Altered Myocardial Contractility and Energetics in Hypertrophied Myocardium

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Alterations of myocardial contractility and energetics were examined in cardiac hypertrophy induced by different types of cardiac overload. Myocardial contractility was estimated by isometric contraction of isolated left ventricular papillary muscles. Myocardial energetics were assessed from ventricular myosin isoenzyme patterns obtained by pyrophosphate gel electrophoresis. Cardiac hypertrophy was induced by endurance swim-training and sustained pressure-overload by abdominal aortic constriction or volume-overload created by an arteriovenous shunt. In swim-trained hypertrophied myocardium, isometric developed tension (T) and \( \frac{dT}{dt_{\text{max}}} \) showed a tendency to increase and response of \( \frac{dT}{dt_{\text{max}}} \) to isoproterenol increased significantly as compared with sedentary rats. Training shifted the left ventricular myosin isoenzyme pattern toward VM-1, which has the highest ATPase activity. In pressure- or volume-overloaded myocardium, \( \frac{dT}{dt_{\text{max}}} \) decreased significantly and mechanical response to isoproterenol also decreased (or tended to decrease in volume-overloaded hearts) as compared with the respective sham-operated controls. In pressure- or volume-overloaded hearts, left ventricular myosin isoenzyme pattern shifted toward VM-3, which has the lowest ATPase activity. These results indicate that alterations in myocardial contractility, mechanical catecholamine responsiveness and myocardial energetics in hypertrophied myocardium do not always display the same trend, but are greatly influenced by the causes or duration of cardiac overload.

CARDIAC hypertrophy induced by sustained mechanical overloads is generally considered to be an adaptive change which serves to maintain pumping function, and is all the more important because of its pathophysiological significance in the transition from normal to failed myocardium. A number of studies of cardiac hypertrophy have been concerned with changes in contractile function\(^1\text{−}^5\) or biochemical alterations, e.g., changes in calcium transport or ATPase activity of sarcoplasmic reticulum\(^6\text{−}^8\) myofibrillar or myosin ATPase activity and myosin isoenzyme patterns\(^9\text{−}^{12}\). In the present study, alterations in myocardial contractility and energetics in hypertrophied myocardium were investigated using rats subjected to swim-training, aortic constriction (pressure-overload) and arteriovenous shunting (volume-overload).

MATERIALS AND METHODS

Nine-week-old male Wistar rats were subjected to swim-training in accordance with the pro-

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**Key words:**
- Isometric contraction
- Myosin isoenzyme
- Swim-training
- Aortic constriction
- AV shunt

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TABLE I  BODY WEIGHT, VENTRICULAR WEIGHT AND MYOCARDIAL MECHANICS

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>T (g/mm²)</th>
<th>dT/dt max (g/mm²·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>396 ± 31</td>
<td>1010 ± 50</td>
<td>2.9 ± 0.9</td>
<td>33.3 ± 6.9</td>
</tr>
<tr>
<td>Swimming (n = 9)</td>
<td>333 ± 19</td>
<td>1070 ± 70</td>
<td>3.2 ± 1.2</td>
<td>37.0 ± 15.4</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. BW = body weight; VW = ventricular weight; T = tension; ns = not significant.

TABLE II  BODY WEIGHT, VENTRICULAR WEIGHT AND MYOCARDIAL MECHANICS

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>T (g/mm²)</th>
<th>dT/dt max (g/mm²·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>655 ± 71</td>
<td>1301 ± 176</td>
<td>2.9 ± 0.6</td>
<td>32.8 ± 7.5</td>
</tr>
<tr>
<td>AC (n = 10)</td>
<td>674 ± 18</td>
<td>1919 ± 132</td>
<td>2.7 ± 0.7</td>
<td>26.3 ± 6.1</td>
</tr>
<tr>
<td>ns</td>
<td>p &lt; 0.001</td>
<td>ns</td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. AC = aortic constriction; BW = body weight; VW = ventricular weight; T = tension; ns = not significant.

TABLE III  BODY WEIGHT, VENTRICULAR WEIGHT AND MYOCARDIAL MECHANICS

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>T (g/mm²)</th>
<th>dT/dt max (g/mm²·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>568 ± 26</td>
<td>1294 ± 45</td>
<td>2.8 ± 0.8</td>
<td>31.9 ± 7.0</td>
</tr>
<tr>
<td>AV shunt (n = 6)</td>
<td>632 ± 46</td>
<td>2050 ± 235</td>
<td>2.4 ± 0.5</td>
<td>23.9 ± 4.8</td>
</tr>
<tr>
<td>p &lt; 0.02</td>
<td>p &lt; 0.001</td>
<td>ns</td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. BW = body weight; VW = ventricular weight; T = tension; ns = not significant.

gramme of Rupp and Jacob. The training time was 10 min on the initial day and was increased by 10 min daily to 180 min per day. A daily swimming time exceeding 90 min was divided into two sessions. The programme was continued for 4 weeks (7 days per week). Pressure-overload was induced by constriction of the abdominal aorta in 10-week-old male Wistar rats using a silver clip of 0.7 mm internal diameter (AC rats). The rats were reared in ordinary cages for 24–26 weeks after the operation. Age-matched sham-operated rats served as controls. Chronically volume-overloaded cardiac hypertrophy was induced by abdominal arteriovenous shunt in 10-week-old male Wistar rats (AV shunt rats). Arteriovenous shunt was created in rats under a microscope in accordance with the procedure of Flaim et al. and Mercadier et al. Myocardial and biochemical changes were investigated ten weeks after the operation.

Mechanical studies were conducted using isolated left ventricular papillary muscles. Left ventricular free walls were used for the determination of myosin isoenzyme patterns. Papillary muscles were stimulated at 32°C with a frequency of 0.2 Hz and a voltage 30% above threshold and were perfused with Tyrode solution containing 1.1 mM Ca²⁺. Developed tension (T) and dT/dt were recorded after the steady state was attained at Lmax. The responses of mechanical parameters to isoproterenol (10⁻⁵ M) and dibutyryl cyclic AMP (DBcAMP) (10⁻⁵ M) were also estimated at Lmax, following the interposition of Tyrode solution for 25–30 min. The response of each parameter was assessed by comparing 2 paired values, one value being that measured in the steady state prior to isoproterenol or DBcAMP administration.

Polyacrylamide gel electrophoresis in the presence of pyrophosphate was performed as described elsewhere. The gel contained 3.8% acrylamide and 0.12% N, N'-methylenebisacrylamide. The electrophoresis buffer was a 20 mM Na₄P₂O₇ solution (pH 8.8) containing 10% glycerol. Native myosin from the left ventricle was extracted with a solution consisting.

Fig. 1. Myocardial mechanical catecholamine responsiveness (left panel) and myocardial β-receptors (right panel) in swim-trained rats. Vertical lines indicate SD. Ns: not significant.

Fig. 2. Myocardial mechanical response to isoproterenol (left panel) and to dibutyl cyclic AMP (DBcAMP) (right panel) in rats with aortic constriction (ACrats). Vertical lines indicate SD.

of 100 mM Na₄P₂O₇ (pH 8.8), 5 mM 1, 4-dithiothreitol, 5 mM EGTA and 5 μg/ml leupeptin. The samples were electrophoresed for 30h at 2°C and a voltage gradient of 13.3 V/cm.

Cardiac membrane preparation and β-adrenoceptor binding assay were performed in accordance with the method of Williams and Lefkowitz using [³H]-dihydroalprenolol (0.825–15 nM). Specific binding of [³H]-dihydroalprenolol was defined by comparing total binding in the absence of propranolol and nonspecific binding in the presence of 1 × 10⁻⁵ M (+) propranolol. The number of binding site and the dissociation constants (Kₐ) were calculated by Scatchard analysis.

Student's t-test was used for statistical comparisons.

RESULTS

Table I shows the comparisons between the body weight, ventricular weight and myocardial mechanics of trained and sedentary rats. The ventricular weight of swim-trained rats was significantly higher than that of the sedentary controls. T and dT/dtmax in the trained group showed a tendency to increase as compared with the corresponding values in the controls, but this increase was not significant. As shown in Table II, the rats with aortic constriction dis-
Fig. 3. Myocardial mechanical response to isoproterenol (left panel) and to dibutyryl cyclic AMP (DdBcAMP) (right panel) in rats with arteriovenous shunt (AV shunt rats). Vertical lines indicate SD. ns: not significant.

Fig. 4. Left ventricular myosin isoenzyme pattern in swim-treated rats. Values indicated are means ± SD.

Fig. 5. Left ventricular myosin isoenzyme pattern in rats with aortic constriction (AC rats). Values indicated are means ± SD.

Fig. 6. Left ventricular myosin isoenzyme pattern in rats with arteriovenous shunt (AV shunt rats). Values indicated are means ± SD.

played a markedly higher ventricular weight than the control rats. There was no significant difference in T between the AC rats and the controls, but dT/dt_max decreased significantly in the former group. The ventricular weight of the AV shunt rats was also markedly greater than that of the controls (Table III). T remained unchanged and dT/dt_max was significantly decreased in the AV shunt rats. Myocardial mechanical responsiveness to isoproterenol was significantly enhanced in the swim-trained rats (Fig. 1). Interestingly, the number of binding sites for myocardial β-receptors showed a tendency to decrease in the trained group, although the dissociation constant was also lower in this group. The relative increase of T and dT/dt induced by isoproterenol was significantly
smaller in the AC rats than in the control rats (Fig. 2). The same tendency was observed with DBCAMP. Myocardial mechanical responses to isoproterenol and DBCAMP were both depressed in AV shunt rats, although not to a statistically significant extent (Fig. 3). Left ventricular myosin isoenzyme patterns shifted toward VM-1, which has the highest ATPase activity, in the hypertrophied myocardium of swim-trained rats (Fig. 4). On the other hand, in pressure- or volume-overloaded hypertrophied myocardium, the pattern shifted toward VM-3, which has the lowest ATPase activity (Fig. 5, 6).

DISCUSSION

In the swim-trained rats, isometric developed tension (T) of isolated left ventricular papillary muscles and dT/dt_{max} showed a tendency to increase, as previously reported by other authors. These results are supported by biochemical evidence, which showed increased Ca transport in the sarcoplasmic reticulum of trained myocardium, although other authors found no changes in Ca binding or uptake. Myocardial mechanical responses to isoproterenol increased significantly in the trained rats despite the fact that myocardial β-receptors showed a tendency to decrease. Myocardial mechanical responses to isoproterenol was enhanced in the trained spontaneously hypertensive rats despite the fact that myocardial β-receptor numbers decreased and receptor affinity remained unchanged. This phenomenon could be attributed to various mechanisms including post-receptor processes. The shift of the myosin isoenzyme pattern toward VM-1 due to training may be an adaptation to provide enhanced cardiac function associated with improved coronary flow and oxygen delivery during exercise. Various hormones, may be involved, e.g., thyroid hormones or catecholamines. Alteration in myosin isoenzyme patterns could be of significance with respect to the increase of mechanical catecholamine responsiveness in the trained rats if the magnitude of the increase in contractility resulting from activation of the cAMP-regulated system varies with the relative concentration of VM-1, as postulated by Winegrad et al. Sustained pressure-overload induced marked cardiac hypertrophy. The nature of the associated mechanical or biochemical changes was opposite to that of the changes which occurred in swim-trained rats. In the present study, isometric developed tension (T) remained unchanged but dT/dt_{max} decreased in pressure-overload hypertrophied myocardium. Depressed dT/dt_{max} could be explained by sarcoplasmic reticular dysfunction (e.g., decreased ATPase activity) in severe cardiac hypertrophy. Myocardial mechanical catecholamine responsiveness was decreased in the AC rats. According to other reports, chronic pressure-overload decreases myocardial β-receptor density although increased β-receptor number has been reported in mild cardiac hypertrophy induced by short-term pressure-overload. Myocardial mechanical response to DBCAMP was also decreased in hypertrophied myocardium in the AC rats. Post-receptor processes might also be involved here, since DBCAMP passes the cardiac surface membrane and manifests a positive inotropic effect without direct stimulation of β-receptors. Decreased myosin ATPase activity or alteration of myosin isoenzyme patterns manifested by increased VM-3 in pressure-overloaded cardiac hypertrophy is thought to be an adaptation which serves to maintain efficient force development, with low oxygen and energy utilization. The present results concerning myosin isoenzymes are compatible with this hypothesis. Chronic cardiac volume-overload by arteriovenous shunting induced marked cardiac hypertrophy, as in the AC rats. Changes in myocardial mechanics were similar to those in the AC rats, which showed that myocardium contracts slowly in isometric contraction. Myocardial mechanical responses to isoproterenol and to DBCAMP in the AV shunted rats were both depressed, as in the AC rats, although this change was not statistically significant as compared with the sham-operated control rats. Shift of ventricular myosin isoenzyme patterns toward VM-3 by chronically volume-overloaded cardiac hypertrophy may be regarded as having the same significance as in pressure-overloaded cardiac hypertrophy.

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