Left Ventricular Structure and Performance in Middle-Aged Rats With Deoxycorticosterone Acetate–Salt Hypertension

Robert J. Tomanek and Peggy A. Barlow

We designed this study to establish the structural and functional characteristics of the hypertrophied left ventricle of middle-aged rats during mineralocorticoid-salt hypertension. Treatment was initiated at 12 months of age, and the rats were studied at either 13 or 15 months of age (after 1 or 3 months of treatment). All rats were unilaterally nephrectomized. One group received deoxycorticosterone acetate (DOCA) injections (30 mg/kg s.c.) biweekly and 1% NaCl drinking water (DOCA salt), and the other group was injected with the vehicle (sesame seed oil) and given tap water to drink (sham). During the first 4 weeks of DOCA-salt treatment, arterial pressure reached its peak and left ventricular enlargement was mainly due to increases of 47% in cardiocyte cross-sectional area in the middle layer of the left ventricular wall. The last 2 months were characterized by an accelerated endomyocardial growth. Because absolute left ventricular mass did not increase during the last 2 months of treatment, we conclude that cellular hypertrophy was accompanied by a focal loss of cardiocytes. Myocardial hydroxyproline concentration was initially elevated by 37% but normalized by the third month of treatment. Intracellularly, myofibril volume percent was not changed, but mitochondria volume percent declined (13% in the midmyocardium and 15% in the endomyocardium) and sarcoplasmic volume density increased by 25% and 39%, respectively, in these regions. Left ventricular hypertrophy was associated with enhanced peak cardiac and stroke indexes, measured during increased preload, after both 1 and 3 months of DOCA-salt treatment. Acceleration of flow, however, was depressed in the rats with left ventricular hypertrophy. After 1 month of treatment, developed pressure (during aortic occlusion) was higher than the controls but returned to control values after 3 months. We conclude that in middle-aged rats with DOCA-salt hypertension cardiocyte hypertrophy is accompanied by cell loss and an expansion of sarcoplasm but that left ventricular function is compensated. Furthermore, our data distinguish between the development and stabilization of left ventricular hypertrophy (i.e., these stages differ with respect to intracellular compartment volume shifts, regional cardiocyte hypertrophy, collagen concentration, and ventricular developed pressure). (Hypertension 1990;15:225–233)

Although deoxycorticosterone acetate (DOCA) in combination with an elevated salt intake has been used as a model of hypertension, relatively few studies have addressed the consequences of this particular model of hypertension on the heart. Like other models of pressure overload, the DOCA-salt model is characterized by moderate left ventricular hypertrophy, increased anatomic intercapillary distance, and increased coronary minimal resistance per gram of tissue. However, the left ventricles of rats treated with DOCA-salt also have characteristics that are not common to all models of pressure overload. Adrenergic activity is apparently enhanced as suggested by increased plasma catecholamines, decreased cardiac norepinephrine concentration, and a higher cardiac norepinephrine turnover rate. Scarring of the epicardial surface and within the myocardium has been noted. Moreover, a susceptibility to myocardial injury and ventricular fibrillation induced by isoproterenol characterize this model.
The importance of avoiding generalizations concerning pressure overload cardiac hypertrophy has become increasingly evident during the last decade, as it has been shown that a number of variables in addition to the increased hemodynamic load influence cardiac hypertrophy. These include, but are not limited to, the time period over which pressure elevation occurs and the duration of the overload as well as the age of the animal. Based on this rationale, we initiated studies that would characterize left ventricular structure and function in DOCA-salt hypertension. Because hypertension in humans is usually manifest in middle age, we induced hypertension in 12-month-old rats and obtained data after 1 or 3 months of treatment (from 13- and 15-month-old rats). This age period was selected because: 1) the hypertrophic response to aortic constriction has been found to be blunted between the ninth and 18th month of life and 2) peak developed pressure/ventricular mass declines in spontaneously hypertensive rats (SHR) after 12 months of age. We studied the rats after two time periods of DOCA-salt treatment to distinguish between the stages of developing and established left ventricular hypertrophy. Structural studies were based on the midmyocardium and endomyocardium because these layers consist of cardiocytes that are in a horizontal plane in the former and in a longitudinal plane in the latter.

**Methods**

**Animals and Experimental Design**

Male Sprague-Dawley rats (Harlan Sprague-Dawley), 12 months old, were unilaterally nephrectomized and after 5–7 days were assigned to either a sham or DOCA-salt group. The latter group received DOCA (30 mg/kg s.c.) dissolved in sesame seed oil (30 mg/ml) semiweekly and 1% NaCl drinking water, which also contained 0.5% KCl and 0.1% MgCl₂. The sham rats were injected with sesame seed oil and were supplied with tap water. All rats received Purina rat chow ad libitum. Data were obtained from 54 rats.

Blood pressure was measured by the tail-cuff plethysmographic method during the first 3 weeks in the 1-month study and during the last 3 weeks in the 3-month study. At the conclusion of the experimental period, we obtained left ventricular function data, blood samples for bioassays (atrial naturetic factor [ANF] and renin), and left ventricular specimens for evaluation of hydroxyproline. The hearts that were not used for the latter were fixed by vascular perfusion and used for morphometric studies.

**Left Ventricular Function**

Left ventricular function was evaluated by measuring 1) indexes of cardiac output at rest and during acute volume expansion (increased preload) and 2) developed ventricular pressure during a brief aortic occlusion (increased afterload). These methods have been previously detailed and are summarized here.

Anesthesia was maintained with methoxyflurane (Metofane) introduced as needed into the air system of a mechanical respirator (Harvard Apparatus, South Natick, Massachusetts). We continuously recorded arterial pressure (via a PE-50 polyethylene cannula tied into the femoral artery) and left ventricular pressure (via PE-10 tubing telescoped to PE-50 tubing and introduced through the right carotid artery). A cannula (PE-50 tubing) was tied into the left jugular vein for the purpose of introducing Tyrode’s solution. After obtaining prethoracotomy pressures, the aortic arch was exposed via sternal incision, and a Carolina Instruments electromagnetic flow probe (King, North Carolina) was placed on the ascending aorta. After a 10–15-minute period of stabilization, aortic flow (referred to here as cardiac output) was recorded. The flowmeter and pressure transducers attached to the femoral and ventricular catheters were interfaced to a Gislon 5/6 polygraph recorder, DC, 75 Hz (Middleton, Wisconsin). Acceleration of flow (dF/dt) and heart rate were also recorded at this time.

After the recording of baseline values, we rapidly volume loaded the heart by infusing Tyrode’s solution (40 ml/min/kg) until cardiac output reached a peak; the increase in cardiac output was sharp, and peak values were attained within 50 seconds after infusion onset. Zero flow was determined by arresting the heart with procaine. Instrument calibration was performed at the conclusion of the recording session by perfusing an isolated aortic segment with whole blood at various flow increments. The above parameters were directly measured and then used to calculate stroke volume, stroke index, and stroke volume/ventricular weight.

Once cardiac output returned to baseline values, the aorta was totally occluded for 3–4 seconds with a surgical suture. This period of increased afterload was used to determine developed pressure, defined as the difference between left ventricular systolic and end-diastolic pressure.

**Plasma Renin Activity and Atrial Naturetic Factor**

Blood samples were collected from anesthetized rats in chilled tubes containing EDTA and were subsequently used for standardized assays for plasma renin activity and ANF. The rats used for these blood samples were not subjected to evaluation of cardiac output or pressure development.

Plasma renin activity was determined with the aid of an iodine-125–labeled angiotensin I radioimmunoassay kit (DuPont Co., Billerica, Massachusetts). This assay was designed to favor the generation of angiotensin I by plasma renin under optimal pH conditions (5.5–6.0). After centrifugation of the blood samples, the resulting plasma samples were frozen. Subsequently, the amount of angiotensin I generated at 37°C was measured by radioimmunoassay. ANF plasma levels were determined by radioim-
munoassay with a rabbit antiserum purchased from Peninsula Laboratories, Belmont, California.

Hydroxyproline

After heart and ventricular weights were determined, a tissue sample from the left ventricular lateral free wall, midway between the apex and base, was excised and frozen in liquid nitrogen. The sample was trimmed so as to exclude the epicardium and endocardium. After the tissue was pulverized in liquid N₂ and dried to a constant weight, the tissue was hydrolyzed (duplicate samples) in 6N HCl, dried, and hydroxyproline content subsequently determined with a Pico-tag amino acid analyzer (Milford, Massachusetts).

Tissue Preservation, Microscopy, and Morphometry

We heparinized the rat, arrested the heart in diastole with procaine (injected via the left ventricular apex), excised the heart, and mounted the ascending aorta to a cannula that was attached to a gravity perfusion apparatus. About 30 ml Locke's solution was perfused through the coronary vasculature at a driving pressure of 120 mm Hg, and without interruption, 100 ml fixative (1.5% glutaraldehyde, 0.2% paraformaldehyde, 0.1 M cacodylate, and 0.03 M CaCl₂, pH 7.4) was perfused. After the heart and ventricles were weighed, tissue samples were excised from the midmyocardium and endomyocardium. The tissue samples were carefully oriented in molds so that cross or longitudinal sections of cardiocytes could be cut.

For the purpose of general histological evaluation and the measurement of cardiocyte cross-sectional areas, we embedded tissues in JB-4 (metacrylate), cut 1.5 μm sections, stained them according to a modification of the periodic acid methenamine silver procedure, and counterstained with light green. Cross-sectional cell areas of 30-40 cells/sample were traced at the level of the nucleus with the aid of a Leitz microprojector (Wetzlar, FRG) at a magnification of x 1,440 and measured with a digitizer. This method correlates well (r=0.93) with that of including all cell profiles. The silver stain demarcated cell outlines of closely packed cells so that their borders were readily distinguished. Areas selected for sampling contained capillary profiles, which were circular rather than elliptical. Smaller specimens were post-fixed in OsO₄, dehydrated, embedded in Spurr's plastic, and sectioned for electron microscopy. These thin sections were stained with uranyl acetate and lead citrate. Cross sections were used for point counting stereology, and longitudinal sections were used for sarcomere length measurements.

Electron micrographs, obtained with a Hitachi 7,000 microscope (Denshi Ltd, Tokyo, Japan) and printed at a final magnification of ×24,000, were used to point count mitochondria, myofibrils, and all other sarcoplasmic structures (sarcoplasm). We used a grid with intercepts every 13 μm and evaluated 10 cells from each specimen site. This method is routinely used in our laboratory and has been shown to have an error of less than 5%. The stereological data are expressed as volume percent (volume density×100).

Longitudinal sections were photographed and also printed at a final magnification of ×24,000. Sarcomere lengths were estimated by averaging the lengths of about 10 sarcomeres/cell and then using the mean of 10 cells from each specimen. Because obliquity of sectioning overestimates sarcomere length, we adjusted the measured values by the following formula:

\[
\text{sarcomere length} = \frac{SL_{n} \times 1.5}{A_{n}}
\]

where \(SL_{n}\) is the measured sarcomere length, \(A_{n}\) is the measured A band length, and 1.5 is the actual A band length. Adequate sample size for all morphometric parameters was confirmed by the progressive means test.

Statistical Methods

Analysis of variance was used for group comparisons of all data. A \(p \leq 0.05\) was selected to denote statistically significant differences between group means.

Results

General Characteristics of Deoxycorticosterone Acetate Model

Figure 1 illustrates the rise in systolic arterial pressures with time. Although the DOCA-salt rats remained normotensive (119±6 mm Hg) during the first week of treatment, the second week of treatment was characterized by a sharp rise in systolic arterial pressure, which reached 173±5 mm Hg compared with 125±5 mm Hg for the controls. With longer treatment, arterial pressures remained elevated; however, the magnitude of the intergroup difference was not as great (i.e., systolic arterial pressures...
averaged over the last 2 months were 149±4 in DOCA-salt and 123±3 mm Hg in the sham rats. These data suggest that the initial increase in arterial pressure during the first 3 weeks was attenuated when treatment was continued for 3 months. Plasma renin activity (Table 1) clearly tended to be lower, although quite variable, in the DOCA-salt group compared with the sham control group; no significant changes were observed with either duration of DOCA-salt treatment. In contrast, plasma ANF (Table 1) was about threefold higher in the DOCA-salt rats after 1 month of treatment compared with their sham controls.

**Anatomic Characteristics**

As seen in Table 2, DOCA-salt treatment led to reductions in body weight after both 1 and 3 months of treatment. Absolute left ventricular weight was significantly elevated after 1 but not after 3 months of treatment. The fact that absolute left ventricular mass did not increase during the last 2 months of treatment is consistent with the further loss of body weight during this time period. When adjusted for body weight (left ventricular weight/body weight ratio) the DOCA-salt group left ventricular mass is larger than that of the sham group in both the 1- and 3-month groups. Right ventricular mass was not altered by treatment at either stage.

**Tissue and Cell Data**

Mean cell cross-sectional area (Table 3) after 1 month of DOCA-salt treatment increased by 46% in the endomyocardium, but the cardiocytes in the epicardial region of the left ventricle for histology, this region in DOCA-salt rats displayed scarring when examined grossly. These data, when considered in aggregate, suggest that cellular enlargement during the last 2 months of DOCA-salt treatment compensated for focal cell loss.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham (n=7)</th>
<th>1-month DOCA-salt (n=6)</th>
<th>p value</th>
<th>% change</th>
<th>Sham (n=6)</th>
<th>3-month DOCA-salt (n=7)</th>
<th>p value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin (ng/ml/hr)</td>
<td>2.47±0.80</td>
<td>1.13±0.87</td>
<td>NS</td>
<td>NS</td>
<td>2.07±0.73</td>
<td>2.11±0.68</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>ANF (pg/ml plasma)</td>
<td>72±27</td>
<td>240±29</td>
<td>&lt;0.001</td>
<td>233</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. DOCA-salt, deoxycorticosterone acetate-salt; NS, nonsignificant (p>0.05); ANF, atrial natriuretic factor.
TABLE 3. Cell and Organelle Morphometry in Left Ventricular Midmyocardial and Endomyocardial Layers

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham (n=9)</th>
<th>1-month DOCA-salt (n=8)</th>
<th>p value</th>
<th>% change</th>
<th>Sham (n=6)</th>
<th>3-month DOCA-salt (n=6)</th>
<th>p value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Midmyocardium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cross-sectional area (mm²)</td>
<td>341±28</td>
<td>500±34</td>
<td>0.003</td>
<td>47</td>
<td>357±38</td>
<td>550±40</td>
<td>0.003</td>
<td>54</td>
</tr>
<tr>
<td>Organelle volume percent (mm³/mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>27.24±0.67</td>
<td>24.81±0.67</td>
<td>0.01</td>
<td>9</td>
<td>27.09±1.04</td>
<td>23.53±1.04</td>
<td>0.02</td>
<td>13</td>
</tr>
<tr>
<td>Myofibrils</td>
<td>57.86±0.60</td>
<td>59.89±0.60</td>
<td>NS</td>
<td>NS</td>
<td>56.65±0.42</td>
<td>55.85±0.42</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcoplasm</td>
<td>14.91±0.72</td>
<td>15.32±0.72</td>
<td>NS</td>
<td>NS</td>
<td>16.45±1.11</td>
<td>20.65±1.11</td>
<td>0.01</td>
<td>26</td>
</tr>
<tr>
<td>Sarcomere length (mm²)</td>
<td>2.11±0.04</td>
<td>2.18±0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endomyocardium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cross-sectional area (mm²)</td>
<td>378±33</td>
<td>420±33</td>
<td>NS</td>
<td>NS</td>
<td>371±42</td>
<td>504±46</td>
<td>0.03</td>
<td>36</td>
</tr>
<tr>
<td>Organelle volume percent (mm³/mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>26.66±0.75</td>
<td>25.53±0.79</td>
<td>NS</td>
<td>NS</td>
<td>26.01±0.89</td>
<td>22.05±0.89</td>
<td>0.005</td>
<td>15</td>
</tr>
<tr>
<td>Myofibrils</td>
<td>59.00±1.36</td>
<td>59.49±1.44</td>
<td>NS</td>
<td>NS</td>
<td>59.05±1.12</td>
<td>57.19±1.12</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sarcoplasm</td>
<td>14.37±1.01</td>
<td>15.01±1.07</td>
<td>NS</td>
<td>NS</td>
<td>14.94±1.54</td>
<td>20.76±1.54</td>
<td>0.01</td>
<td>39</td>
</tr>
<tr>
<td>Sarcomere length (mm²)</td>
<td>2.14±0.04</td>
<td>2.12±0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. DOCA-salt, deoxycorticosterone acetate-salt; NS, nonsignificant (p>0.05).

Cardiomyocytes after 1 month and in the endomyocardium after 3 months of treatment (Figure 3). These changes correspond to the primary sites of cellular hypertrophy during the early and late stages of treatment, respectively. Sarcoplasmic volume density (Table 3) increased rather markedly with DOCA-salt administration with significant elevations noted at 3 months in both midmyocardium (25%) and endomyocardium (39%). This expansion of the sarcoplasmic compartment was not due to edema as its capacity was not altered and no other histological evidence of edema was observed.

Sarcomere length (Table 3), which we measured after 1 month of DOCA-salt treatment, was nearly identical in the sham and experimental rats in both regions of the ventricle from which specimens were

![Figure 2](http://hyper.ahajournals.org/Downloaded from http://hyper.ahajournals.org/)

**Figure 2.** Micrographs of endomyocardial specimens obtained from sham (Panel a) and rats treated with deoxycorticosterone acetate-salt (Panels b and c). Micrograph from sham rat displays normal morphology without evidence of fibrosis. Fibrosis surrounding two arterioles is evident in panel b. Arterioles also have thicker media than those of sham rats. Fibrotic region with both large (hypertrophic) and small cardiocyte profiles is seen in panel c. Such focal regions were limited to subendocardium and were not observed in the midmyocardium. Scale bars=25 μm.
obtained. Moreover, the values were very consistent from animal to animal, as indicated by standard error of the mean values, which approximate 2% of the mean.

Hydroxyproline concentration in the left ventricular free wall showed a significant increase after 1 month of DOCA-salt treatment but fell to sham levels after 3 months (Figure 4). Thus, the increase in hydroxyproline concentration appeared to be transient.

Left Ventricular Function

Indexes of cardiac output at rest were similar for the DOCA-salt and sham groups (Table 4). However, DOCA-salt treatment, as seen in Table 5, increased peak cardiac and stroke indexes after either 1 or 3 months. In contrast, dF/dt was reduced by treatment. When adjusted for heart mass, stroke volume was similar in the DOCA-salt and sham groups.

Developed pressure (the difference between systolic and end-diastolic pressures during a brief aortic occlusion) is illustrated in Figure 5. During the development of LVH (first month of treatment) the left ventricle of the DOCA-salt-treated rat was able to develop a greater pressure than that of the sham control rat. However, this enhancement did not persist as the developed pressure means in the 3-month groups are virtually identical.

Discussion

This study is the first to characterize both the structural and functional status of the heart during mineralocorticoid-induced hypertension. Importantly, this study has focused on 1) mid-life, a period of the life span that often encompasses the onset of hypertension, and 2) two time periods (the development of hypertension and ventricular enlargement and the stabilization of these parameters). While our data show that the left ventricle is able, in general, to adapt to this type of hypertension, they also reveal that some structural and functional abnormalities develop in this model.

Our findings regarding the general characteristics of the DOCA-salt model are consistent with previous reports. The markedly elevated ANF plasma levels noted in our DOCA-salt rats 1 month after initiation of treatment have been reported previously. Plasma renin activity in this model of hypertension is lower than in normotensive controls, whereas in renal hypertension plasma renin activity is initially elevated most markedly in two kidney, one-clip hypertension. Although plasma renin activity was not significantly depressed in our DOCA-salt rats, a trend toward lower values was evident. The lower body
weights in the DOCA-salt rats that are noted in this study have been reported by several groups.3-7,19

Left Ventricular Structure

As shown by the changes in cardiocyte cross-sectional area, it can be concluded that during the first 4 weeks of DOCA-salt administration nearly all of the hypertrophy occurred in the midmyocardium. During the last 2 months of treatment, the cardiocytes in the midmyocardium showed virtually no growth, but the cardiocyte cross-sectional area increased in the endomyocardium. Preferential growth in one myocardial region at a given time point is not uncommon. A selective hypertrophy has been noted in the midmyocardium of renal hypertensive rats21 and in the epimyocardium in thyroxine-induced LVH.22 In SHR the hypertrophy is initially more pronounced in the endomyocardium, but with time the process accelerates in the epimyocardium.23 These findings suggest that the time course as well as the model of LVH are important considerations in regional cardiocyte growth.

As previously shown,7 myocardial scarring, both on the ventricular surface and within the myocardium, occurs in this model of LVH. The degenerative changes in a few endomyocardial myocytes closely linked with foci of fibrosis that we noted suggest scar replacement of necrotic cardiocytes. Such fibrosis is in concert with the hydroxyproline concentration enhancement during the development of LVH. However, the amount of fibrosis is clearly a focal phenomenon as hydroxyproline concentration (which was based on the myocardium exclusive of the endocardial and epicardial regions) falls to control levels as hypertrophy stabilizes (2-3 months). This fibrosis is clearly associated with a loss of cardiocytes. This conclusion is based not only on the observable degeneration of cardiocytes but on the data, which indicate that absolute left ventricle mass was unchanged with DOCA-salt treatment (at 3 months) while mean cardiocyte cross-sectional area increased by 39-54%. If cardiocyte length increased, then cardiocyte loss would be even greater than if cardiocyte length remained unchanged (i.e., cell loss would be underestimated). However, pressure overload is not associated with any significant increase in cell length.24,25 Cell loss and focal fibrosis are not unique to the DOCA-salt model of hypertension. We have recently demonstrated that similar changes occur in the left ventricles of 15- and 24-month-old rats with one-kidney, figure 8 wrap renal hypertension (R.J. Tomanek, M.A. Aydelotte, unpublished observations).

An important finding in this study is that left ventricular mass increased in middle-aged rats by similar magnitudes noted in much younger DOCA-salt rats. Left ventricular weight/body weight ratio in the DOCA-salt rats treated for 1 month was 36% higher than in the sham rats. This magnitude of LVH is similar to the 32-42% LVH found in 4-5-month-old rats treated for 9-18 weeks.2,3,13 These data support the view that young adult and middle-aged rats develop similar magnitudes of LVH when treated chronically with DOCA-salt. However, if treatment commences at the time of weaning, LVH is much greater (i.e., about 61%).11 Considering these findings, it appears that while young rats demonstrate a greater magnitude of ventricular enlargement in response to DOCA-salt hypertension, young adult

---

TABLE 5. Peak Left Ventricular Function During Increased Preload (Acute Volume Expansion)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham (n=8)</th>
<th>1-month DOCA-salt (n=12)</th>
<th>p value</th>
<th>% change</th>
<th>Sham (n=8)</th>
<th>3-month DOCA-salt (n=7)</th>
<th>p value</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac index (ml/min/kg)</td>
<td>368±23</td>
<td>449±18</td>
<td>0.006</td>
<td>22</td>
<td>485±24</td>
<td>589±26</td>
<td>0.006</td>
<td>21</td>
</tr>
<tr>
<td>Stroke index (ml/beat/kg)</td>
<td>1.07±0.08</td>
<td>1.35±0.07</td>
<td>0.008</td>
<td>26</td>
<td>1.51±0.10</td>
<td>2.01±0.11</td>
<td>0.002</td>
<td>33</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>24±3</td>
<td>19±3</td>
<td>NS</td>
<td>NS</td>
<td>14±3</td>
<td>22±3</td>
<td>0.04</td>
<td>57</td>
</tr>
<tr>
<td>dF/dt (ml/sec²)</td>
<td>879±65</td>
<td>670±53</td>
<td>0.01</td>
<td>24</td>
<td>910±71</td>
<td>699±76</td>
<td>0.03</td>
<td>23</td>
</tr>
<tr>
<td>SV/LVW (ml/g)</td>
<td>0.50±0.03</td>
<td>0.48±0.03</td>
<td>NS</td>
<td>NS</td>
<td>0.61±3</td>
<td>0.65±3</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DOCA-salt, deoxycorticosterone acetate-salt; LVEDP, left ventricular end-diastolic pressure; NS, nonsignificant (p>0.05); dF/dt, acceleration of flow; SV/LVW, stroke volume/left ventricular weight.

---

FIGURE 5. Left ventricular developed pressure (systolic minus end-diastolic) recorded during a brief total occlusion of the ascending aorta. Values illustrated are mean±SEM. Number of rats per group is given in parentheses. DOCA-S, deoxycorticosterone acetate-salt.
and middle-aged rats undergo similar degrees of ventricular growth.

Quite independent of the magnitude of cardiocyte hypertrophy, shifts in the volume densities of mitochondrial and sarcoplasm became statistically significant in both left ventricular regions only after 3 months of treatment. However, mitochondrial/myofibrils volume ratio became significantly elevated in the midmyocardium during the first month of treatment but required a longer period to attain significance in the endocardium. A close look at the organellar volume data indicates that the most marked changes are an increased sarcoplasmic volume during the last 2 months of treatment, which corresponded to a decrease in mitochondrial volume ratio. The expansion of sarcoplasm is not a characteristic of most rodent models of pressure overload (e.g., renal hypertension,16,21 aortic constriction,26 and SHR26,27). Interestingly, this expansion does not occur during the first month of DOCA-salt-induced growth and, therefore, is not associated with the initial adaptations to mineralocorticoid treatment and increasing arterial pressure levels. Considering that myofibrillar volume percent does not change, while mitochondrial volume percent decreases slightly, it is evident that sarcoplasmic volume expands during the second and third months of hypertension by a greater magnitude than either of the major cell organelles. That this increase is not due to cellular edema is evident from dry weight percentages of tissues and from the ultrastructural cell characteristics.

Left Ventricular Function

Most studies have shown that pressure overload, in the absence of congestive heart failure, does not necessarily lead to deterioration of left ventricular function if the hypertrophy is not excessive.10 Well-compensated ventricular function has been demonstrated in several rodent models of hypertension: SHR.14,28,29 Dahl hypertension,30 and renal hypertension.20 Although shortening and relaxation times, measured in isolated papillary muscles, may be prolonged in rats with LVH secondary to increased afterload, tension development is normal;11 this statement is valid even for 1-year-old SHR.32 Thus, the frequently cited abnormalities or impaired myocardial mechanics do not necessarily translate into an impairment of left ventricular function, especially in rodents32; rather, as noted by Bing et al,31 the myocardial hypertrophy may compensate for the prolongation of shortening velocity allowing "apparently normal" heart function.

As shown by Heller,33 not only is papillary muscle active tension normal in young rats after DOCA-salt treatment, but time to peak tension and one half relaxation time are also unaltered. However, in papillary muscles of 1-year-old rats studied during isometric contractions, the DOCA-salt LVH is associated with prolonged action potentials, contraction times, and electrical and mechanical refractory periods.34 Thus, age appears to influence mechanical properties in this model.

Normal left ventricular global function in rats with LVH under resting conditions is a common finding in several models of pressure overload, including DOCA-salt.23 The latter has been shown to have normal dP/dt and cardiac index after 9–18 weeks of treatment. That stroke index and cardiac index were elevated during the first but not the last 2 months of treatment in our study suggests that these changes were transient and related to the developmental stage of hypertension and LVH. It has been concluded that a rise in cardiac output in mineralocorticoid hypertension is transient and, therefore, unnecessary for the development of hypertension.35 Such conclusions are consistent with observations on both dogs and humans that indicate an initial rise in cardiac output followed by a return to normal values.39 This variation in cardiac output may underlie the differences in blood pressure at 1 and 3 months (i.e., a greater elevation at 1 month). This initial rise in cardiac output may also explain why the degree of hypertension was higher after 1 compared with 3 months of treatment.

Because ventricular dysfunction may only be manifest when the ventricle is challenged, normal ventricular function at rest is not a surprising finding. Accordingly, we evaluated ventricular function under both increased preload and afterload. A well-compensated ventricular function is suggested by the higher peak stroke and cardiac indexes in the DOCA-salt groups during enhanced preload (volume expansion). Further evidence for compensation is the finding that peak stroke volume matches left ventricular mass (peak stroke volume/g left ventricular weight is similar in the DOCA-salt and sham groups). However, we need to add a caveat; such data must be interpreted in the light of the experimental conditions. Although DOCA-salt rats remained hypertensive throughout the 3-month experimental period, their arterial blood pressures were virtually normal under the conditions in which we obtained measures of cardiac output (anesthetized, open chest preparation). Therefore, afterload was similar for the experimental and sham rats. Thus, it is possible that peak ventricular function in the presence of increased afterload may have been compromised. Although developed pressure, a good measure of the force-generating ability of the ventricle, was higher during the first month of DOCA-salt treatment, it decreased to the levels of the sham rats at some point during the last 2 months of treatment. This finding demonstrates that the duration or stage of the pressure overload in this model of LVH is a factor in the functional adaptability of the left ventricle to high afterload. Age may play a role in limiting the compensatory function of the ventricle, as it has been shown that in genetic hypertension (SHR) peak developed pressure no longer increases in proportion to left ventricular enlargement after 12 months of age.14 Current
studies in progress in our laboratory are aimed at resolving this important issue.

Acknowledgments

We gratefully acknowledge the technical assistance of Donna Farley who performed the plasma renin activity and atrial natriuretic factor assays (Dr. Dianna Van Orden's laboratory) and Suzan Hays-Goldsmith and Susan Pedigo who performed the hydroxyproline assays (Protein Structure Facility).

References

11. Isoyama S, Wei JY, Izumo S, Fort P, Schoen FJ, Grossman W: Age-dependent DOCA-S hyper trophy • deoxycorticosterone • cardiac output • collagen

Key Words • mineralocorticoid hypertension • cardiac hypertrophy • deoxycorticosterone • cardiac output • collagen


Downloaded from http://hyper.ahajournals.org/ by guest on July 13, 2017
Left ventricular structure and performance in middle-aged rats with deoxycorticosterone acetate-salt hypertension.
R J Tomanek and P A Barlow

doi: 10.1161/01.HYP.15.2.225

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/2/225

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/