SUMMARY  The changes in ventricular isomyosin composition and Ca$^{2+}$-activated ATPase activity occurring with regression of both hypertension and cardiac hypertrophy were investigated by using polyacrylamide gel electrophoresis under nondenaturing conditions, heavy chain peptide mapping, and an enzymatic assay. Eight control male Wistar rats and 14 two-kidney, one clip (Goldblatt II) hypertensive rats were studied from the fifth week of age. At 10 weeks of age, five Goldblatt II rats and four normotensive controls were killed. Five other Goldblatt II rats underwent nephrectomy of the ischemic kidney, which resulted in subsequent normalization of blood pressure. The remaining four control, four Goldblatt II rats, and five nephrectomized rats were killed at 15 weeks of age. Both the 10- and 15-week-old hypertensive rats had a significantly higher ($p<0.001$) biventricular weight to body weight ratio than the age-matched controls ($3.84 \pm 0.76 \times 10^{-2}$ vs $2.75 \pm 0.25 \times 10^{-2}$; $5.93 \pm 2.26 \times 10^{-2}$ vs $2.65 \pm 0.17 \times 10^{-2}$). The 15-week-old nephrectomized rats had a biventricular weight to body weight ratio ($2.90 \pm 0.25 \times 10^{-2}$) close to that of age-matched controls and significantly lower ($p<0.05$) than that of age-matched hypertensive rats. In both the 10- and 15-week-old hypertensive rats left ventricular myosin Ca$^{2+}$-activated ATPase activity was significantly lower ($p<0.001$) than in the age-matched controls ($0.44 \pm 0.03$ vs $0.59 \pm 0.06$; $0.24 \pm 0.05$ vs $0.48 \pm 0.05$). Conversely, in nephrectomized rats activity was similar to that of age-matched controls ($0.46 \pm 0.04$) and significantly higher ($p<0.001$) than that of age-matched hypertensive rats. The expression of "slow" isoenzymes of myosin V$_2$ and V$_3$ was evident in the hypertensive animals, which displayed a decrease of the "fast" V$_1$ isoenzyme. Through peptide mapping of myosin heavy chains, an additional band was found in both groups of hypertensive animals that was absent in the age-matched controls. Nephrectomized 15-week-old rats showed a ventricular isomyosin composition and peptide mapping similar to that of age-matched controls. In conclusion, with normalization of blood pressure, complete reversal of cardiac hypertrophy was achieved and the biochemical and molecular properties of left ventricular myosin were fully restored. (Hypertension 8: 1143-1148, 1986)

KEY WORDS  cardiac hypertrophy • renovascular hypertension • myosin • pressure overload

In the last few years, considerable advances have been made in the knowledge of the pathophysiology of cardiac hypertrophy caused by pressure overload. A great deal of research has been carried out in different animal models, especially in the rat. These studies have clarified the mechanisms and the importance of the decreased speed of muscle shortening$^{1-3}$ that is found in overload-induced cardiac hypertrophy in the rat. This reduction is due to a shift in the ventricular myosin isoenzymes toward the "slow" isoforms V$_2$ and V$_3$,$^{4-7}$ accompanied by a decrease in ventricular myosin Ca$^{2+}$-activated ATPase activity$^{8,9}$ and in oxygen consumption.$^{10-12}$ These changes are part of a compensatory process that allows the ventricular myocardium to maintain its function and preserve energy at the same time.$^{13,14}$ However, after long-standing exposure to a pressure overload, decompensation occurs through mechanisms that have not as yet been clarified.

Although several studies have been conducted on the progression of cardiac hypertrophy in hypertensive rats$^{1-4,31,12}$ and rabbits,$^{9,10}$ little is known about the changes in myosin isoenzyme distribution and ventric-
ular ATPase activity levels during the regression of overload-induced cardiac hypertrophy. Previous observations in two-kidney, one clip (Goldblatt II) hypertensive rats demonstrated a reversal to normality of the myocardial contractile properties and the Ca2+-activated ATPase activity after both hypertension and cardiac hypertrophy had regressed.10 Since these effects might be due to the reversal to normality of ventricular isomyosin composition, we studied the ventricular isomyosin pattern in Goldblatt II hypertensive rats after normalization of blood pressure induced by the removal of the clipped kidney and the subsequent regression of cardiac hypertrophy. This could also represent the key feature for understanding the changes that occur in the hypertrophic myocardium as a consequence of drug administration. Indeed, the modifications in the pattern of isomyosins during antihypertensive therapy could be due to the removal of overload per se or to a direct effect of the drugs. This latter hypothesis is supported by the recent observations that some antihypertensive drugs are capable of inducing a shift in the pattern of the ventricular isomyosins.16,17

Materials and Methods

Study Design

Twenty-two male Wistar rats were studied between the fifth and the 15th week of age. All the animals were born from cousin nests, kept in the same environment, and placed two by two in separate cages at controlled temperature. They were fed a standard diet and water ad libitum. At 5 weeks of age, 14 were made hypertensive by the two-kidney, one clip (Goldblatt II) procedure: after intraperitoneal injection of pentobarbital (Pentothal), 30 mg/kg, the left renal artery was constricted with a silver clip (inner diameter, 0.20 mm). To define the progression of cardiac hypertrophy 5 weeks from the application of the clip and the related changes in isomyosin composition, five 10-week-old Goldblatt II rats were examined. At the same time, five other Goldblatt II rats underwent the removal of the ischemic kidney. These animals were killed under ether anesthesia at 15 weeks of age together with the remaining four hypertensive rats. Four control rats were killed at 10 weeks of age and four at 15 weeks. Blood pressure and heart rate were measured periodically by photoplethysmography of the caudal artery (OTE Biomedica Equipment, Padova, Italy).

After death, the rats’ hearts were removed, the atria were excised, and the ventricles were weighed to establish the biventricular weight to body weight ratio as an index of cardiac hypertrophy. Samples from the left ventricle (600–800 mg each) were immediately processed for myosin purification according to published procedures.18,19

Ca2+-Activated ATPase Activity

Myosin Ca2+-activated ATPase activity was assayed as previously described.20 The assay mixture contained 25 mM KCl, 10 mM CaCl2, 2.5 mM adenosine 5′-triphosphate (ATP), and 50 mM tris(hydroxymethyl)aminomethane (TRIS) HCl, at pH 7.6. The protein concentration was 0.1 mg/ml in a final volume of 1 ml. The reaction was started by adding ATP at 25°C for 5 minutes. The liberated inorganic phosphate was measured as described by Lanzetta et al.21

Peptide Mapping of the Heavy Chains

Peptide mapping of the electrophoretically purified myosin heavy chains was performed according to Cleveland et al.22 with the following modifications: 150 mm wide, 6 mm high, and 0.75 mm thick strips containing the visualized heavy chains were cut from a 5% acrylamide slab. The digestion with Staphylococcus aureus V8 protease and the second electrophoresis were carried out in a linear gradient of polyacrylamide (ranging between 15 and 20%), 220 mm long and 1 mm thick. The strips were laid over a 20-mm long stacking gel; a 20% glycerol solution in equilibration buffer was used to cover the strips. Protease, 12 μg, in 130 μl equilibration buffer containing 10% glycerol and bromophenol blue dye was overlaid. The electrophoresis was initially carried out at constant current of 12.5 mA. To avoid heating the slab and therefore distorting the bands, the power supply was set at 200 V maximum. The running buffer was 62.9 mM TRIS HCl (pH 8.3), 480 mM glycine, and 0.25% sodium dodecyl sulfate. The bands were visualized by staining the gels in 0.1% Coomassie brilliant blue.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis of purified myosin under nondenaturing conditions was performed on samples from each animal. Cooelectrophoretic experiments were performed using myosins from nephrectomized rats and age-matched controls and pure Vj isomyosin from fetal rats. The electrophoresis was carried out according to Hoh et al.23 and D’Albis et al.24 in the presence of 0.04 M Na2PO4, 0.002 M ethylenediaminetetraacetic acid, 0.05% mercaptoethanol (vol/vol) at pH 8.8. Cylindrical polyacrylamide gels (6.5 × 0.5 cm) containing 3.88% acrylamide (wt/vol) and 0.12% N,N′-methylene-bisacrylamide (wt/vol) were prepared. Each gel was loaded with approximately 2 to 4 μg of protein, and the electrophoresis was carried out at 2°C for 16 hours at a voltage gradient of 10 V·cm−1. The gels were stained with Coomassie blue in perchloric acid25 and destained with 5% methanol and 7% acetic acid by diffusion.

Slides (magnification 2 ×) were taken from each gel. The relative amounts of the myosin isoenzymes were determined by densitometry of the electrophoretic bands, using the Omnicron alpha TM 500 Image Analysis System (Bausch & Lomb, Rochester, NY, USA).

Analysis of Data

The effects and the interactions of aging, hypertension, and nephrectomy on biventricular weight to body weight ratio, myosin Ca2+-activated ATPase activity, and the relative amounts of the myosin isoenzymes...
were evaluated by analysis of variance combined with orthogonal contrasts. The coefficients for the orthogonal contrasts were determined as follows:

1. Aging: $3\bar{x}_1 + 3\bar{x}_2 - 2\bar{x}_3 - 2\bar{x}_4 - 2\bar{x}_5$
2. Hypertension: $1\bar{x}_1 + 1\bar{x}_2 + 1\bar{x}_3 - 1\bar{x}_4 + 0\bar{x}_5$
3. Interaction between aging and hypertension: $1\bar{x}_1 - 1\bar{x}_2 - 1\bar{x}_3 + 1\bar{x}_4 + 0\bar{x}_5$
4. Nephrectomy: $0\bar{x}_1 + 0\bar{x}_2 + 1\bar{x}_3 + 1\bar{x}_4 - 2\bar{x}_5$

The data concerning blood pressure were evaluated by one-way analysis of variance.

Results

As reported in Figure 1, the blood pressure of Goldblatt II rats rose after the operation and remained significantly elevated throughout the study period. In the five Goldblatt II rats in which the ischemic kidney was removed 5 weeks after the application of the clip, a significant fall in blood pressure ($p<0.001$) was observed within 2 days after nephrectomy. In these animals blood pressure remained at normotensive levels throughout the remainder of the study.

No significant difference in heart rate and in body weight was observed at any time among the different groups of animals. In addition, despite a smaller size, the ischemic kidney of Goldblatt II rats appeared normal at gross inspection and at microscopic examination.

Both the 10-week-old and 15-week-old hypertensive rats had a significantly higher ($p<0.001$) biventricular weight to body weight ratio than the age-matched controls (Table 1). On the contrary, at 15 weeks of age the nephrectomized rats displayed a biventricular weight to body weight ratio very close to that of the age-matched controls and significantly lower ($p<0.05$) than that of age-matched hypertensive animals. The data reported in Table 1 show that the observed changes in ventricular mass were related only to hypertension or nephrectomy.

In both the 10-week-old and 15-week-old hypertensive rats, left ventricular myosin Ca$^{2+}$-activated ATPase activity was significantly lower ($p<0.001$) than that in the age-matched controls (Table 2). On the contrary, in nephrectomized rats it was similar to that of age-matched controls and significantly higher ($p<0.001$) than that in the age-matched hypertensive animals. The observed differences in ATPase levels were not only to hypertension and nephrectomy (see Table 2) but also to aging, as previously reported.

Polyacrylamide gel electrophoresis of the purified myosin under nondenaturing conditions showed the presence of pure V$_1$ myosin isoenzyme of the nomenclature of Hoh et al. in both the 10-week-old and 15-week-old controls (Figure 2, Lanes A and C; Table 3). In our laboratory this finding represents the physiological age-related pattern. The expected shift toward the "slow" isoforms V$_2$ and V$_3$ with significant decrease of V$_1$ ($p<0.001$) was observed in the 10- and 15-week-old hypertensive rats, with a more pronounced expression of V$_2$ and V$_3$ in older animals (see Figure 2, Lanes B and D; Table 3). Nephrectomized rats were found to have the same ventricular myosin pattern as that of the age-matched controls, which was characterized by a significantly ($p<0.001$) higher amount of the V$_1$ isoform in comparison to that in age-matched hypertensive controls (see Figure 2, Lane E; Table 3). The coelectrophoretic experiments provided additional evidence for the identification of the isoenzymes (Figure 3).

Through peptide mapping of electrophoretically purified heavy chains, a complex pattern of proteolytic bands was observed (Figure 4). Controls of any age and nephrectomized rats showed the same pattern. In both the 10-week-old and 15-week-old hypertensive rats an additional band was present. This band was always absent in the control animals as well as in the nephrectomized rats.

Discussion

In the Goldblatt II rats, a 5-week period of hypertension was sufficient to induce a significant increase in ventricular mass that was accompanied by a significant decrease in ventricular myosin Ca$^{2+}$-activated ATPase activity. This latter finding was due to the reduction of the "fast" V$_1$ isoform of myosin with a shift toward the "slow" isoforms V$_2$ and V$_3$. The structural change occurring in ventricular myosin was further confirmed by peptide mapping of myosin heavy chains. This type of response to pressure overload is known to be of adaptive importance and became even more pronounced after the 10-week period of hypertension, during which a further increase in cardiac hypertrophy.
TABLE 1. Biventricular Weight to Body Weight Ratio in Goldblatt II Hypertensive, Nephrectomized, and Control Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>10 weeks of age</th>
<th>15 weeks of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 4)</td>
<td>Goldblatt II hypertensive (n = 5)</td>
</tr>
<tr>
<td>BVW/BW</td>
<td>2.75 ± 0.25</td>
<td>3.84 ± 0.76</td>
</tr>
</tbody>
</table>

Values are expressed as n × 10⁻² (mean ± SD). BVW/BW = ratio of biventricular weight to body weight; NS = not significant.

Analysis of variance: $F_{4,17} = 7.24, p < 0.001$.

BVW/BW as affected by 1) aging: $t = -1.18, p = NS$; 2) hypertension: $t = -6.49, p < 0.001$; 3) aging-hypertension: $t = -0.03, p = NS$; 4) nephrectomy: $t = 2.35, p < 0.05$.

TABLE 2. Left Ventricular Myosin Ca²⁺-Activated ATPase Activity in Goldblatt II Hypertensive, Nephrectomized, and Control Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>10 weeks of age</th>
<th>15 weeks of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 4)</td>
<td>Goldblatt II hypertensive (n = 5)</td>
</tr>
<tr>
<td>Ca²⁺-activated ATPase activity (µmol Pᵢ/mg protein/min)</td>
<td>0.59 ± 0.06</td>
<td>0.44 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD. Pᵢ = inorganic phosphate.

Analysis of variance: $F_{4,17} = 30.75, p < 0.001$.

Ca²⁺-activated ATPase activity as affected by 1) aging: $t = 6.08, p < 0.001$; 2) hypertension: $t = 18.48, p < 0.001$; 3) aging-hypertension: $t = 8.04, p < 0.001$; 4) nephrectomy: $t = -4, p < 0.001$.

was observed. The hypertensive Goldblatt II rats in which the ischemic kidney was removed showed an immediate fall in blood pressure that during the following 5 weeks of the study resulted in the normalization of cardiac mass and the restoration of both biochemical and structural properties of ventricular myosin. The reversal to the V₁ isoform explains the restored biochemical and contractile properties of myocardium reported by Capasso et al. under the same conditions.

At variance with the spontaneously hypertensive rats, changes in ventricular mass in Goldblatt II hypertensive rats depend on overload rather than on changes occurring in catecholamines or in the renin-angiotensin system. Therefore, it is also likely that the reversal to the V₁ pattern observed after the decrease of ventricular mass depends on the removal of the overload per se. This mechanism is in keeping with the physiological and adaptational importance attributed to the myosin isoenzymic shifts in the presence of different working loads.

Our results might also be relevant in the evaluation of changes in ventricular isomyosin composition caused by some antihypertensive drugs. Changes were recently reported in hypertensive rats treated with an angiotensin converting enzyme-inhibitory drug. Moreover, we found that the administration of propranolol leads to a shift toward "slow" isomyosins in normotensive rats. This shift could occur through different mechanisms, including hemodynamic changes, reflex sympathetic stimulation, cardiac output modifications, biochemical alterations of cardiac muscle cells, or thyroid hormone level variations. Some of these mechanisms are known to influence not only ventricular isomyosin composition but also the degree of regression of cardiac hypertrophy independent of blood pressure lowering (for review, see Reference 30). Further studies should deal with the choice of antihypertensive drugs, not only in light of the blood pressure lowering but also in relation to the specific mechanism involved.
TABLE 3. Relative Amounts (%) of the Three Myosin Isoenzymes (V1, V2, V3) as Assessed by Densitometry

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>10 weeks of age</th>
<th>Control</th>
<th>Goldblatt II hypertensive</th>
<th>15 weeks of age</th>
<th>Control</th>
<th>Goldblatt II hypertensive</th>
<th>Goldblatt II nephrectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V1</td>
<td>100</td>
<td>51.9</td>
<td>100</td>
<td>20.7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>—</td>
<td>31.9</td>
<td>—</td>
<td>43.1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>—</td>
<td>16.2</td>
<td>—</td>
<td>36.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>V1</td>
<td>100</td>
<td>60.0</td>
<td>100</td>
<td>47.9</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>—</td>
<td>32.0</td>
<td>—</td>
<td>33.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>—</td>
<td>8.0</td>
<td>—</td>
<td>19.1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>V1</td>
<td>100</td>
<td>60.7</td>
<td>100</td>
<td>33.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>—</td>
<td>25.5</td>
<td>—</td>
<td>39.7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>—</td>
<td>13.8</td>
<td>—</td>
<td>26.8</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>V1</td>
<td>100</td>
<td>69.9</td>
<td>100</td>
<td>35.8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>—</td>
<td>27.1</td>
<td>—</td>
<td>32.9</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>—</td>
<td>3.0</td>
<td>—</td>
<td>31.3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>V1</td>
<td>60.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>32.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>6.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD

|     |     |     |                         |     |     |                         |
| V1  | 100±0| 60.58±6.37| 100±0| 34.47±11.14| 100±0|
| V2  | 29.86±3.32| 37.18±5.07| 28.35±7.26| —     |
| V3  | 9.36±5.33| 28.36±7.26| —     | —     |

Dashes indicate absence of the isoenzyme. NS = not significant.
Analysis of variance: \( F_{4,17} = 122, p < 0.001 \).
Relative amounts of V1 isomyosin as affected by 1) aging: \( t = 0.87, p = \text{NS} \); 2) hypertension: \( t = 19.19, p < 0.001 \);
3) aging-hypertension: \( t = -4.77, p < 0.001 \); 4) nephrectomy: \( t = -10.25, p < 0.001 \).

pressure lowering effect and reversal of cardiac hypertrophy, but also in light of the possible changes in myosin isoenzymes and related biochemical, metabolic, and contractile modifications occurring in the myocardium.

FIGURE 3. Cooelectrophoretic experiments. A. a = myosin from 15-week-old control rats; a' = fetal rat myosin (pure V3); a'' = a + a'. B. b = myosin from 15-week-old control rat; b' = myosin from 15-week-old nephrectomized rat; b'' = b + b'.

FIGURE 4. Staphylococcus aureus V8 peptide mapping of the ventricular myosin heavy chains. Lane a: 10-week-old control rats; Lane b: 15-week-old control rats; Lane c: 15-week-old Goldblatt II nephrectomized rats; Lane d: 10-week-old Goldblatt II hypertensive rats; Lane e: 15-week-old Goldblatt II hypertensive rats. The arrows indicate the peptide present in hypertensive animals that is absent in both control and nephrectomized rats.
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