Age-related Differences in the Expression of Proto-oncogene and Contractile Protein Genes in Response to Pressure Overload in the Rat Myocardium

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Abstract

Cardiac adaptation to hemodynamic stress involves both quantitative (hypertrophy) and qualitative (pattern of gene expression) changes. Our previous studies have shown that advancing age in the rat is associated with diminished capacity to develop left ventricular hypertrophy in response to either ascending aortic constriction (AoC). In this study, we examined whether the expression of protooncogenes and contractile protein genes in response to AoC differs between adult (9-mo-old) and old (18-mo-old) rats. RNA was isolated from the left ventricles of AoC animals of both age groups subjected to a similar hemodynamic stress. Immediately after AoC, the levels of the ventricular expression of c-fos and c-jun protooncogenes were markedly lower in the old rats than in the adult animals. 5 d after the operation, the ratio of β- to α–myosin heavy chain mRNAs increased significantly after AoC in both age groups. In contrast, AoC was associated with a marked reduction in the levels of mRNAs encoding sarcoplasmic reticulum Ca^{2+}-ATPase (by 69%) and cardiac calsequestrin (by 49%) in the old rats but not in the adults. The mRNAs encoding atrial natriuretic factor and skeletal α-actin increased in response to AoC only in the adult rats. There were no significant differences in expression of the cardiac α-actin mRNA among the experimental groups. These data suggest that (a) the expression of protooncogenes in response to acute pressure overload is significantly reduced in the aged rats and (b) the pattern of expression of the contractile protein gene in response to AoC in the old rats differs qualitatively as well as quantitatively from that in younger animals. These age-related differences may play a role in the higher frequency of heart failure in the aged during hemodynamic stress. (J. Clin. Invest. 1992. 89:939–946.) Key words: actin • myosin heavy chain • calsequestrin • Ca^{2+}-ATPase • atrial natriuretic factor

Introduction

The phenomenon of senescence is of fundamental importance for the function of cardiac muscle because, like neurons, cardiac myocytes are terminally differentiated cells and their life span is as long as that of the whole organism. Since adult cardiac cells are unable to divide, the ability of the heart to adapt to increased hemodynamic load, either from hypertension, valvular heart disease, or loss of myocardium due to ischemic heart disease, is critically dependent on its capacity to develop hypertrophy (1–3). When functional demands exceed adaptive limits, heart failure ensues (3, 4).

It is well known that, when subjected to similar hemodynamic stress, old patients develop heart failure more frequently than their younger cohorts (5, 6). This higher incidence of heart failure in old patients might be due to a diminished capacity for cardiac hypertrophy in response to a given stress. It has been shown that the extent of cardiac hypertrophy induced by exercise is substantially less in the senescent than in young adult animals (7). Our previous studies have confirmed that the capacity for left ventricular (LV)[1] hypertrophy in response to pressure or volume overload diminishes with advancing age (8, 9).

Cardiac adaptations to hemodynamic stress also involve altered pattern of gene expression. Changes in expression of a variety of cardiac genes in response to overload have been summarized as the acute induction of protooncogenes, followed by the reinduction of fetal isoforms of contractile proteins (10–12) such as β–myosin heavy chain (MHC) (13, 14) and skeletal α-actin (10, 15). In addition, pressure overload has been shown to markedly increase ventricular mRNA levels of atrial natriuretic factor (ANF) (10, 16, 17) and to significantly decrease the mRNA levels of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (18–20). Thus, hemodynamic stress changes the levels of these mRNA species similar to those observed during the fetal stage (10, 11, 18).

Normal myocardial aging also involves both quantitative and qualitative alterations (21, 22). Morphologically, interstitial fibrosis and myocyte hypertrophy have been observed in the left ventricles of normal aged rats (22, 23). Physiological studies of LV papillary muscle preparations have revealed that the duration of tension and the time to half-relaxation were prolonged in aged rats compared with in their younger cohort (21, 24). The prolongation of myocardial contraction has been

attributed to a decline of Ca^{2+}-activated myosin-ATPase activity, secondary to a decrease in the percentage of α-MHC or V_1 isozyme (25, 26). The abnormal myocardial relaxation has been thought to reflect age-related decreases in Ca^{2+}-uptake by the SR (27, 28).

In the present study, we asked the following questions. First, can the aging process affect changes in cardiac gene expression in response to aortic constriction (AoC)? Second, do qualitative changes in the pattern of gene expression occur in response to an ascending AoC in the old animals, in spite of the absence of quantitative hypertrophic responses (8)? These two questions may be important because they may be related both to fundamental mechanisms concerning the effects of aging on regulation of gene expression and to clinical problems concerning the age-related differences in the frequency of heart failure. To answer these questions, we examined expression of genes encoding two protooncogenes (c-fos and c-jun), two SR proteins (SR Ca^{2+}-ATPase and cardiac calsequestrin), two isoforms of sarcomeric proteins (skeletal and cardiac α-actins and α- and β-MHC) and a secreted protein (ANF) in the LV myocardium of the adult (9-mo-old) and old (18-mo-old) rats with or without pressure overload.

Methods

Materials

Two age groups (9 and 18 mo old) of male Fisher 344 rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were obtained under contract with the National Institute of Aging. Average life span of the males of this colony is ~ 24 mo. The rats of this species do not gain body weight between 9 and 18 mo of age, and their blood pressure does not change during this time period (8).

Operative procedures

Operative procedures for producing an ascending AoC were described in detail previously (8). Briefly, each rat was anesthetized either with brevital (50 mg/kg i.p.) or with methohexital sodium (60 mg/kg i.p.). The rats were intubated and respiration was controlled by a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA) with room air. The left thorax was opened at the third intercostal space to expose the ascending aorta. The ascending aorta was carefully dissected free from the pulmonary artery and surrounding tissues, and a surgical thread (1–0 silk) was drawn under the ascending aorta. A 16-gauge needle (1.6 mm o.d.) was placed alongside the ascending aorta, and the aorta and needle were tied tightly together with the thread. The needle was then removed, leaving the ascending aorta constricted to a diameter of 1.6 mm. The thorax was closed with a silk suture, whereas the lungs were inflated with positive end-expiratory pressure. For sham-operated groups, the identical procedure was performed, except that induction of AoC was not done. As reported previously (8), operative mortalities of 9- and 18-mo-old rats with AoC were 15 and 23%, respectively.

RNA measurement and analyses

RNA analyses were performed in groups of 56 animals ($n = 28$ each for 9- and 18-mo-old rats) that had undergone the operations described above, creating AoC ($n = 34$) or performing a sham operation ($n = 22$). Rats were killed at 30, 45, 60, 90, and 180 min after AoC for the study of protooncogenes and at 5 d for the study of contractile protein gene expression. The left ventricle was carefully dissected from the atria and right ventricle and stored in liquid nitrogen until use. Total cellular RNA was extracted by the guanidinium–hot phenol method. The RNA concentration in the LV myocardium was determined by spectrophotometry, with the absorbance at 260 nm. The ratio of the absorbance at 280 nm was >1.9 in all the samples.

RNA blot analysis. 20-μg samples of total cellular RNA from each animal were size fractionated by electrophoresis on a 1.0% agarose–formaldehyde gel and transferred to nylon or nitrocellulose filters. The filters were prehybridized in the solution mix (containing 50% formamide, 5× standard saline citrate [SSC; 1× = 0.15 M NaCl, 0.015 M sodium citrate], 5× Denhardt's solution, 0.2% SDS, 0.025 M sodium phosphate, and 250 μg/ml calf thymus DNA) at 42°C for ≥ 2 h and hybridized with specific DNA probes in the same solution mix plus 10% dextran sulfate, at 42°C, for 16 h. For the 18-S rRNA oligonucleotide probe, double-distilled water was substituted for formamide. At the end of hybridization, the filters were washed serially, with the final wash in 0.1× SSC–0.2% SDS at 65°C for 15–90 min, except for the skeletal α-actin probe (in 0.5× SSC–0.1% SDS at 55°C for 15 min) and the 18-S rRNA probe (in 3× SSC at room temperature for 30 min). DNA probes used in this study were as follows: (a) c-fos: a 2.1-kb EcoRI fragment of the mouse c-fos cDNA clone pGEM-fos (a generous gift from Dr. M. E. Greenberg); (b) c-jun: a 2.1-kb fragment of the mouse c-jun cDNA (29); (c) SR Ca^{2+}-ATPase: a 0.7-kb PstI fragment generated from the carboxy terminal and 3' untranslated region of the cDNA clone pCA, specific for the rabbit cardiac–slow twitch skeletal muscle SR Ca^{2+}-ATPase (30); (d) cardiac calsequestrin: a 1.9-kb EcoRI fragment from a cDNA clone IC3A, containing the entire coding region and 3' untranslated region of a canine cardiac calsequestrin (31); (e) ANF: a synthetic 84-nucleotide-long oligonucleotide complementary to the entire coding sequence of the rat ANF (32); (f) skeletal α-actin: a synthetic oligonucleotide complementary to the first 57 nucleotides of the 3' untranslated region of the mouse skeletal α-actin cDNA (33); (g) cardiac α-actin: a synthetic oligonucleotide complementary to the first 55 nucleotides of the 5' untranslated region of the mouse actin cDNA (33); (h) glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a 1.3-kb PstI fragment from cDNA pUC-GAPDH13, containing the entire coding region and a part of 3' untranslated region of the rat GAPDH (34); and (i) 18-S rRNA: a synthetic oligonucleotide (5'-ACGGTAT-CTGATCGTCTTCGA-3') complementary to the rat 18-S rRNA (35). The cDNA probes were labeled by random priming method with [^{32}P]dCTP (3,000 Ci/mmol, New England Nuclear, Boston, MA) and the synthetic oligonucleotide probes with T_4 polynucleotide kinase and [^{32}P]γATP (3,000 Ci/mmol, New England Nuclear) (36).

The blots were exposed on x-ray films with intensifying screens for 1–7 d at −80°C. Relative amounts of each mRNA were determined by laser densitometry in the linear response range of the x-ray films. The densitometric scores of specific mRNAs were "normalized" by that of GAPDH mRNA, which encodes a constitutively expressed glycolytic enzyme, as an internal control. Normalization by 18-S ribosomal RNA signals or by the cardiac α-actin signals produced similar results. The mean value of the normalized scores of mRNA from sham-operated, adult (9-mo-old) animals was arbitrarily determined as 1.0 for each mRNA species.

S1 nuclease mapping. S1 mapping analysis was performed as previously described (14). Briefly, 20 μg of total RNA was hybridized in 80% formamide at 42°C for 16 h with a 3' end-labeled, single-stranded probe, generated from the 3' end PstI fragment of NB3, a cDNA specific for the rat β-MHC (14). This 347-nucleotide-long probe contains 180 nucleotides of common coding sequence at the carboxy terminal of the α- and β-MHC, in addition to the entire 3' untranslated sequence of the β-MHC gene, which diverges completely from the α-MHC gene. This probe also contains 43 nucleotides of oligo (dT) tails. S1 nuclease digestion was done with 150 U of enzyme (New England Nuclear) and the digestion products were size-separated on 7% polyacrylamide–8.3 M urea-sequencing gel. The gel was dried and exposed on an x-ray film at −80°C as mentioned above. The relative amounts of α- and β-MHC mRNAs were quantitated by laser densitometry in the linear range of the x-ray film.

Statistical analyses

All data are expressed as means±SEM. The statistical significance of differences in mean values between two groups was assessed by the

unpaired Student's t-test with Bonferroni's correction or Fisher's exact test if overall significance was demonstrated by analysis of variance (37). Significance was accepted at $P < 0.05$ level.

Results

The present study is a direct extension of our previous study concerning the hemodynamic and morphological changes in response to AoC in the rat LV myocardium (8). Table I summarizes the results of the hemodynamic and morphological parameters in this model. The degrees of the severity of pressure overload imposed on the left ventricles were similar in both age groups (LV peak systolic pressure in mmHg: sham 91±3 and AoC 127±13 for the adult; sham 95±9 and AoC 124±14 for the old). This pressure overload caused a moderate but significant LV hypertrophic response (23% increase in LV dry weight in 4 wk) in the adult rats but no responses in the old rats. At histological level, the mean value of myocyte width in the sham-operated old animals was much larger than that in the sham-operated adults (29.2±1.4 vs. 20.4±0.8 μ). AoC increased the mean value of myocyte width significantly (from 20.4±0.8 to 25.9±0.9 μm) in the adult rats but did not cause a further increase in this index in the old. These age-related differences in LV hypertrophic responses to AoC were also evident in the LV myocardial RNA concentration, which also increased significantly (21% over sham) in the adult, but by only 4% in the old. Thus, at the moderate pressure overload used in this model, LV hypertrophy occurred in the adult but not in the old rats.

In this study, we asked whether the aging process affects changes in cardiac gene expression in response to AoC, in spite of the absence of quantitative hypertrophic response in the old animals. First, we examined expression of two "immediate early" genes, c-fos and c-jun protooncogenes, in response to acute pressure overload. As shown in Fig. 1, c-fos, mRNA was markedly induced at 90 min after AoC in 9-mo-old rats, but its expression was significantly reduced in 18-mo-old animals. The induction of c-fos was transient, inasmuch as its level of expression returned to near the baseline by 180 min after AoC. This age-related difference in c-fos expression was also observed when the animals were killed at earlier time points (30, 45, and 60 min) after AoC (data not shown). Expression of c-jun in response to AoC was also reduced in the old rats compared with the adults at 90 min after AoC. Thus, the old hearts

appear to have an attenuated nuclear response to acute pressure overload, as determined by the two-protooncogene induction.

Next, we examined expression of various contractile protein genes in response to AoC. One of the well-studied biochemical markers of hypertrophy in the rat is increased β-MHC/α-MHC ratio. To determine the levels of α- and β-MHC mRNAs, we performed S1 nuclease mapping analysis (Fig. 2). S1 digestion produced two protected bands of 180 and 304 nucleotides, corresponding to α- and β-MHC mRNAs, respectively. In 9-mo-old animals, the level of β-MHC mRNA was significantly higher than in young (2–3-mo-old) animals (14). The ratio of β/α MHC mRNAs was similar between the 9- and 18-mo-old sham-operated rats. In response to AoC, the β/α ratio increased to a similar degree in both age groups. In the adult rats, this increase in the β/α ratio seemed to be achieved primarily by an increase in the β-MHC mRNAs, whereas in the old rats the same increase appeared to be achieved primarily by a decrease in the α-MHC mRNA. Thus, in response to pressure overload, the β/α ratio increased in both age groups, but possibly by different mechanisms.

We also examined changes in the level of the SR Ca^{2+}-ATPase mRNA in response to AoC. Northern blot analysis has detected an mRNA species of $\sim$ 4 kb in the rat LV myocardium (Fig. 3, top row). Densitometric analysis of the hybridizing signal from each animal revealed that the level of the SR Ca^{2+}-ATPase mRNA, corrected by GAPDH signals, remained unchanged after AoC in the adult rats but decreased markedly (by 69%, $P < 0.01$) in the old rats (Fig. 4, left). Slot blot analysis of RNA yielded a very similar result (data not shown). Similar results were also obtained when the SR Ca^{2+}-ATPase signals were corrected either by the α-cardiac actin signals or by the 18-S ribosomal signals (data not shown).

The aging process alone, at least until age 18 mo, did not alter expression of the SR Ca^{2+}-ATPase gene, because there was no significant difference in the level of this mRNA species between the sham-operated adult and old rats. However, at very advanced age (30 mo), the SR Ca^{2+}-ATPase mRNA was reported to be decreased by 60% compared with young (4-mo-old) animals (38).

We then analyzed expression of cardiac calsequestrin, a high capacity and medium affinity Ca^{2+}-binding protein localized at the terminal cisternae portion of cardiac SR (31). Little is known concerning regulation of the cardiac calsequestrin

Table I. Changes in Hemodynamic and Morphological Parameters and RNA Concentration in the LV Myocardium in Response to AoC in the Adult (9-mo-old) and old (18-mo-old) Rats

	Adult		Old	
	Sham	AoC	Sham	AoC
LVPSP (mmHg)	91±3	127±13*	95±9	124±14‡
LVEDP (mmHg)	4.6±0.8	5.6±0.9	5.1±1.0	6.0±0.6
LV dry weight/body weight (mg/g)	0.47±0.01	0.58±0.03‡	0.49±0.01	0.49±0.01
LV dry weight/tibial length (mg/cm)	39±1	44±2‡	39±1	39±2
Myocyte width (μm)	20.4±0.8	25.9±0.9‡	29.2±0.6	29.2±1.4
RNA Concentration (μg/g wet wt)	963±22	1,168±31‡	980±10	1,021±16

Data are means±SE. * $P < 0.05$, ‡ $P < 0.01$ compared with sham of the same age group. See reference 8 for more detailed hemodynamic and morphological data. LV, left ventricular; PSP and EDP, peak systolic and end-diastolic pressures, respectively. Adults were 9 mo and old rats were 18 mo old.
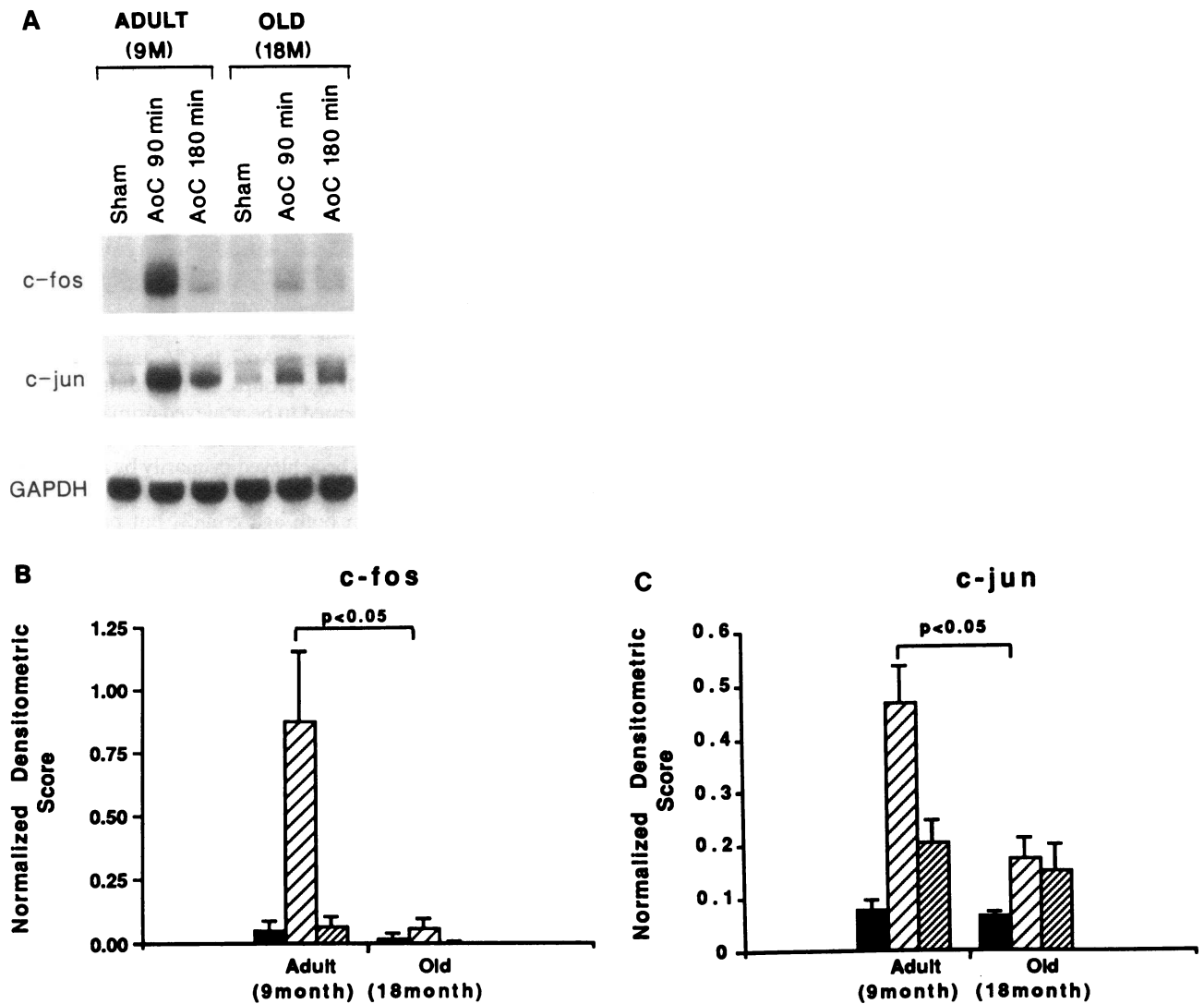
A

ADULT (9M) OLD (18M)

Sham | AoC 90 min | AoC 180 min | Sham | AoC 90 min | AoC 180 min

c-fos

c-jun

GAPDH

B c-fos

Normalized Densitometric Score

1.25
1.00
0.75
0.50
0.25
0.00

$p<0.05$

Adult (9month)   Old (18month)

C c-jun

Normalized Densitometric Score

0.6
0.5
0.4
0.3
0.2
0.1
0

$p<0.05$

Adult (9month)   Old (18month)

Figure 1. Expression of c-*fos* and c-*jun* mRNA in response to acute pressure overload. (*A*) RNA blot hybridization. The same blot was hybridized with c-*fos*, c-*jun*, and GAPDH cDNA probes. (*B* and *C*) Normalized densitometric scores. The signals of c-*fos* and c-*jun* were corrected by those of GAPDH. The numbers of samples are $n = 3$ for sham in each group; $n = 6$ for sham in each group; $n = 6$ for AoC in each group. ■, Sham; □, AoC 90 min; ▨, AoC 180 min.

gene, particularly in pathologic states such as cardiac hypertrophy and failure. Northern blot analysis (Fig. 3, *2nd row*) indicated that a single cardiac calsequestrin mRNA species of 2.9 kb was expressed in the rat LV myocardium. The level of cardiac calsequestrin mRNA expression did not change in response to AoC in the adult rats but decreased significantly (by 49%, $P < 0.05$) in the aged rats (Fig. 4, *right*). There was no age-related difference in expression of this SR protein gene in the sham-operated animals.

Fig. 5 shows the representative RNA blot analyses of mRNAs encoding ANF and skeletal and cardiac α-actins in the sham-operated and AoC rats of both age groups, and Fig. 6 summarizes changes in the relative abundance of mRNAs by densitometric analysis. By RNA blot analysis (Fig. 5, *top row*), LV expression of ANF mRNA (~ 1.0 kb) was barely detectable. However, the mean level of ANF mRNA increased in response to AoC in the adult rats. The level of ANF mRNA was already high in the sham-operated old rats. AoC failed to cause

a further significant increase in the level of this mRNA in the old rats (Fig. 6 *A*).

The mean level of skeletal α-actin mRNA increased in response to AoC in the adult rats (Fig. 5, *2nd row*, and Fig. 6 *B*). In the old sham-operated rats, the level of skeletal α-actin mRNA was not different from that in the adult sham-operated rats and AoC did not increase expression of this mRNA species. The level of cardiac α-actin mRNA was not different among the experimental groups. (Fig. 5, *3rd row*, and Fig. 6 *C*).

Discussion

This study demonstrated that the pattern of expression of cardiac gene expression in response to pressure overload differs markedly between the adult and aged animals. Expression of two immediate early genes, c-*fos* and c-*jun*, in response to AoC is markedly attenuated in the old rats. In contrast, expression of some cardiac genes (SR Ca^{2+}-ATPase, cardiac calsequestrin,
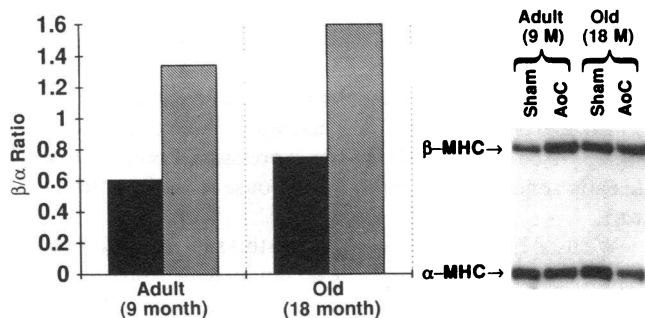
Figure 2. Expression of the α- and β-MHC mRNAs in the LV myo-cardium. S1 nuclease mapping analysis was performed with a 3' end-labeled, single-strand probe, generated from the 3' end PstI fragment of NB3, cDNA-specific for the rat β-MHC (3, 10). Each lane represents the pooled sample from five animals of each group. S1 digestion created two protected bands of 180 and 304 nucleotides, corresponding to α- and β-MHC mRNAs, respectively. Relative amounts of the α- and β-MHC mRNAs were determined by laser densitometry and the ratio of β-MHC to α-MHC (β/α) was calculated and shown in the bar graph. The β/α ratio increased similarly in both age groups in response to AoC. ■, Sham; ▨, AoC.

Figure 4. Changes in the levels of mRNAs encoding the SR Ca^{2+}-ATPase (left) and cardiac calsequestrin (right) in response to AoC. The relative abundance of the SR Ca^{2+}-ATPase mRNA (normalized by the level of GAPDH mRNA) remained unchanged in response to AoC in the adult rats, but decreased markedly (by 69%, $P < 0.01$) in the old rats. The level of cardiac calsequestrin mRNA also decreased significantly (by 49%, $P < 0.05$) after AoC in the old rats but not in the adult. $n = 5$ for each group. §$P < 0.01$ adult vs. old animals. ■, Sham; ▨, AoC.

and MHCs) changed in response to AoC in the old rats, in spite of the absence of LV hypertrophic response in this group of animals. On the other hand, the levels of mRNAs encoding ANF and skeletal α-actin did not change after AoC in the old animals. These data suggest that there exists a dissociation be-
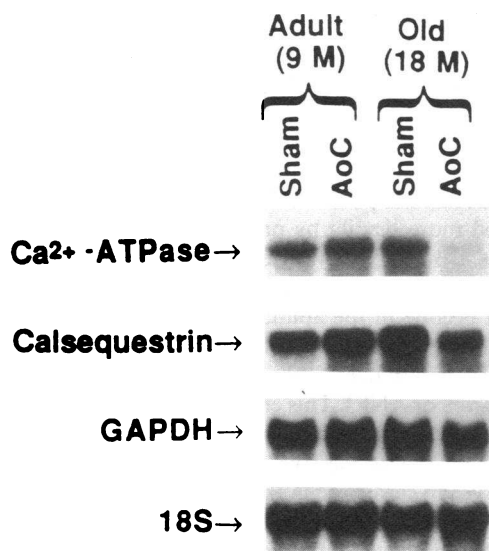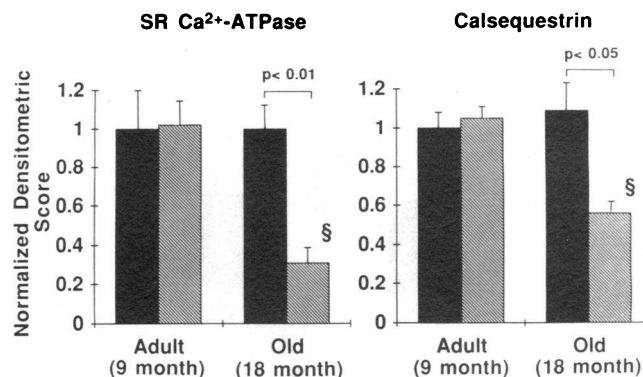
tween the quantitative (hypertrophy) and qualitative (patterns of gene expression) responses of the LV myocardium to the hemodynamic stress and aging on cardiac gene expression were complex and varied with individual genes studied, suggesting that underlying molecular mechanisms for the altered expression of these genes may also be complex.

Recent studies have shown that senescent human cultured fibroblasts failed to proliferate in response to serum (39) or platelet-derived growth factor (40). Interestingly, expression of c-fos gene failed to increase by serum stimulus in the senescent cells (39). These findings are reminiscent of our results because the old myocardium had very diminished expression of c-fos and c-jun and failed to develop hypertrophy in response to

Figure 3. Expression of mRNAs encoding the SR Ca^{2+}-ATPase and cardiac calsequestrin in the rat LV myocardium. RNA blot hybridization was carried out with cDNA clones specific for the rabbit cardiac–slow twitch skeletal SR Ca^{2+}-ATPase (27) and canine cardiac calsequestrin (25). The SR Ca^{2+}-ATPase cDNA probe (pCA) hybridized with an mRNA species of ∼ 4 kb. The cardiac calsequestrin cDNA probe (IC3A) produced a single band of ∼ 2.9 kb. In contrast to the canine heart (25), the additional band of 2.2 kb was not detected with the ICA3 clone in the rat LV myocardium. The same blot was hybridized with a cDNA for GAPDH (29) and a synthetic oligonucleotide for 18-S rRNA as internal controls. Each lane represents the pooled sample from five animals of each group. Expression of the SR Ca^{2+}-ATPase and calsequestrin mRNAs decreased markedly in the old AoC animals but remained unchanged in the adult animals.
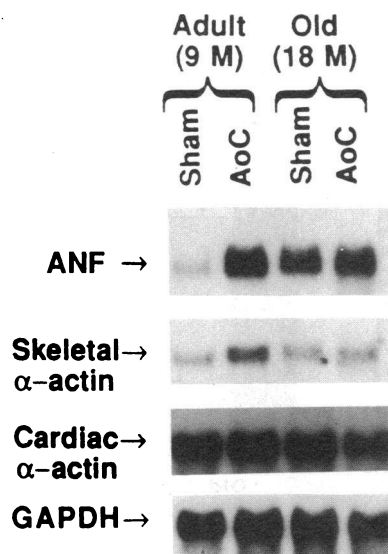

Figure 5. Representative RNA blot analysis of the expression of mRNAs encoding ANF and skeletal and cardiac α-actins in the rat LV myocardium. Each lane represents the pooled samples of five animals of each group. Synthetic oligonucleotides were used for the hybridization. The mean level of ANF mRNA (1.0 kb in size) more than doubled in response to AoC in the adult rats. In contrast, ANF mRNA was already high in the LV myocardium from the old sham-operated rats and did not increase further after AoC. The mean level of skeletal α-actin mRNA (1.8 kb) also elevated by twofold in the adult rats with AoC but showed no changes in the old animals. The level of cardiac α-actin mRNA (1.8 kb) was not different among the experimental groups.
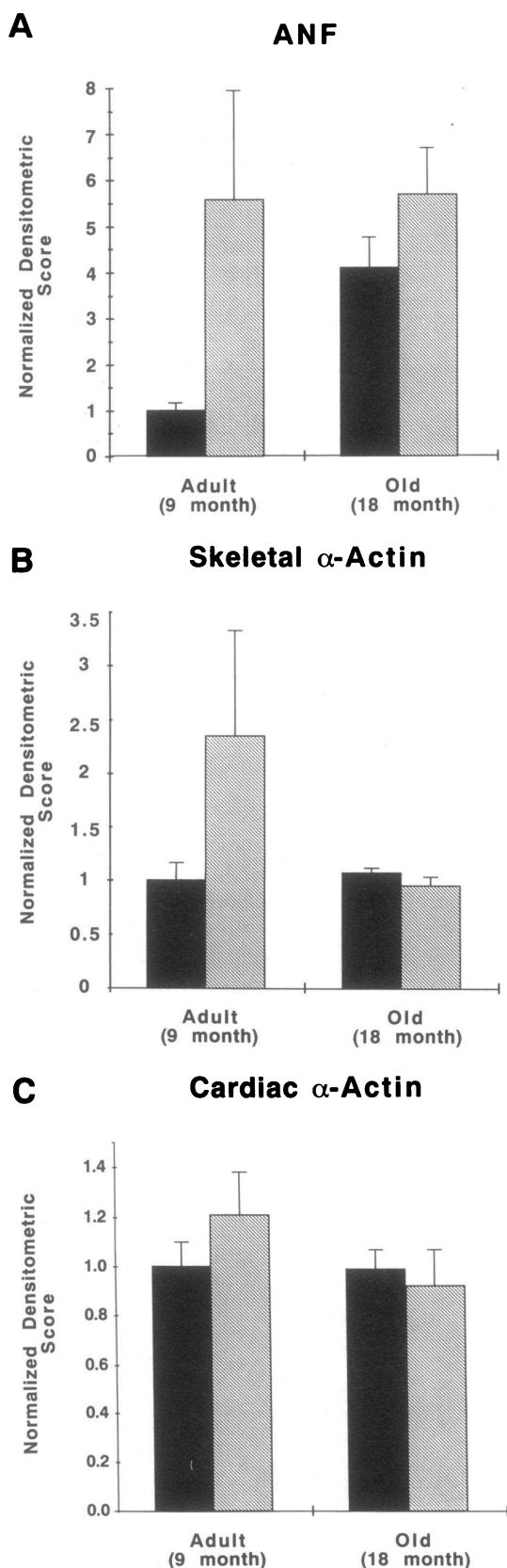
A

ANF

B

Skeletal α-Actin

C

Cardiac α-Actin

Figure 6. Densitometric analysis of the levels of mRNAs encoding ANF (*A*) and skeletal (*B*) and cardiac α-actin (*C*) in the LV myocardium. The relative abundance of the specific mRNA species was normalized by the level of GAPDH mRNA in each animal. $n = 5$ for each group. ■, Sham; ▨, AoC.

AoC. Such similarity may imply that common mechanisms might underlie the aging process of the cell growth system in cardiocytes and other cell types. It should be noted, however, that at present there is no formal proof that expression of protooncogene is causatively linked to cardiac hypertrophy. It also remains to be determined whether expression of other immediate early genes is also reduced in response to AoC in the aged heart.

We used Fisher 344 rats because in this species body weight, heart weight, and blood pressure do not increase from the adult stage to old age. In contrast, Sprague-Dawley and Long-Evans rats develop age-related cardiac enlargement. Fisher 344 rats are also known to have significant age-related myocyte loss (22). Consistent with this finding, our histological study showed that the mean value of myocyte width in the sham-operated old animals was significantly larger than that in the adult sham-operated rats (29.2 ± 0.6 vs. 20.4 ± 0.8 μm). Thus, advancing age alone appears to cause hypertrophy at the cellular, but not at the organ, level to compensate for myocyte loss accompanying the aging process.

AoC increased the mean value of myocyte width from 20 to 26 μm in the adult rats but did not cause a further increase in this index in the old. On the other hand, the biochemical phenotype of the stressed myocardium of the old animals is very similar to that of the advanced hypertrophy in the young (10–20). This indicates that the senescent myocytes are able to sense increased load and change the pattern of gene expression. However, they are unable to increase their size further, at least not in response to the moderate pressure overload used in this study. It is possible that the senescent myocytes may be approaching the limit in size during the normal aging process. In this regard, it is noteworthy that hypertrophied ventricles of young rats (produced by AoC) had very diminished expression of c-*fos* and c-*jun* in response to acute pressure overload in vitro compared with those in nonhypertrophied ventricles (41).

An alternative interpretation of the age-related differences might be considered. It is possible that proliferation of fibroblasts is triggered more readily by pressure overload in aged than in adult rats. Dilution of myocyte RNA with nonmyocyte RNA in the total RNA preparation could create the spurious appearance of a decline in the abundance of certain mRNA species (Ca^{2+}-ATPase or calsequestrin) and mask overload-induced increases in abundance of other species (β-MHC, skeletal actin, c-*fos*, c-*jun*, or ANF). This effect may not be detected by the GAPDH or 18-S RNA control signals. However, it is highly unlikely that this is the sole cause of the age-related differences observed in cardiac gene expression, because (*a*) the decrease in the Ca^{2+}-ATPase and calsequestrin mRNAs in the aged rats with AoC was evident even when corrected by the level of the cardiac actin mRNA, a myocyte-specific gene product; (*b*) the age-related differences in protooncogene expression in response to AoC were observed much earlier than proliferation of fibroblast could take place; and (*c*) there is no evidence for exuberant proliferation of fibroblasts sufficient to dilute the cardiac-specific mRNAs by $\sim 50\%$ in the old heart preparations, since the total RNA content increased by only 4% after AoC in the old rats.

In contrast to previous studies (18–20), expression of the SR Ca^{2+}-ATPase in our study remained unchanged in response to AoC in the adult rats. This is most likely due to a moderate degree of pressure overload (a mean of 33-mmHg increase in

LV systolic pressure) used in our study. Ventricular dry weight/ body weight ratio increased by > 40% in other studies (18, 19), but by only 23% in ours. It has been demonstrated that the level of the SR Ca^{2+}-ATPase mRNA does not change in mild to moderate LV hypertrophy in young rats (20). However, more severe pressure overload was not feasible in this study, because operative mortality was unacceptably high (> 50%) in the aged rats.

We examined expression of the cardiac genes only at mRNA level, and it is possible that some translational or post-translational controls may be present in regulation of these genes. However, previous studies have shown that the mRNA and protein levels of the SR Ca^{2+}-ATPase (18, 20), ANF (16), and MHCs (14) changed in a parallel fashion in the LV myocardium subjected to pressure overload. Although yet to be proven formally, it is likely that the changes in the mRNA levels in our study are likely to be reflected to those of the corresponding proteins.

At present, we do not know whether our findings are unique to this experimental model or are more general phenomena. If we can extrapolate our findings to human subjects, the present study suggests the possibility that the diminished ability to induce proto-oncogenes in response to hemodynamic overload, as well as the age-related differences in cardiac gene expression, may contribute to the higher frequency of heart failure in old patients than in young cohorts subjected to similar hemodynamic stress. Although an acute obstruction of ascending aorta is extremely rare in the clinical setting, other kinds of acute hemodynamic overload, such as acute myocardial infarction and acute mitral regurgitation, are very common in aged patients. Altered expression of a variety of cardiac gene expression might be related to systolic and diastolic dysfunction, which is observed often in old patients.

Acknowledgments

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