Cardiac dysfunction in aging conscious rats: altered cardiac cytoskeletal proteins as a potential mechanism

Samuel C. Lieber,1,2* Hongyu Qiu,1* Li Chen,1 You-Tang Shen,1 Chull Hong,1 William C. Hunter,1,2 Nadine Aubry,1,2 Stephen F. Vatner,1 and Dorothy E. Vatner1

1Cardiovascular Research Institute and Department of Cell Biology & Molecular Medicine, University of Medicine & Dentistry of New Jersey, New Jersey Medical School, Newark; and 2Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, New Jersey

Submitted 11 February 2008; accepted in final form 27 May 2008

Lieber SC, Qiu H, Chen L, Shen YT, Hong C, Hunter WC, Aubry N, Vatner SF, Vatner DE. Cardiac dysfunction in aging conscious rats: altered cardiac cytoskeletal proteins as a potential mechanism. Am J Physiol Heart Circ Physiol 295: H860–H866, 2008. First published June 20, 2008; doi:10.1152/ajpheart.00146.2008.—The objective of this study was to test the hypothesis that the mechanism mediating left ventricular (LV) dysfunction in the aging rat heart involves, in part, changes in cardiac cytoskeletal components. Our results show that there were no significant differences in heart rate, LV pressure, or LV diameter between conscious, instrumented young [5.9 ± 0.3 mo (n = 9)] and old rats [30.6 ± 0.1 mo (n = 10)]. However, the first derivative of LV pressure (LV dP/dt) was reduced (8,309 ± 790 vs. 11,106 ± 555 mmHg/s, P < 0.05) and isovolumic relaxation time (τ) was increased (8.7 ± 0.7 vs. 6.3 ± 0.6 ms, P < 0.05) in old vs. young rats, respectively. The differences in baseline LV function in young and old rats, which were modest, were accentuated after β-adrenergic receptor stimulation withdobutamine (20 μg/kg), which increased LV dP/dt by 170 ± 9% in young rats, significantly more (P < 0.05) than observed in old rats (115 ± 5%). Volume loading in anesthetized rats demonstrated significantly impaired LV compliance in old rats, as measured by the LV end-diastolic pressure and dimension relationship. In old rat hearts, there was a significant (P < 0.05) increase in the percentage of LV collagen [2.4 ± 0.2 vs. 1.3 ± 0.2%, α-tubulin (92%), and β-tubulin (2.3-fold)], whereas intact desmin decreased by 51%. Thus the cardiomyopathy of aging in old, conscious rats may be due not only to increases in collagen but also to alterations in cytoskeletal proteins.

at the level of the single myocyte (27) led us to hypothesize that material property changes in aging hearts may be due to more than just changes in collagen. Therefore, the third goal of this work was to measure cellular protein changes in the LV of young and old Fischer 344 × Brown Norway F1 hybrid (F344×BN) rats, focusing on intracellular proteins, e.g., tubulin and desmin. Although cytoskeletal and membrane proteins have been examined in other states of LV dysfunction (7, 8, 13, 20), their effects in aging remain to be elucidated.

MATERIALS AND METHODS

Animals. F344×BN rats were obtained from the National Institute on Aging (NIA). Physiologic studies were conducted on young [5.9 ± 0.3 mo old; n = 9] and old rats [30.6 ± 0.1 mo old (aged, not senescent) n = 10] (26, 28). A parallel group of young (5.5 ± 0.2 mo old; n = 7) and old Fischer 344 × Brown Norway (F344×BN) rats (30.3 ± 0.3 mo old; n = 7) were used only for tissue harvest, and samples were prepared for immunoblotting (Western blot analyses), pathology (collagen measures), and immunohistochemistry. All procedures and protocols were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey-New Jersey Medical School and followed the National Institutes of Health guidelines for the care and use of laboratory animals.

Implantation of instrumentation. The animals were anesthetized with ketamine and acepromazine (65 mg/kg ip and 2 mg/kg ip, respectively). A left intercostal thoracotomy was performed. A miniature solid-state pressure gauge (P1.5; Konigsberg Instruments; Passadena, CA) was inserted into the LV for the measurements of LV pressure and the first derivative of LV pressure (LV dP/dt). A Micro-Renathane tubing (Braintree Scientific) venous catheter (7-in. length) was placed for drug injection, whereas a Rena Pulse tubing (Braintree Scientific) catheter (8-in. length) was placed in the ascending aorta for aortic pressure measurements. LV pressure or aortic pressure was measured using a 1.4-F micromanometer (Millar Instruments) under anesthesia as described above. The right carotid artery was exposed via a midline ventral incision and dissected free from neighboring structures. A pair of ultrasonic crystals were placed on the opposing LV surfaces to measure LV diameter and fractional shortening. Animals were allowed to recover for 1–2 wk before the initiation of experiments.

Hemodynamics. Rats were placed in a restraining box designed for conscious studies. The fluid-filled catheter was connected to a pressure transducer (DTX Plus; Becton Dickinson) and calibrated with a manometer. The implanted instrumentation was connected to an amplifier with an electronic filter setup. Data were displayed and recorded on a computer through a data acquisition system (Notocord).

**THE EFFECTS OF AGE ON CARDIAC FUNCTION** have been explored extensively in anesthetized rats (16, 24, 33, 37, 44). Almost all studies demonstrate the development of cardiomyopathy with age, characterized by depressed left ventricular (LV) systolic and diastolic function, LV hypertrophy (LVH), and increased collagen in the heart (21, 23). The first goal of this study was to determine the extent to which LV function was depressed in conscious, chronically instrumented rats. A second goal was to examine the extent to which LV diastolic compliance was altered in the aging rat heart. LV dysfunction with age has largely been attributed to altered excitation-contraction mechanisms, a shift in myosin heavy chain (MHC) protein from a faster to a slower isoform, and an increase in collagen in the heart (22). Our recent finding that stiffness increases with age

* S. C. Lieber and H. Qiu contributed equally to this work.

Address for reprint requests and other correspondence: D. E. Vatner, Dept. of Cell Biology & Molecular Medicine, UMDNJ-New Jersey Medical School, 185 South Orange Ave., MSB G-609, Newark, NJ 07103 (e-mail: vatnerdo@umdnj.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
with a 1,000-Hz sampling rate. The aortic pressure measured with a fluid-filled catheter was used to cross-calibrate the offset for the Konigsberg, where the LV systolic pressure is matched with the systolic arterial pressure as previously described (9). Baseline physiological measurements were then recorded and parameters calculated with Notocord software, including dP/dt, heart rate, LV end-diastolic pressure (EDP), end-systolic pressure (ESP), the Weiss isovolumic relaxation time constant (τ), end-diastolic diameter (EDD), and end-systolic diameter (ESD). LV fractional shortening was calculated as \((\text{EDD} - \text{ESD})/\text{EDD}) \times 100\). Responses to dobutamine (5, 10, 15, and 20 μg/kg iv) were also examined in conscious young and old rats as previously described (9).

Additional young and old F344xBN rats were used for assessment of LV compliance, i.e., the LV pressure-dimension relationship. The basic surgical procedure and anesthesia were as described above. While LV pressures and dimensions were recorded continuously, acute volume loading was performed using intravenous infusion of saline to increase LVEDP by \(\sim 15\) mmHg.

**Western blot analysis.** Extracts for cytoskeletal and membrane proteins were prepared from frozen LV tissue from young and old F344×BN rats in a 2% SDS buffer containing 10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, and Sigma protease inhibitor (3 μl/ml), as previously described (30). Proteins were analyzed by Western blotting as previously described (46). In brief, equal amounts of each sample were loaded onto gels for SDS-PAGE using the Bio-Rad Minigel system. A 10% gel was used for β1-integrin (20 μg/well), α-tubulin (10 μg/well), β-tubulin (20 μg/well), and desmin (10 μg/well); an 8% gel was used for α-actinin (10 μg/well) and vinculin (10 μg/well); and a 6% gel was used for talin (10 μg/well). Gels were transferred to nitrocellulose membranes with a wet transfer cell. The blots were probed with primary antibodies and incubated overnight at 4°C. The following antibodies were used: β1-integrin, 610468 (Becton Dickinson); α-tubulin, T6199 (Sigma); β-tubulin, T7816 (Sigma); desmin, D8281(Sigma); α-actinin, A5044 (Sigma); vinculin, V9133 (Sigma); and talin, T3287 (Sigma). The primary antibody was applied at a concentration of 1:1,000 for all proteins. Secondary antibodies coupled to horseradish peroxidase were used. The immunopositive bands were visualized by using Western Lightning chemiluminescence reagent (PerkinElmer). All Western blot exposures were in the linear range of detection, and the intensities of the resulting bands were quantified using Quantity One software on a GS-800 densitometer (Bio-Rad). Data were normalized by reprobing the plots for

Fig. 1. Sample waveforms of left ventricular (LV) pressure (LVP; A), LV diameter (LVD; B), aortic pressure (AOP; C), and the first derivative of LVP (dP/dt; D) acquired from young and old conscious rats.
β-actin at 1:50,000 concentration and rocking at room temperature for 1 h.

Measurement of advanced glycation end products. Advanced glycation end products (AGEs) were determined as previously described (35). Briefly, defatted LV tissues (n = 5/group) were minced and washed with cold saline, resuspended in 0.5 M acetic acid, and digested for 24 h at 4°C in the presence of 1 mg/ml pepsin (Sigma). Samples were centrifuged at 15,000 g for 45 min. The resulting supernatant was considered the pepsin-soluble fraction, whereas the pellet was washed with cold saline, resuspended in 0.2 M Tris·HCl, pH 7.4, and incubated for 24 h at 37°C with 300 U/ml collagenase type VII (Sigma) containing 100 mM CaCl2. After centrifugation at 15,000 g for 45 min, the supernatant was regarded as the collagenase-soluble fraction. Both the pepsin-soluble fraction and the collagenase-soluble fraction samples were subjected to fluorescence emission spectra at 370-nm excitation and 440-nm emission wavelengths (Hitachi F-3010). The quantity of glycation-induced fluorescence was expressed as the relative fluorescence per milligram of dry weight of the tissue.

Histological analysis. Histological analyses of the heart sections for collagen were conducted as described previously (15). LV specimens were fixed with 10% neutral buffered formalin, embedded in paraffin, and sectioned at 6-μm thickness. Interstitial fibrosis was evaluated by picric acid Sirius red staining. The positively stained (red) fibrotic area was measured and expressed as a percentage of total area.

Statistics. All data are means ± SE. Statistical significance was determined by calculating a probability value (P) with a Student’s t-test, where values of P < 0.05 were considered significant. The regression for the LVEDP-diameter relationship was calculated using linear regression.

RESULTS

Hemodynamic measurements in conscious, chronically instrumented rats. An example of phasic waveforms of LV function in a young and old conscious rat is shown in Fig. 1. There were no significant differences in LV systolic pressure, LVEDP, mean arterial pressure, and heart rate between young and old conscious rats. However, there was a significant (P < 0.05) decrease in LV +dP/dt (11,106 ± 555 vs. 8,309 ± 790 mmHg/s) and LV −dP/dt (−7,133 ± 328 vs. −5,647 ± 492 mmHg/s). The LV isovolumic relaxation time constant τ was significantly (P < 0.05) reduced in old (8.9 ± 0.7 ms) compared with young rats (6.3 ± 0.6 ms). There was no difference in LVEDD between young and old rats. However, LV fractional shortening was significantly (P < 0.05) reduced in old (18.8 ± 2.1%) compared with young rats (26.4 ± 1.6%). The differences in baseline LV function were accentuated after volume loading was shifted significantly (P < 0.05) to the left in the old rats, demonstrating that the LV was stiffer. Regression analysis of linear data demonstrated a difference (P < 0.05) in the slopes.

Cellular matrix proteins. There were significant (P < 0.05) increases in α-tubulin (92%) and β-tubulin (2.3-fold) and a decrease in intact desmin (51%) in old rats (Fig. 4). The desmin degradation products increased twofold in the myocardium of old rats.

Table 1. Baseline hemodynamic values in young and old conscious F344×BN rats

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
<th>n</th>
<th>Value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP, mmHg</td>
<td>106±2</td>
<td>103±5</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5.2±1.3</td>
<td>7.0±1.3</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>11,106±555</td>
<td>8,309±790</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−7,133±328</td>
<td>−5,647±492</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Weiss τ, ms</td>
<td>6.3±0.6</td>
<td>8.7±0.7</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>LV EDD, mm</td>
<td>7.57±0.50</td>
<td>7.80±0.20</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>26.4±1.6</td>
<td>18.8±2.1*</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>383±9</td>
<td>376±15</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE measured in young and old Fischer 344 × Brown Norway (F344×BN) rats. LVSP, left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; +dP/dt, first derivatives of LV pressure; Weiss τ, Weiss isovolumic relaxation time constant; LV EDD, LV end-diastolic diameter. *P < 0.05 vs. young rats.
old rats, which might explain the reduction of the intact desmin protein (Fig. 4C). There were no significant changes in \( \beta _{1} \)-integrin, vinculin, \( \alpha _{1} \)-actinin, and talin (Fig. 5).

**Extracellular matrix proteins.** There was a significant \((P < 0.05)\) 34% increase in the ratio of LV to tibial length in old rats \((213 \pm 6.3 \text{ mg/cm})\) compared with young rats \((160 \pm 4.7 \text{ mg/cm})\). There was an 81.9% increase \((P < 0.05)\) in collagen deposition in the LV (Fig. 6) in old rats as shown by immunohistostaining. AGEs in the old rat LV were significantly \((P < 0.05)\) increased compared with those in young rat LV (Fig. 6).

**DISCUSSION**

We examined the effects of aging on LV hemodynamics in conscious, chronically instrumented rats and found significant differences in systolic and diastolic LV function in the old rats, directionally consistent with most prior studies in aging rats (5, 37). There were significantly greater differences in response to \( \beta _{1} \)-AR stimulation with dobutamine in old compared with young rats (Fig. 2). In part, this could be attributed to the additional LV dysfunction induced by \( \beta _{1} \)-AR desensitization, which is known to occur with age (16, 22, 33, 39, 44). Chronic sympathetic stimulation may support LV function in old rats, but reserve LV function is lost due to \( \beta _{1} \)-AR desensitization (22, 24, 39).

Although baseline LV stiffness, as assessed by LVEDP and diameter, did not change with aging, LV stiffness increased with volume loading (Fig. 3). The relationship between LVEDP and dimension we observed in old compared with young rats resembles closely the data published by Asif et al. (3) in anesthetized dogs. A prior echocardiography study by Walker et al. (43) found diastolic dysfunction in aging rats \((3–6 \text{ mo old})\), and Rozenberg et al. (36) and Cappelli et al. (6) found diastolic dysfunction in aging rat papillary muscle. Whether LV stiffness increases with aging in response to stress (e.g., increased LV end-diastolic volume) has been controversial. For example, Kass (17) showed in elderly patients that LV stiffness increases with volume load, as assessed using the LV diastolic pressure-volume component of the LV pressure-volume loop; the original data were included in an article by Brenneaux and Williams (10). Similar data on the diastolic portion of the pressure-volume load were found by Pacher et al. (33) in aging rat hearts with increased preload. Brenner et al. (5) did not find increased LV stiffness with aging in normal rat hearts with a simple increase in LV volume but, in contrast, found increased LV diastolic stiffness with ischemia-induced increases in LV volume.

One explanation for the increased LV stiffness is an increase in LV collagen. We found that LV collagen was increased in the old rats, consistent with what has been described by others previously (1, 12). In addition, the conjugation of AGEs was significantly higher in the LV of old rats than in young rats (Fig. 6), which is also consistent with previous studies (19, 25). Further evidence supporting the AGE hypothesis is also provided by the observation that the AGE cross-link breaker can reverse age-related increases in myocardial stiffness (2, 3).

The major finding of the current investigation demonstrates that cellular proteins change with aging, which furthers the...
novel hypothesis that a component of cardiac dysfunction, which occurs with aging, results from changes occurring at the myocyte level (27). Western blot analysis showed a significant 92% increase in β2-tubulin (Fig. 4A) and a 2.3-fold increase in β1-tubulin (Fig. 4B). The generally accepted role of the microtubular network is to serve as an intracellular transport system for proteins and lipids (14); however, its role in cell mechanics and the development of aging cardiomyopathy has not been identified previously.

Although the microtubules (or rather, tubulin) have been implicated in β2-AR signaling, the role of tubulin in the regulation of β2-AR pathway in adult cardiac muscle is still a subject of debate. Rasenick’s group (45) has shown that free tubulin can transfer GTP to a subunit of G proteins. Transfer is initially to Gαi and then to Gαs; thus, depending on the experimental conditions, tubulin either inhibits or activates adenylyl cyclase. Malan et al. (29) also recently proposed an effect of proliferated microtubulin on the Gi signaling pathway through the muscarinic receptor. It also was reported that microtubulin, disrupted by colchicine, attenuates the response to β2-AR stimulation (11, 29), and the proliferation of tubulin has been implicated in the depression of contractility in cardiac hypertrophy (42).

We also observed a decrease in the intermediate filament desmin (Fig. 4C). Desmin maintains myocyte cytoskeletal architecture through connections to the Z disk, contractile apparatus, subsarcolemmal cytoskeleton and the nuclei, and cellular organelles (34). Desmin’s function has been demonstrated in several studies using desmin knockout mice, which develop hypertrophy and eventually suffer from cardiomyopathy accompanied by myocyte death and extracellular matrix

---

**Fig. 5.** Comparison of expression levels of β1-integrin, vinculin, α-actinin, and talin in young and old LV rat samples. There were no significant differences in the expression of these proteins.

**Fig. 6.** Comparison of collagen in young and old LV rat samples. The collagen concentration (top) and advanced glycation end products (AGEs; bottom) were significantly increased in old rat LV (*P < 0.05). OD, optical density.
changes (32, 34, 40). The effect of desmin on cardiac global function has been studied in Langendorff preparations, where the LV weight was found to increase along with higher diastolic pressure and lower developed pressures (34). At the cellular level it has been suggested that desmin may be involved in supporting sarcomere alignment and force transmisión (34), where the contractile apparatus generates less active force without desmin (4). Studies also have indicated that desmin influences the position, movement, and activity of mitochondria in cardiac muscle (18, 31), which in turn could explain their abnormal function and appearance in transgenic mice (32, 34, 40).

Interestingly, many of the changes in proteins observed in the current study also have been observed in LVH and heart failure (13). For example, there are several cardiomyopathies attributed to alterations in desmin: animals with desmin knockout develop severe cardiomyopathy (32, 34, 40). In addition, alterations in tubulin have been proposed as the cause of LV dysfunction in LVH (38, 41, 42). Therefore, it appears that the cardiomyopathy of the aging rat heart shares some of the same features that occur in the cardiomyopathy of cardiac hypertrophy and failure, most importantly, the alterations in cytoskeletal proteins.

ACKNOWLEDGMENTS

We appreciate the helpful editorial assistance provided by Lauren Danridge.

GRANTS

This work was supported in part by National Institutes of Health Grants AG014121, HL033107, HL059139, HL069752, AG027211, AG023137, HL069020, and AG023567.

REFERENCES