Ventricular Myosin and Creatine-Kinase Isoenzymes in Hypertensive Rats Treated With Captopril

Paolo Pauletto, Luigino Nascimben, Diana Piccolo, Sandra Secchiero, Giorgio Vescovo, Gianluigi Scannapieco, Luciano Dalla Libera, Ugo Carraro, Achille C. Pessina, and Cesare Dal Palù

In the myocardium, myosin and creatine kinase isoforms possess different capacities for using O₂ and energy-rich phosphates. We studied electrophoretically the distribution of these isoforms in 19 hypertensive rats (two-kidney, one clip model of hypertension) and in age-matched controls. After 6 weeks of hypertension, seven rats were treated with captopril (2 mg/kg daily) for 4 weeks, six were left hypertensive for another 4 weeks, and the remaining rats were killed under ether anesthesia. In the latter, ventricular mass was significantly higher than in controls; V₃ isomyosin was 32.3±6.8% versus 0%, and both creatine kinase-MB and -BB were increased at the expense of creatine kinase-MM (creatine kinase-MB=29±2.8% vs. 14.7±1.8%, p<0.001; creatine kinase-BB=3.1±0.6% vs. 1.7±0.8%, p<0.001). After 10 weeks of hypertension, ventricular mass, V₃ isomyosin, and both creatine kinase-MB and -BB isoforms were found to be persistently higher than in controls. At the same time, captopril-treated rats showed reduced but not normalized blood pressure levels, normalized ventricular mass, and prevalence of the V₁ isomyosin (56.9±22% vs. 47.9±23.8% in normotensive controls, p=NS). However, higher levels of creatine kinase-MB and -BB were still found in these rats in comparison with the normotensive controls (creatine kinase-MB=22.4±5.4% vs. 15.8±2.8%, p<0.025; creatine kinase-BB=2.3±0.1% vs. 1.8±0.3%, p<0.02). Therefore, despite the normalization in ventricular mass and isomyosin pattern, captopril-treated rats partly maintain the adaptive changes in creatine kinase isoenzymes that lead to a better use of energy-rich phosphates (Hypertension 1989;14:556–562).

In some mammalian species, such as the rat, chronic cardiac overload results in left ventricular hypertrophy, which is accompanied by adaptive changes at the molecular level involving isoenzymes of both myosin and creatine kinase.¹ The preferential synthesis of the β-type myosin heavy chains at the expense of the α-type has been previously reported as a typical feature of ventricular hypertrophy.²,³ This change occurs through transcriptional regulation at the level of two distinct genes, each coding for the specific type of myosin heavy chains.⁴ In hypertensive rats, the development of ventricular hypertrophy is therefore accompanied by a shift from the myosin V₁ isoenzyme (α-chains homodimer) toward the V₃ isoenzyme (β-chains homodimer). This latter represents the fetal form of myosin. The prevalence of V₃ implies a reduction in velocity of muscle shortening, Ca²⁺-activated adenosine triphosphatase (ATPase) activity, and oxygen consumption,⁶–⁸ which allows the hypertrophied myocardium to maintain its pumping function and saves energy at the same time.¹,⁸ Another mechanism enabling the hypertrophied myocardium to save energy is thought to occur through redistribution within the four creatine kinase isoenzymes.⁹–¹¹ This enzymatic system consists of four electrophoretically distinct isoforms. The mitochondrial isofrom, which is located on the mitochondrial membrane, transforms creatine into phosphocreatine. Phosphocreatine is then used at the cytosolic level by the other three isoforms (MM, MB, and BB) as an energy source for ion transport and myofibril contraction.¹² In the rat hypertrophied myocardium, preferential synthesis of the B monomer occurs resulting in an increased expression of MB and BB isoenzymes.⁹,¹¹
This change would imply a more efficient use of phosphocreatine, the level of which decreases in compensated hypertrophy and even more in failure.9,13 Captopril, a converting enzyme inhibitor, has been reported to influence the expression of ventricular isomyosins in hypertensive rats by inducing a preferential synthesis of the \( V_1 \) isoenzyme.14 Because ventricular myosin and creatine kinase isoforms represent two of the major systems controlling energy production and consumption in cardiac muscle, we decided to study the effect of captopril on both.

Materials and Methods

Study Design

Nineteen six-week-old Wistar rats were made hypertensive by clipping one renal artery (inner diameter, 0.20 mm) according to the Goldblatt II procedure. To define the progression of cardiac hypertrophy, six rats were killed under ether anesthesia 6 weeks after clipping together with seven age-matched controls; six rats were left hypertensive for a further 4 weeks; seven other rats were given captopril 2 mg/kg/day for 4 weeks, delivered by Alzet minipumps (Alzet Co., Palo Alto, California) implanted subcutaneously in the scruff of the neck. At the end of the study period, the two latter groups of rats were killed together with five normotensive age-matched controls.

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 weighed to establish the biventricular weight/body weight ratio as an index of cardiac hypertrophy. Samples from the left ventricle (600–800 mg each) were immediately processed either for myosin purification, according to previously published procedures,15 or for creatine kinase assay after they had been frozen in liquid nitrogen.

Polyacrylamide Gel Electrophoresis of Ventricular Myosin

Polyacrylamide gel electrophoresis of purified myosins was performed under nondenaturing conditions on samples of myocardium taken from each rat. The electrophoresis was carried out according to Hoh et al16 and D’Albis et al17 as modified and detailed by Dalla Libera et al.15 The gels were stained with Coomassie blue in perchloric acid and destained with 5% methanol and 7% acetic acid by diffusion.

The relative amounts of the myosin isoenzymes were then determined by laser densitometry of the electrophoretic bands with the Omnicron alpha TM 500 Image Analysis System (Bausch & Lomb, Rochester, New York) as previously described.18

Enzymatic Assay of Ventricular Creatine Kinase

Samples of myocardium (20–30 mg each) were homogenized for 10 seconds with a mechanical homogenizer (Polytron, Kinematica, Lucerne, Switzerland) at 2–4°C in the presence of 0.1 M potassium phosphate buffer, 1 mM EDTA, 1 mM \( \beta \)-mercaptoethanol (final concentration: 5 mg wet wt/ml). Aliquots were removed to assess the noncollagen protein concentration.

After centrifugation (1,000 rpm for 10 minutes) total creatine kinase activity was measured at 37°C according to the guidelines of the German Society for Clinical Chemistry19 using the Merck CK-Nac System (Merck, Darmstadt, FRG). Serial dilutions made on different samples from rat myocardium showed that the reaction was linear between 0.0054 and 0.54 \( \Delta A/min \) at 334 nm. Where necessary, samples were appropriately diluted. Values for total enzyme activity were expressed as International Units (IU) per milligram of protein.

The distribution of the four creatine kinase isoenzymes was determined by measurement of the creatine phosphate–dependent colorimetric reaction after electrophoretic separation of isoenzymes according to Burlina.20 The electrophoresis was carried out at 180 V for 25 minutes by using cellulose-acetate strips embedded in barbital buffer (Electra Bl Buffer, pH 8.3–8.7, Helena Laboratories, Beaumont, Texas). The concentration of substrates was not limiting for any of the isoenzymes in that they were assayed under the same conditions used for the measurement of total creatine kinase activity. The relative amount of each isoenzyme was determined by densitometric measurement of the electrophoretic bands at 550 nm using EDC equipment (Helena Laboratories, Beaumont, Texas).

Analysis of Data

Blood pressure levels of the different groups of rats were analyzed by multivariate analysis of variance. In rats treated with captopril, blood pressure levels before and after treatment were also analyzed by Student’s \( t \) test for paired data. Data concerning changes in ventricular weight and in myosin and creatine kinase isoenzymes were evaluated by Student’s \( t \) test for unpaired data, by linear regression analysis, or by analysis of variance where appropriate.

Results

As reported in Table 1, blood pressure in Goldblatt II rats rose after the operation and remained significantly higher than that recorded in controls throughout the study period (Table 1).

The seven Goldblatt II rats that received captopril from the sixth week of hypertension showed an immediate and significant fall in blood pressure when compared with the values the same rats had before treatment (185.0±12.9 mm Hg vs. 147.1±17.2 mm Hg; \( p<0.01; \) Student’s \( t \) test for paired data). However, in these rats blood pressure remained significantly higher than that found in normotensive controls throughout the study period (Table 1).
TABLE 1. Systolic Blood Pressure in Renovascular Hypertensive Rats, in Age-Matched Controls, and in Hypertensive Rats Treated With Captopril From the Sixth Week of Hypertension

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks of hypertension</th>
<th>3 (n=12)</th>
<th>6 (n=12)</th>
<th>7 (n=5)</th>
<th>10 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>111.6±9.8</td>
<td>108.7±8.8</td>
<td>110.0±7.9</td>
<td>113.6±8.0</td>
</tr>
<tr>
<td>RHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril-treated</td>
<td></td>
<td>171.9±13.5</td>
<td>177.8±12.6</td>
<td>189.1±12.0</td>
<td>200.8±13.5</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. Analysis of variance: F(9, 85)=21.7; p<0.001. RHR, renovascular hypertensive rats.

No significant differences in heart rate or body weight were observed among the different groups of rats. Both the 6-week-hypertensive and 10-week-hypertensive rats showed a significantly higher biventricular weight/body weight ratio than the age-matched controls (Table 2). However, the hypertensive, captopril-treated rats displayed a biventricular weight/body weight ratio very close to that of the age-matched controls and significantly lower than that of age-matched hypertensive rats not given captopril (Table 2).

Polyacrylamide gel electrophoresis of the purified myosin under nondenaturing conditions showed the presence of V1 (76.8±6.6%) and V2 (23.1±6.6%) but no expression of V3 in the 12-week-old controls (Figure 1). This finding represents the expected age-related pattern in rats from our breeding. A shift toward the "slow" V2 and V3 isoforms was found in the age-matched rats but made hypertensive at the sixth week of age. In these rats, the relative amount of the three isomyosins was almost identical (V1=36.4±4.6%; V2=31.1±6.3%; V3=32.3±6.8%) with a significant decrease in V1 in comparison with controls (Figure 1).

In hypertensive rats, the percentage of the β-type of myosin heavy chain was directly correlated with the degree of cardiac hypertrophy as expressed by the biventricular weight/body weight ratio (r=0.72; p<0.05).

After 10 weeks of hypertension, Goldblatt II rats showed a further increase in the percentage of V3 (47.6±11.9%) (Figure 2). In the control rats, the percentage of V3 was 26.6±18.9%. The large standard deviation is because the physiological age-related appearance of V3 was detected in three of five rats only. However, the amount of V3 was

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Bar graph showing relative amounts (%) of the three ventricular isomyosins (V1, V2, and V3) in 12-week-old rats after 6 weeks of renovascular hypertension and in age-matched controls (mean±SD).
significantly lower than that found in the hypertensive rats (Figure 2).

Rats that were treated with captopril from the sixth week of hypertension showed a complete restoration of ventricular isomyosin pattern. In particular, their percentage of $V_3$ ($19.2\pm18.1\%$) was significantly lower than that of untreated hypertensive rats (Figure 2). Again, three of seven captopril-treated rats did not show any expression of $V_3$ isomyosin. Captopril-treated rats also showed a decreased percentage of $V_3$, which was not statistically significant ($19.2\pm18.1\%$ vs. $26.6\pm18.9\%$) when compared with the normotensive age-matched controls. An increase in $V_1$ percentage was also noted when compared with normotensive controls ($56.9\pm22\%$ vs. $47.9\pm23.8\%$). In 10-week-hypertensive rats, the percentage of $\beta$ myosin heavy chains correlated with the degree of cardiac hypertrophy even more closely than in 6-week-hypertensive rats ($r=0.95; p<0.001$). No correlation was found in captopril-treated rats between these two parameters.

Table 3 shows the expression of the different creatine kinase isoenzymes after 6 weeks of hypertension. No differences could be found between hypertensive rats and controls both in total enzymatic activity and in percentage of mitochondrial isoenzyme, whereas a shift toward the B monomer of creatine kinase at the expense of the M monomer was always present (Table 4).

Rats that had captopril treatment since the sixth week of hypertension showed an increase in the percentage of MM isof orm and a parallel decrease in the percentage of MB and BB, which was statistically significant for BB only when compared with that of the hypertensive rats. However, a significantly higher amount of both the creatine kinase–MB and –BB isoenzymes was still present in captopril-treated rats in comparison with controls (Table 4). The ratio of B to M monomer was $0.14\pm0.03$ in control rats, $0.27\pm0.03$ in hypertensive rats ($p<0.001$ vs. normotensive controls), and $0.21\pm0.04$ in captopril-treated rats ($p<0.01$ vs. hypertensive rats; $p<0.005$ vs. normotensive controls).

In both groups of hypertensive rats as well as in captopril-treated rats, a poor correlation was found between percentage of creatine kinase B monomer and degree of cardiac hypertrophy.

**Discussion**

Although progression of cardiac hypertrophy and the concomitant changes in myosin and creatine kinase isoenzymes have been extensively studied,\textsuperscript{1-11} there are only a few studies on isomyosins during regression\textsuperscript{14-18} and none on creatine kinase isof orms.

As in previous works\textsuperscript{3,14,18} on renovascular hypertensive rats, this study showed an increase in ventricular mass paralleled by a shift toward the $V_3$ isomyosin. A direct correlation both at 6 and at 10 weeks of age was found between the degree of cardiac hypertrophy and the magnitude of this shift. At the same time, the increase in ventricular mass was accompanied by a redistribution in the relative amounts of the different creatine kinase isoenzymes with an increase in the B monomer at the expense of the MM isof orm was evident in hypertensive rats in comparison with controls.

After 10 weeks of hypertension, a similar picture was observed in hypertensive rats and in controls. Again, no changes were observed between the two groups in total creatine kinase activity and in percentage of mitochondrial isoenzyme, whereas a shift toward the B monomer of creatine kinase at the expense of the M monomer was always present (Table 4).

**Table 3.** Total Activity of Left Ventricular Creatine Kinase and Percentage of Various Isoenzymes After 6 Weeks of Hypertension in Renovascular Hypertensive Rats and in Age-Matched Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n=7$)</th>
<th>RHR ($n=6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (IU mg/prot.)</td>
<td>6.8±2.2</td>
<td>6.5±1.3</td>
</tr>
<tr>
<td>MITO (%)</td>
<td>22.5±1.7</td>
<td>21.7±3.6</td>
</tr>
<tr>
<td>MM (%)</td>
<td>61.1±2.2</td>
<td>46.0±3.6</td>
</tr>
<tr>
<td>MB (%)</td>
<td>14.7±1.8</td>
<td>29.0±2.8</td>
</tr>
<tr>
<td>BB (%)</td>
<td>1.7±0.8</td>
<td>3.1±0.6</td>
</tr>
</tbody>
</table>

Values are mean±SD. Analysis of variance: $F(7, 44)=496.2; p<0.001$. CK, creatine kinase; MITO, mitochondrial isof orm; NS, not significant; RHR, renovascular hypertensive rats.

\textsuperscript{1}p<0.01; \textsuperscript{2}p<0.001.
the M monomer. Such a change resulted in a percentage increase of both creatine kinase-MB and -BB at the expense of the creatine kinase-MM in both groups of hypertensive rats. At variance with studies carried out in SHR and in rats with aortic constriction,11 we could not find a direct correlation between the percentage of B monomer and the degree of cardiac hypertrophy either after 6 weeks or after 4 further weeks of hypertension. As for the mitochondrial isoform and the total enzymic activity, no appreciable changes were observed in 6-week- and 10-week-hypertensive rats compared with the normotensive age-matched controls. It is worthwhile noting that an increase in B monomer of creatine kinase without significant changes in total activity and in percentage of the mitochondrial isoform has previously been reported by Ingwall9 and by Younes et al11 in other animal models of cardiac hypertrophy at the compensated stage (i.e., SHR and aortic banding in rats and dogs). Patients with aortic stenosis and preserved ventricular function had similar findings but a significant decrease in total enzymic activity was also present.10

Captopril treatment decreased but did not normalize blood pressure. In fact, significantly higher values were found in captopril-treated rats compared with the normotensive age-matched controls. This is likely to be due to the relatively low doses of captopril we used, similar to those used in humans. Sen and Young14 found higher doses of captopril and were able to normalize blood pressure in hypertensive rats. A normalization of ventricular mass was achieved after 4 weeks of treatment despite the incomplete blood pressure control, supporting the hypothesis that in this animal model angiotensin II may play an important but independent role in modulating the increase in ventricular mass.22 A substantial restoration of ventricular isomyosin pattern was also obtained after captopril treatment. In fact, these rats showed a significantly lower percentage of V, in comparison with the age-matched hypertensive controls and a slight but not significant decrease also when compared with the age-matched normotensive controls. At the same time, a parallel increase in V3 was evident.

To our knowledge, there are only two studies published on the effect of converting enzyme inhibitors on contractile proteins.14,21 and they both showed that a preferential synthesis of V3 occurs both in renovascular hypertensive and in normotensive rats. Although the mechanisms involved are unknown, this possibility could be of interest in humans whose potentiality of shifting toward V3 is very high. Human ventricular myocardium is in fact mainly composed of V3.23,24 Other drugs like β-blockers14,25 and amiodarone26 have been reported in rats to induce a preferential expression of V3 and therefore are unlikely to induce significant changes in ventricular isomyosin patterns in humans.

At variance with the above mentioned studies,14,21 in our experience captopril seems to restore the normal ventricular isomyosin pattern rather than inducing a specific increase in V3. In other words, in our hypertensive rats the regression of left ventricular hypertrophy achieved by captopril is accompanied by changes in isomyosins that resemble those observed after removal of the ischemic kidney.18 In addition, we previously found captopril unable to modify the ventricular isomyosin pattern in normal rats treated with the same doses used in this study.27 However, it should be pointed out that this discrepancy with the previously quoted studies may only be apparent and may well be dependent on the lower doses we used (i.e., 2 mg/kg vs. 50 mg/kg body wt) or on the relatively shorter duration of treatment (i.e., 4 weeks vs. 6 weeks). We cannot rule out the possibility that higher doses or a more prolonged treatment may further increase the expression of the V3 isoenzyme that was not statistically significant in our experiment.

As for the effect of captopril on the expression of the different creatine kinase isoenzymes, captopril-treated rats showed a certain trend toward the normalization of the percentage of creatine kinase-MM, -MB, and -BB isoforms. However, a significantly higher amount of creatine kinase-MB and

<table>
<thead>
<tr>
<th>Group</th>
<th>CK IU mg/prot.</th>
<th>MITO (%)</th>
<th>MM (%)</th>
<th>MB (%)</th>
<th>BB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=5)</td>
<td>6.3±2.2</td>
<td>21.5±4.8</td>
<td>61.1±1.7</td>
<td>15.8±2.8</td>
<td>1.8±0.3</td>
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<tr>
<td>RHR (n=6)</td>
<td>6.9±3.1</td>
<td>23.0±5.8</td>
<td>46.6±2.7</td>
<td>27.3±4.6</td>
<td>3.1±0.4</td>
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<tr>
<td>Captopril-treated (n=7)</td>
<td>6.3±3.7</td>
<td>22.1±4.7</td>
<td>53.1±6.1</td>
<td>22.4±5.4</td>
<td>2.3±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SD. Analysis of variance: F(11, 60)=243; p<0.001. CK, creatine kinase; MITO, mitochondrial isoform; NS, not significant; RHR, renovascular hypertensive rats. □ p<0.005; ○ p<0.025; △ p<0.02; ● p<0.01; ▲ p<0.001.
BB was still present in captopril-treated rats in comparison with the age-matched normotensive controls. The shift toward the B monomer is believed to play a beneficial role. In fact, in cardiac hypertrophy as well as in ischemic heart disease, reduced myocardial levels of both creatine phosphate and adenosine triphosphate (ATP) have been described. Because the affinity of the B monomer for creatine phosphate is higher than that of the M monomer, this may imply a more efficient use of creatine phosphate in the generation of ATP. Moreover, the B monomer has an intrinsically slower reaction rate than the M monomer, and this could lead to a reduced rate of creatine phosphate consumption.

No significant changes were observed in total enzymic activity or in the percentage of mitochondrial activity both during progression of ventricular hypertrophy and after captopril-induced regression. This is not surprising in that a decrease of total creatine kinase activity and of the mitochondrial isoenzymes have been found only in rats with overt cardiac failure caused by long-standing hypertension.

The persistence of relatively large amounts of MB- and BB-isoenzymes despite the regression of cardiac hypertrophy may be because of the persistence of higher blood pressure levels in comparison with the normotensive controls. It is known that high blood pressure leads to a relative myocardial hypoxia through structural and functional changes in myocardial microvasculature that also take place in the absence of cardiac hypertrophy and epicardial coronary disease. We can hypothesize that this situation also occurs under our experimental conditions leading to a preferential synthesis of the B monomer of creatine kinase as observed in patients with ischemic heart disease without myocardial hypertrophy. This may imply that in our model the relative myocardial hypoxia, not just the myocardial hypertrophy, plays a role in the regulation of creatine kinase gene expression. This does not seem to be the case with isomyosins. It may also be that a different threshold of blood pressure is required to trigger the induction of different genes coding for myosin and for creatine kinase or that a longer time is needed for creatine kinase gene inactivation with the regression of cardiac hypertrophy. It is worthwhile noting that nonsynchronous induction of the genes coding for different contractile proteins has recently been described in a model of pressure overload–induced cardiac hypertrophy.

Another possibility could be that captopril has a direct effect on the creatine kinase system. Because, to our knowledge, this is the first study to evaluate changes occurring in the creatine kinase isoenzymes with regression of cardiac hypertrophy and no data exist in the literature on the effect of other drugs, this possibility remains to be explored.

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ventricular isomyosins with regression of cardiac hypertrophy. Hypertension 1986;8:1143–1148

KEY WORDS • hypertrophy • renovascular hypertension • myosin • creatine kinase • converting enzyme inhibition • cardiac hypertrophy
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