2+}-ATPase of the SR and the resulting reduction in the rate of calcium uptake by the myocardial SR of old animals.\textsuperscript{7,8} These changes could constitute the biochemical basis for the slower rate of relaxation observed in the heart muscle with aging.

The age-dependent reduction in the levels of this specific mRNA should result in lower levels of the corresponding protein. However, there are no studies published so far that have quantified the Ca\textsuperscript{2+}-ATPase protein itself. Our findings are thus consistent with decreased calcium pump function caused by decreased synthesis of Ca\textsuperscript{2+}-ATPase rather than a change in the specific activity of this enzyme.

Several other recent studies have also described the effect of age on the expression of specific mRNA species.\textsuperscript{14} Most of the studies have shown decreasing values of different specific mRNA with aging.\textsuperscript{14} However, one of the mRNA species that has been studied in detail is albumin. Recently, Horbach et al\textsuperscript{15} showed that the cell-free synthesis of albumin by rat liver increased 35% between 12 and 24 months of age, and the levels of albumin mRNA increased approximately 80% between these two ages.
In addition, the present study confirmed earlier observations of a reduction of serum T₄ levels with aging although circulating T₃ concentrations remain relatively unchanged.16-19 This observation is part of a large amount of data supporting the concept that major changes in thyroid function occur with aging in rats, including a reduced thyroid uptake of radiolabeled iodide, a probable reduction in the thyroid release of the hormone as well as changes in the release of thyroid-stimulating hormone from the pituitary, and a reduction in tissue responsiveness to administered thyroid hormone.20

The marked influence that thyroid status has on cardiac function is well established, especially as related to the activity of the SR by changing the rate of calcium transport across SR membranes as well as the calcium-dependent ATP hydrolysis,21 and also, as recently reported, by modifying the mRNA levels of the slow Ca²⁺-ATPase of the SR.22 In our study, the marked influence of thyroid hormone on mRNA for Ca²⁺-ATPase was demonstrated by a rapid increase in the levels of mRNA for this enzyme to T₃ administration in hypothyroid rats.

In our investigation, a significant correlation between the serum levels of T₄ and the corresponding relative levels of mRNA was observed when all animals were analyzed together. Even though our findings are consistent with previous observations, suggesting a functional relation between thyroid hormones and Ca²⁺-ATPase activity, we should point out that the magnitude of the difference of T₄ levels between young and old rats was relatively small and also that no correlation was observed between mRNA and the levels of T₃, which is the most biologically active thyroid hormone. Although partial desensitization to circulating thyroid hormones with aging cannot be ruled out, our data suggest, but do not prove, that the aging process itself is the primary cause for the decrease in the mRNA for Ca²⁺-ATPase. However, a contribution of age-induced changes in the thyroid status and alterations in the SR Ca²⁺-ATPase mRNA levels by a thyroid hormone-mediated mechanism cannot be excluded.

In summary, our results indicate that the mRNA for slow Ca²⁺-ATPase decreases with age in rats and may lead to a decrease in the amount of the Ca²⁺-ATPase protein itself. Further studies will be necessary to determine whether the observed changes in mRNA levels are dependent exclusively on the aging process or if the thyroid status contributes to this alteration to some extent.

**References**


**Table 1. Quantification of Sarcoplasmic Reticulum Ca²⁺-ATPase and Myosin Light Chain Messenger RNA Levels by Densitometric Scanning**

<table>
<thead>
<tr>
<th></th>
<th>Old rats</th>
<th></th>
<th>Young rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>Densitometric reading</td>
<td>Animal</td>
<td>Densitometric reading</td>
</tr>
<tr>
<td>SR Ca²⁺-ATPase/MLC₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.785</td>
<td>2</td>
<td>5.800</td>
</tr>
<tr>
<td>3</td>
<td>0.692</td>
<td>4</td>
<td>3.329</td>
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<tr>
<td>5</td>
<td>0.891</td>
<td>6</td>
<td>3.893</td>
</tr>
<tr>
<td>7</td>
<td>0.366</td>
<td>8</td>
<td>5.872</td>
</tr>
<tr>
<td>9</td>
<td>1.748</td>
<td>10</td>
<td>2.178</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>0.896±0.23*</td>
<td></td>
<td>4.214±0.717</td>
</tr>
</tbody>
</table>

The signals and autoradiograms of Northern blots were quantitated by densitometric scanning. The values from these readings are indicated. SR Ca²⁺-ATPase, sarcoplasmic reticulum calcium ATPase; MLC₂, myosin light chain; SR Ca²⁺-ATPase/MLC₂, ratio of these two messenger RNAs.

*p<0.01.

**Table 2. Effect of Aging on Serum Thyroxine and 3,5,3'-Triiodothyronine Levels in Male Fischer 344 Rats**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Age (months)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4 (n=9)</td>
<td>30 (n=9)</td>
<td></td>
</tr>
<tr>
<td>T₄ (µg/dl)</td>
<td>4.3±0.7*</td>
<td>3.1±0.8</td>
<td></td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>68.6±5.6</td>
<td>67.3±10.6</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean±SD. n, Number of animals; T₄, thyroxine; T₃, 3,5,3'-triiodothyronine.
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**Key Words**

- \( \text{Ca}^{2+} \)-ATPase
- sarcoplasmic reticulum
- age effects
Age-induced decreases in the messenger RNA coding for the sarcoplasmic reticulum Ca2(+) ATPase of the rat heart.

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