Myocardial Mechanical and Myosin Isoenzyme Alterations in Streptozotocin-Diabetic Rats

Nobuakira Takeda, M.D., Izuru Nakamura, M.D., Toshio Hatanka, M.D., Tadanari Ohkubo, Ph.D., and Makoto Nagano, M.D.

**SUMMARY**

Fifteen week old male Wistar rats \( (n=7) \) were made diabetic by intravenous injection of streptozotocin \((50 \text{ mg/kg})\). Age-matched, untreated male Wistar rats \( (n=9) \) served as controls. Hearts were removed after 5–6 weeks of diabetes, and the isometric developed tension \((T)\) of isolated left ventricular papillary muscles and its first derivative \((dT/dt)\) were measured at a frequency of 0.2 Hz. During testing, the muscles were perfused with Tyrode's solution \((\text{Ca}^{2+} \text{ concentration was half of normal Tyrode's solution, pH 7.4, 32°C, bubbled with 95% O}_2 \text{ and 5% CO}_2)\). In addition, the left ventricular isoenzyme pattern, which is related to myocardial energetics, was determined by pyrophosphate gel electrophoresis. There was no significant difference in isometric developed tension between diabetic and control rats \((\text{DM: 2.90±0.89 vs controls: 2.87±0.85 g/mm², mean±SD)})\), but in diabetic rats, \(dT/dt\)max decreased significantly as compared with controls \((\text{DM: 23.5±4.2 vs controls: 31.9±7.9 g/mm²-s, } p<0.05)\). Myocardial mechanical responses to isoproterenol \((10^{-7} \text{ M})\) and dibutyryl cyclic AMP \((10^{-5} \text{ M})\) also decreased in diabetic rats. The left ventricular myosin isoenzyme pattern shifted toward VM-3 in diabetic rats \((\text{VM-3: DM: 74.9±10.7 vs controls: 9.5±4.1 }%, \ p<0.001)\). These results indicate that diabetes influences myocardial contractility and changes cardiac energetics. Post-receptor processes may play a role in myocardial mechanical responses to catecholamines in streptozotocin-diabetic rats.

**Additional Indexing Words:**
Isometric developed tension  Papillary muscle  Isoproterenol  Dibutyryl cyclic AMP  Pyrophosphate gel electrophoresis

A decrease in cardiac function has been observed in alloxan- or streptozotocin-induced experimental diabetes.\(^1\)–\(^4\) However, Fein et al\(^5\) reported that the developed tension of isolated left ventricular papillary muscles

\(^1\)–\(^4\) From the Department of Internal Medicine, Aoto Hospital, Jikei University School of Medicine, Tokyo, Japan.

Address for reprints: Nobuakira Takeda, M.D., Department of Internal Medicine, Aoto Hospital, Jikei University School of Medicine, Aoto 6–41–2, Katsushika-ku, Tokyo 125, Japan.

Received for publication May 29, 1987.

Accepted April 11, 1988.
was not affected in diabetic rats, although relaxation was delayed and shortening velocity was depressed. Myocardial biochemical changes in diabetes have been reported, though, reflected as a decrease in Ca\(^{2+}\)-binding and Na\(^{+}-\)K\(^{-}\)-ATPase in the sarcolemma,\(^{6,7}\) a decrease in Ca\(^{2+}\)-uptake and Ca\(^{2+}\)-ATPase in the sarcoplasmic reticulum,\(^{8-10}\) a decrease in myofibrillar, actomyosin and myosin ATPase activity,\(^{11-14}\) and an alteration in the myocardial myosin isoenzyme pattern.\(^{11,14}\) In the present study, the isometric developed tension of isolated left ventricular papillary muscle was examined using streptozotocin-induced diabetic rats. Myocardial mechanical catecholamine responsiveness was also examined, because changes in sympathetic activity, including a decrease in numbers of myocardial beta-receptors, were reported in diabetic animals.\(^{15-18}\) We have already reported a discrepancy between myocardial mechanical catecholamine responsiveness and myocardial beta-receptor number.\(^{19,20}\) In order to see whether factors other than the number of myocardial beta-receptors play a role in mechanical catecholamine responsiveness, myocardial mechanical responses to dibutyryl cyclic AMP (DBcAMP) were also examined.\(^{21}\) In addition, changes in the left ventricular myosin isoenzyme pattern, which is related to the energetics of muscle contraction, were examined by pyrophosphate gel electrophoresis.

**Materials and Methods**

Fifteen week old male Wister rats were used in this experiment. Rats were made diabetic by intravenous administration of streptozotocin (50 mg/kg), and after 5–6 weeks the hearts were excised. Fasting blood glucose was measured by the glucose oxidase method one day before the hearts were excised. Mechanical studies were performed using isolated left ventricular papillary muscles. The left ventricular free walls were used for determination of the myosin isoenzyme pattern. Dissected papillary muscles were suspended vertically between a fixed lever and force transducer, using silk ligatures and small steel hooks. Papillary muscles were stimulated at a frequency of 0.2 Hz and with a voltage 30% above threshold, while being perfused with 32°C Tyrode’s solution (consisting of 25.0 mM glucose, 130.0 mM NaCl, 20.0 mM NaHCO\(_3\), 1.2 mM NaH\(_2\)PO\(_4\), 4.1 mM KCl, 1 mM CaCl\(_2\), and 1.5 mM MgCl\(_2\), bubbled with 95% O\(_2\) and 5% CO\(_2\), pH of 7.4). The Ca\(^{2+}\) concentration was half that of normal Tyrode’s solution to avoid saturation of tension development in the midst of the response to isoproterenol or DBcAMP.\(^{19,20}\) Muscles were stimulated to contract for a one-hour equilibration period, and set to the muscular length of L\(_{\text{max}}\). After a steady state was obtained at L\(_{\text{max}}\), developed tension (T), dT/dt, time to peak tension (TPT), and total contraction time
(TGT), were recorded. Subsequently, mechanical responses to isoproterenol (10^{-7} M) were estimated. Following perfusion with Tyrode's solution for 30-35 min, the responses to DBcAMP administration (10^{-5} M) were also measured. The response of each parameter was obtained by comparing two paired values; (1) the steady-state prior to isoproterenol or DBcAMP administration and (2) the maximum value after isoproterenol or DBcAMP administration. The length and diameter of the muscle fibers were measured with a graticule, using a microscope. The estimation of developed tension and measurement of papillary muscle size were used to set the muscle length constantly at Lmax.

Polyacrylamide gel electrophoresis was carried out in the presence of pyrophosphate, as described elsewhere.22)-24) The gel contained 3.8% acrylamide and 0.12% N,N'-methylene-bis-acrylamide. The electrophoresis buffer was 20 mM Na_4P_2O_7 (pH 8.8) in the presence of 10% glycerol. Native myosin from the left ventricle was extracted with a solution consisting of 100 mM Na_4P_2O_7 (pH 8.8), 5 mM 1,4-dithiothreitol, 5 mM EGTA, and 5 µg/ml leupeptin. Electrophoresis was carried out for 30 hr at 2°C, and a voltage gradient of 13.3 V/cm. Gels were stained with Coomassie brilliant blue R-250, and were destained with 7% acetic acid. The relative amounts of myosin isoenzymes were obtained from densitometric tracings of the gels, using a scan densitometer (Gelman DCD-16) at 575 nm.

Statistical comparisons were carried out using Student's t-test.

RESULTS

Seven diabetic and 9 normal rats were examined. The mean body and ventricular weights of diabetic rats were about 37% and 28% smaller, respectively, than those of the controls, but the ventricular weight to body weight ratio was significantly higher in the diabetic group (Table I). The fasting

Table I. Comparisons of Body Weight, Ventricular Weight, Papillary Muscle Size, and Fasting Blood Glucose

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>VW (mg)</th>
<th>VW (mg)</th>
<th>Papillary muscle size</th>
<th>BG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BW (g)</td>
<td>L (mm)</td>
</tr>
<tr>
<td>DM (n=7)</td>
<td>345.7±30.1</td>
<td>956.9±90.6</td>
<td>2.78±0.27</td>
<td>5.5±0.6</td>
<td>0.86±0.17</td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>532.8±34.9</td>
<td>1331.2±95.2</td>
<td>2.41±0.19</td>
<td>5.7±0.3</td>
<td>0.93±0.21</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
<td>ns</td>
<td>ns</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

BW=body weight; VW=ventricular weight; L=length; CSA=cross sectional area; BG=blood glucose. Values are means±SD. ns=not significant.
Table II. Comparisons of Myocardial Mechanics

<table>
<thead>
<tr>
<th></th>
<th>DT (g/mm²)</th>
<th>RT (g/mm²)</th>
<th>dT/dt max (g/mm²·s⁻¹)</th>
<th>TPT (msec)</th>
<th>TCT (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (n=7)</td>
<td>2.90±0.89</td>
<td>1.02±0.38</td>
<td>23.5±4.2</td>
<td>168.6±14.4</td>
<td>589.3±49.7</td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>2.67±0.85</td>
<td>0.98±0.22</td>
<td>31.9±7.9</td>
<td>132.8±11.5</td>
<td>498.3±44.9</td>
</tr>
</tbody>
</table>

DT=developed tension; RT=resting tension; TPT=time to peak tension; TCT=total contraction time. Values are means±SD. ns=not significant.

Fig. 1. Representative traces of isometric contraction. T=developed tension; dT/dt=rate of tension rise.

Blood glucose of diabetic rats was significantly higher than for controls (Table I). Table II shows comparisons of myocardial mechanics and Fig. 1 shows representative traces of isometric contraction. The isometric developed tension of isolated left ventricular papillary muscle was almost the same in both groups, but the dT/dt max of the diabetic rats was 26% lower than for con-
Vol. 29
No. 4
MYOCARDIAL ALTERATIONS IN DIABETIC RATS 459

Fig. 2 (left). Comparisons of tension and dT/dt max in response to isoproterenol (10⁻⁷ M) between diabetic and control rats. Vertical lines indicate the standard deviation (SD).

Fig. 3 (right). Representative contractile responses of left ventricular papillary muscles in the presence of isoproterenol (10⁻⁷ M). The muscle length was Lmax.

cords. The time to peak tension (TPT) and total contraction time (TCT) of the diabetic group were prolonged by 27% and 18%, respectively. These measures of myocardial mechanics were similar to those of other reports.²₅,²₆ Figure 2 shows myocardial mechanical responses to isoproterenol, and Fig. 3 shows representative records. Changes in developed tension and dT/dtmax in response to isoproterenol administration were significantly smaller in diabetic rats than controls (DM vs controls, ΔT: 8.0±4.9 vs 17.6±5.0%, p<0.005, ΔdT/dtmax: 9.8±3.9 vs 23.3±7.9%, p<0.005). These results indicate a depressed myocardial catecholamine responsiveness in diabetic rats, in agreement with the results of Foy et al²⁷ for atria of diabetic rats. As shown in Fig. 4 (Fig. 5 shows representative records), myocardial mechanical responses to DBcAMP administration revealed a response pattern similar to isoproterenol (DM vs controls, ΔT: 6.6±4.1 vs 14.7±3.9%, p<0.005, ΔdT/dtmax: 8.0±4.6 vs 17.4±5.1%, p<0.005).

The left ventricular myosin isoenzyme pattern shifted significantly to-
Fig. 4 (left). Comparisons of $\Delta$ tension and $\Delta dT/dt_{max}$ in response to dibutyryl cyclic AMP ($10^{-4}$ M) between diabetic and control rats. Vertical lines indicate SD.

Fig. 5 (right). Representative contractile responses of left ventricular papillary muscles in the presence of dibutyryl cyclic AMP ($10^{-4}$ M). The muscle length was $L_{max}$.

Fig. 6. Left ventricular myosin isoenzyme distribution obtained by pyrophosphate gel electrophoresis. Relative amounts of isoenzymes were obtained from densitometric tracings. The left panel shows representative densitometric profiles. Values are means±SD. ns = not significant.
wards VM-3 in diabetic rats (VM-3: DM vs controls=74.9±10.7 vs 9.5±4.1%, p<0.001) (Fig. 6).

**DISCUSSION**

In the present study, the isometric developed tension was normal in isolated left ventricular papillary muscle in diabetic rats, but the dT/dtmax decreased, and TPT and TCT were prolonged significantly. These mechanical changes can be explained in terms of myocardial biochemical changes which have already been reported by other authors, such as a decrease in sarcolemmal Ca$^{2+}$-binding and Na$^{+}$-K$^{+}$-ATPase, a decrease in sarcoplasmic reticular Ca$^{2+}$-uptake and Ca$^{2+}$-ATPase, decreases in myofibrillar, actomyosin, and myosin ATPase activity, and an alteration of myocardial myosin isoenzyme pattern towards VM-3. Myocardial mechanical responses to isoproterenol decreased in diabetic rats. This finding may be related to the decreased number of myocardial beta-receptors in diabetic rats. Moreover, it has been indicated in previous studies that post-receptor processes may also play a role in myocardial mechanical responsiveness to catecholamines. Myocardial mechanical responses to DBcAMP were also depressed in diabetic rats in the present study. As DBcAMP is thought to pass through the myocardial surface membrane and to exert its positive inotropic effect without directly stimulating the beta-receptors, processes other than those occurring at the myocardial beta-receptors may play a role in mechanical catecholamine responsiveness.

The ventricular myosin of rats is separable into three isoenzymes, VM-1, 2, 3, which differ in both electrophoretic mobility and ATPase activity. In pressure-overloaded cardiac hypertrophy, the ventricular myosin isoenzyme pattern shifts towards VM-3. This alteration of the pattern is thought to be an adaptive change of the myocardium to economically maintain force of contraction. In the present study, the left ventricular myosin isoenzyme pattern shifted significantly towards VM-3, with no significant change in developed tension. This shift of the myosin isoenzyme pattern in diabetic rats may have the same significance as in pressure-overloaded myocardium. Moreover, the predominant VM-3 pattern in the myocardium of diabetic rats may be a factor in depressed myocardial mechanical catecholamine responsiveness, since some authors have reported that myocardium with a predominant VM-1 pattern is more sensitive to catecholamines.
REFERENCES

22. Hoh JFY, McGrath PA, Hale PT: Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. J Mol Cell Cardiol 10: 1053,


