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EXP EXPERIMENTAL CARDIOLOGY

Cardiac myosin phenotype remodelling following adrenomedullectomy and chronic 6-hydroxydopamine in male Sprague-Dawley rats

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BACKGROUND: An increase was previously found in relative beta myosin heavy chain (MyHC) in the right ventricle of rats following thoracic spinal transection. It was hypothesized that the MyHC remodelling that was observed might be due, in part, to autonomic influences on the right ventricle.

OBJECTIVE: To evaluate cardiac myosin phenotype following 21 days of reduced sympathetic activity.

METHODS: Adult male Sprague-Dawley rats underwent either adrenomedullectomy/chemical sympathectomy (SX) or sham operation/sham injection (CN). Twenty-one days following surgery, the animals were sacrificed and both ventricles were harvested. The ventricles were denatured and run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis for identification of MyHC isoforms.

RESULTS: SX resulted in a significant decline in catecholamines. In the right ventricle, beta MyHC ratio was twofold higher in SX animals than in CN rats, but there was no difference between groups in beta MyHC concentration in the left ventricle (P<0.05). Uniquely, we found a decrease in relative alpha MyHC in the right ventricle but no change in the myocardin phenotype in the left ventricle.

CONCLUSIONS: These data potentially indicate that MyHC concentrations in the left ventricle are less sensitive than the right ventricle to decreased sympathetic activity.

Key Words: Alpha myosin heavy chain; Beta myosin heavy chain; Catecholamines; Sympathectomy

M odulation of the myosin heavy chain (MyHC) composition of the adult myocardium can occur as a result of a variety of stimuli including thyroid hormone, diet, fasting, glucocorticoids and mechanical load (1-5). The sympathetic nervous system also exerts regulatory influences on myocardial contractile function, and blocking neural input into the heart causes profound changes in protein expression in the heart. Given that alterations in autonomic activity are common in aging, spinal injury, diabetes, and in diseases that affect the cardiovascular system, it would be prudent to understand how changes in autonomic activity may affect the relative MyHC content in the heart (6-8).

To date, much of the literature examining the chronic effect of changes in sympathetic nerve activity on MyHC expression in the heart of intact animals and humans has focused on the left ventricle rather than the right ventricle. Previous work from the authors’ laboratory found that chronic spinal injury results in differential remodelling in MyHC gene expression in the right and left ventricles (9). Given the paucity of data comparing changes in MyHC isoform composition between the left and right ventricles as a result of reduced sympathetic nerve activity, the purpose of the present study was to evaluate cardiac myosin phenotype remodelling in the right and left ventricles following 21 days of reduced sympathetic activity.

MATERIALS AND METHODS
All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center.

Subjects
A total of 16 adult male Sprague-Dawley rats were divided into control (CN) and sympathectomized (SX) groups. They were housed singly in a colony room maintained on a 12 h light to 12 h dark cycle, with dark onset at 19:00 h. All animals had continuous access to food and water throughout the experiments.

Surgery
Adrenomedullectomies were performed on nine rats. To perform these procedures, the rats were anesthetized with an intramuscular injection of ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively). The adrenal glands were approached via two 1 cm vertical incisions on the dorsum for visualization of the adrenals. After each adrenal was exposed, a circumferential incision was made on the adrenal, and the medulla was extirpated by gently pressing the surrounding tissue with two cotton tip applicators (10). Sham surgeries only entailed bilateral surgical exposure of the adrenal glands. The muscle and skin was then sutured closed in layers. Rats received buprenorphine (2.5 mg/kg) at the completion of surgery and every 12 h thereafter for two days. Following surgery, animals were allowed unlimited amounts of rat chow and water. The SX animals were given unlimited 1% NaCl drinking water.

Chemical sympathectomy
One week following adrenomedullectomy, the animals underwent chemical sympathectomy to destroy the terminal nerve endings of the sympathetic nervous system. By using these two procedures, catecholamine release was reduced from both the
Weights, total heart weights, left and right ventricle weights, right to left ventricle weights at the time of sacrifice are presented in Table 1. No significant differences in body weights, heart weights, ventricle weights, and ratios of right to left ventricle weights at the time of sacrifice were observed between the groups, nor was there any change in heart rate. Similarly, there were no myosin phenotype differences in the left ventricle MyHC phenotype between the groups.

### RESULTS

Body weights, heart weights, ventricle weights, and ratios of right to left ventricle weights at the time of sacrifice are presented in Table 1. There were no significant differences between the two groups in any of these variables.

There was a significant 62% decrease in plasma adrenaline in the SX group compared with the CN group (20.16±6.84 pg/mL versus 53.61±12.95 pg/mL; P<0.05). Likewise, there was a significant 71% decline in noradrenaline in the SX group as compared with the CN group (23.48±4.33 pg/mL versus 80.67±12.77 pg/mL; P<0.05).

The results of the changes in the relative MyHC compositions in the right and left ventricles from both groups are presented in Figures 1 and 2, respectively. There was a significant increase in the beta MyHC isoform in the right ventricle of the SX group (14%±3%) as compared with the CN group (8%±2%; P<0.05), with a significant and concurrent decrease in the relative concentration of the alpha MyHC isoform (CN: 92%±2%; SX: 86%±3%; P<0.05). There was no change in the left ventricle MyHC phenotype between the groups.

### DISCUSSION

The purpose of the present study was to evaluate cardiac myosin phenotype remodelling in the right and left ventricles following 21 days of reduced sympathetic activity. We found a significant decrease in the relative amount of the alpha MyHC isoform with a concomitant increase in the relative amount of beta MyHC isoform in the right ventricle of the SX animals as compared with the right ventricle of the CN group. However, there were no myosin phenotype differences in the left ventricle between the groups, nor was there any change in heart weight.

These findings are in agreement with data previously reported from the authors’ lab using a chronic spinal transection model in rats. We previously found an eightfold increase in relative beta MyHC in the right ventricle of rats following 60 days of spinal transection (9). There is some evidence that humans and animals have a chronic reduction in sympathetic activity following thoracic level spinal transection; therefore, we hypothesized that the MyHC isoform remodelling may in part be due to autonomic influences on the right ventricle (8,9). The data in rats subjected to reduced sympathetic activity in the present study agree with the hypothesis from the previous study. Thus, we believe that the results we observed in the right ventricle are consistent with a decline in sympathetic nerve activity to the heart.

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>CN</th>
<th>SX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>452±24</td>
<td>412±42</td>
</tr>
<tr>
<td>Total heart weight (g)</td>
<td>1.34±0.17</td>
<td>1.23±0.20</td>
</tr>
<tr>
<td>Left ventricle weight (g)</td>
<td>0.90±0.05</td>
<td>0.81±0.14</td>
</tr>
<tr>
<td>Right ventricle weight (g)</td>
<td>0.18±0.04</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>Right to left ventricle weight ratio</td>
<td>0.20±0.05</td>
<td>0.19±0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SD. CN Control group; SX Sympathectomized group

Peripheral nervous system and the adrenal gland. The neurotoxin 6-hydroxydopamine (6-OHDA) was used to destroy the noradrenergic nerve terminals (11). Once accumulated in neurons, 6-OHDA is believed to auto-oxidize, causing the degeneration of catecholamine-containing neurons (12). 6-OHDA was dissolved in a 0.9% NaCl/0.1% ascorbic acid solution immediately before injection (13). Animals were given one dose of 6-OHDA (40 mg/kg) via injection into the tail vein. Control animals were given an equal volume of the NaCl/ascorbic acid solution.

Preparation and study of the hearts

Two weeks following the 6-OHDA injections, the animals were anesthetized with an intramuscular injection of ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively). The heart was quickly dissected from the chest cavity. The atria and blood vessels were removed, and the right ventricle free wall was separated from the left ventricle and septum. Both the right and the left ventricle were weighed and frozen. Tissue samples were taken from the apex of the right and the left ventricle and processed according to Wang (14). Samples were immediately placed in cold pyrophosphate relaxing buffer (2 mM Na4P2O7+ low salt buffer [LSB]: 0.1 M KCl, 2 mM MgCl2, 2 mM ethyleneglycoltetra-acetic acid [EGTA], 10 mM Tris-maleate, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 mM diothiophenetol [DTT], pH 6.8). Each sample was then ground and centrifuged cold (4°C) at 3500 rpm for 15 min. The supernatant was decanted, and the pellet was resuspended with LSB and centrifuged cold at 3500 rpm for 15 min. Following multiple washes with LSB, the pellet was resuspended and stirred with Triton-X-100 Buffer (Sigma Chemical Co, USA) (0.5% weight per volume plus LSB). The samples were then washed repeatedly with LSB and resuspended with LSB and cold glycerol. The samples were then washed with 5 mM Tris solution, pH 8.0. The pellets were then resuspended with 2:1 5 mM Tris solution and sodium dodecyl sulfate (SDS) sample buffer (30 mM Tris, 3 mM ethylenediaminetetra-acetic acid, 3% weight per volume SDS, 120 mM DTT, 30% weight per volume glycerol, pH 8.0) at 50°C and placed in a water bath at 60°C for 60 s. A few grains of bromophenol blue were added and the samples were frozen. These extracts were then run on a 5% SDS-polyacrylamide gel electrophoresis with an acrylamide to bisacrylamide ratio of 100:1 for 12 h at 3.5 milliamps for separation and identification of MyHC isoforms (15,16). Relative concentrations of MyHCs were measured using a Zenith Soft Laser Scanning densitometer (Model SLR 2D/1D, Biomed Instruments, USA).

Measurement of plasma catecholamine levels

Catecholamines were quantified as previously described (17). Briefly, blood for catecholamines was collected in syringes containing 20 µL of 9% EDTA and 6% glutathione. 3,4-Dihydroxybenzylamine (DHBA) was added to plasma as an internal standard, and catecholamines were absorbed onto alumina at pH 8.5 and eluted with perchloric acid (0.1 M). The processed samples were quantified by high-performance liquid chromatography using a chromatographic analyzer with a catecholamine column and an electrochemical detector (Bioanalytic Systems, USA). This system has a detection limit of 20 pg for DHBA, adrenaline and noradrenaline.

Statistical analysis

Unpaired t tests were used to determine differences in body weights, total heart weights, left and right ventricle weights, right to left ventricle weight ratio, adrenaline and noradrenaline content, and relative alpha and beta MyHC composition in the left and right ventricles. Alpha was set a priori at P<0.05. All values are reported as mean ± SD.
Additionally, the changes we observed in the right ventricle in this study are consistent with other studies that have shown an increase in the beta isoform with reduced sympathetic activity to the heart. Wade et al (18) showed that six weeks of daily propranolol injections at a dose of 30 mg/kg resulted in significant increases in beta isoform expression in both the left and the right ventricles of adult rats, while Pauletto et al (19) observed the shift to the beta isoform of the left ventricle in rats treated with propranolol at a dose of 10 mg/kg for eight weeks. However, Wade et al (18) reported a threefold change and Pauletto et al (19) reported a significant change in the relative concentration of beta MyHC, while the present study reported only a twofold change. In the current study, sympathetic activity was significantly reduced compared with control animals but not completely eliminated. Other evidence that sympathetic nerve activity was reduced is that our animals exhibited chronic diarrhea, which is consistent with impaired autonomic function. Furthermore, the propranolol treatment lasted twice as long in the study by Wade et al (18) than in the current study.

Another factor potentially contributing to an increase in relative beta MyHC is chronic stretch of the myocardium. Using desoxycorticosterone acetate salt rats with left renal banding, Buttrick et al (20) and Yokota et al (21) showed an increase in the relative concentration of beta MyHC in the right ventricle. Additionally, in a model of pulmonary hypertension induced by monocrotaline injection, Tanaka et al (22) showed an increase in beta isoform content in the right ventricle. Although we did not measure blood pressure in the current study, chemical sympathectomy has been reported to result in lower blood pressure than normal (23,24). Furthermore, because there was no change in heart weight, it is unlikely that the change in cardiac MyHC isoform observed in this study was due to hypertension.

Additionally, it is unlikely that hypothyroidism, caloric restriction, glucocorticoids or exercise contributed to the results in this study. Although we did not measure thyroid hormone levels, to our knowledge, hypothyroidism is not a known side effect of adrenomedullectomy and 6-OHDA administration. All of the animals had free access to food and, although food consumption was not measured, there did not appear to be a decline in the amount of food eaten by the animals on a daily basis; therefore, caloric restriction was unlikely. The rats in this study did not receive glucocorticoids, nor were they exercised. Therefore, the most likely explanation for the relative MyHC remodelling in the right ventricle is a chronic decrease in sympathetic activity following adrenomedullectomy and 6-OHDA administration.
REFERENCES


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