ARE DIABETIC HEARTS MORE RESISTANT TO ISCHEMIA/REPERFUSION INJURY?

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The present study was designed to investigate the cardiac impairment during ischemia/reperfusion injury in rats with streptozotocin-induced diabetes vs controls. Male rats were divided into three groups: controls, one-week diabetic rats (1wDM) and four-week diabetic rats (4wDM). In the isolated working heart, left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and maximum positive and negative dP/dt were measured before ischemia and after 20 min of global ischemia followed by 30 min reperfusion. In the pre-ischemic state, hearts of 4wDM showed a significant (p<0.05) depression of the maximum positive and negative dP/dt compared with those of controls and 1wDM. There were no significant differences in LVDP and LVEDP among the three groups. The incidence of reperfusion-induced ventricular fibrillation (VF) was 75% for controls, 15% for 1wDM rats, and 27% for 4wDM rats. In hearts without reperfusion-induced VF, there were no differences in the three groups, between the pre- and post-ischemic values in LVDP, LVEDP, and maximum positive and negative dP/dt. These findings suggest that diabetic hearts exhibit no susceptibility to ischemia/reperfusion injury.

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MYOCARDIAL abnormalities occurring as a result of diabetes, including myocardial infarction and heart failure, have been described in various clinical and experimental settings. Although diabetes has been found to be associated with an increased mortality from vascular disease, evidence suggests that myocardial abnormality may play a more important role than the acceleration of coronary atherosclerosis. Studies of cardiac function in diabetic humans and animals indicate that the cardiac reserve reduced; however, the pathogenetic mechanisms remain controversial.

Although the restoration of blood flow arrests the progression of necrosis, paradoxically it is accompanied by functional derangements including a prolonged contractile impairment and various ventricular arrhythmias. Clinically, reperfusion-induced arrhythmias are sometimes observed in cases of percutaneous transluminal coronary recanalization (PTCR) or percutaneous transluminal coronary angioplasty (PTCA), etc. Experimental arrhythmias can also be produced readily in a variety of preparations, but the mechanisms of the genesis of

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reperfusion-induced arrhythmias are not yet clear.\textsuperscript{11}

Several isolated heart studies suggest that diabetic hearts are more sensitive than normal hearts to anoxic or ischemic injury; that is, the diabetic myocardium may be more susceptible to ischemic injury, with an increased extent of injury for a given area at risk\textsuperscript{12–14}. On the other hand, a few investigators have reported that, although diabetes produced abnormalities of cardiac function, ischemic injury was not increased in diabetic hearts, demonstrating a greater resistance to ischemia/reperfusion injury.\textsuperscript{15}

In the present investigation, we determined characteristic abnormalities of cardiac function at different time periods following the induction of diabetes as compared with normal control, and examined ischemia/reperfusion injury by determining the recovery of mechanical function and reperfusion-induced arrhythmias using the isolated perfused working heart preparation.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats were used for all studies. Diabetes was induced by a single tail vein injection of streptozotocin at a dose of 50 mg/kg (Sigma chemical Co, St.Louis, Mo). Diabetic animals were divided into 2 groups. One group was studied at 1 week (1wDM; n = 13) and the other was studied 4 weeks of diabetes (4wDM; n = 11). Untreated age-matched rats (n = 16) served as the control group.

**Perfusion methods**

Animals were anesthetized with sodium pentobarbital (50 mg/kg) and injection with heparin sodium (200 IU). Thirty seconds later, the hearts were quickly excised and placed in cold (4 °C) perfusion medium until the contraction had ceased. Each heart was then cannulated via the aorta and washed for 5 min of retrograde perfusion at constant pressure (75 cm H₂O) with a nonproteinous, Krebs-Henseleit bicarbonate buffer (37 °C, pH 7.4), which was oxygenated with 95% O₂-5% CO₂. The perfusate contained (in mmol/l) 118.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.5 NaEDTA, 5.5 glucose and was filtered through a membrane with a 5 μm pore size before using. After perfusion washing, the perfusion was switched to a working-heart apparatus as described Neely et al.\textsuperscript{16} The hearts were allowed to stabilize for a period of 15 min in this recirculating system. The height of the left atrial reservoir was consistently kept at 10 cm H₂O and that of the aortic column at 75 cm H₂O. A stable preparation was defined as having a sinus rate of at least 200 beats/min, a coronary flow of at least 9 ml/min, and absence of arrhythmias at 5 min before ischemia. Only stable preparations were used in this study. Global ischemia was induced by cross-clamping the aortic perfusion tube and left atrial cannula for 20 min at 37 °C. Reperfusion was induced by declamping reversely, and perfu-

### TABLE I

**BODY AND HEART WEIGHTS AND PLASMA GLUCOSE LEVELS IN CONTROL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS**

<table>
<thead>
<tr>
<th></th>
<th>control (n=16)</th>
<th>1wDM (n=13)</th>
<th>4wDM (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>324±5</td>
<td>260±12</td>
<td>242±4**</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.36±0.04</td>
<td>1.22±0.07</td>
<td>1.15±0.05**</td>
</tr>
<tr>
<td>Heart/Body weight ratio (mg/g)</td>
<td>4.20±0.14</td>
<td>4.74±0.22</td>
<td>4.76±0.21</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>104±4</td>
<td>466±16**</td>
<td>647±33**</td>
</tr>
</tbody>
</table>

Diabetic rats were sacrificed at 1 (1wDM) or 4 (4wDM) weeks after injection of streptozotocin. Values are mean±SE. **p<0.01 compared with control.
singing with the same oxygenated buffer. Recovery of ventricular function was followed for 30 min. Arrhythmias were defined and quantified in accordance with the "Lambeth conventions".17

**Physiological measurements**

The electrocardiogram was recorded via two silver electrodes attached to the ventricular apex and the aortic cannula. A polyethylene catheter was placed into the left ventricle via the left atrium for the measurement of left ventricular pressure. Heart rate (HR), left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and maximum positive and negative dP/dt were recorded with an FF-030T transducer (Nihon Koden, Tokyo) and an RM-6000 polygraph system (Nihon Koden, Tokyo).

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***Incidence of reperfusion-induced ventricular fibrillation in control (open bar), one-week diabetic (hatched bar), and four-week diabetic (stippled bar) hearts, which were subjected to 20 min of global ischemia and 30 min of reperfusion. After 20 min of ischemia, the hearts were reperfused for 30 min as a working heart preparation by raising the hydrostatic afterload pressure to 75 cm H2O. Left ventricular fibrillation was initiated within 3 min of reperfusion. *p<0.05, **p<0.01 compared with control.

Statistical analysis

The results were presented as mean±SE. Statistical analysis was carried out by analysis of variance with Duncan's test. The probability was considered significant if less than 0.05.

RESULTS

General features of the experimental rats

The characteristics of the control and diabetic animals used in these experiments are shown in Table I. Both body weight and heart weight in the 4wDM group were significantly lower than in the control group. Heart weight/body weight ratio in the diabetic group was 13% greater than that of the control group, not a significant difference. These findings were in agreement with previous reports\textsuperscript{14,18} Blood glucose in the diabetic group was roughly three times that of the control group.

Hemodynamics of control and diabetic animals in pre-ischemic state

The heart rate obtained from various groups was as follows: control 267±11; 1wDM 243±7; 4wDM 238±7. The results indicate that HR in diabetic rats shows a tendency to decrease (Fig. 1A). These were no significant differences in LVDP and LVEDP between the three groups (Fig. 1B, C). Hearts of 4wDM rats showed a significant depression in the left ventricular maximum positive and negative dP/dt as compared with those of controls and 1wDM (Fig. 1. D, E).

Incidence of ventricular fibrillation

The incidence of reperfusion-induced ventricular fibrillation was 75% for controls, 15% for 1wDM rats, and 27% for 4wDM rats (Fig. 2). Reperfusion arrhythmias were markedly reduced in diabetic hearts. Ventricular fibrillation was initiated within 3 min of reperfusion. The duration of ventricular fibrillation was 5.4±1.5 min.

Effects of ischemia and reperfusion on hemodynamics

It is widely recognized that ventricular fibrillation may affect cardiac functions. Consequently, Table II indicates the effect of ischemia/reperfusion on hemodynamic properties in hearts without reperfusion-induced ventricular fibrillation from control and diabetic rats. There were no significant differences between pre- and post-ischemic values of various cardiovascular parameters in the three groups.

DISCUSSION

This study was designed to estimate cardiac function in rats made diabetic by the intravenous injection of streptozotocin as compared with normal rats, and to test whether the diabetic myocardium is more susceptible than the normal myocardium to ischemia/reperfusion.

The results obtained in the present investigation demonstrate certain characteristics of cardiac function in diabetic rats. There were no differences between normal and 1-week diabetic hearts for left ventricular pressure, maximum positive and negative dP/dt. In 4-week diabetic rat hearts, maximum positive and negative dP/dt were significantly depressed compared with 1-week diabetic or controls. Litwin et al have described significant changes in various parameters of heart function in rats after 3-4 weeks.
TABLE II  EFFECT OF GLOBAL ISCHEMIA AND REPERFUSION ON HEMODYNAMICS OF CONTROL AND STREPTOZOTOCIN-INDUCED DIABETIC HEARTS

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (beats/min)</th>
<th>LVDP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV dP/dt (mmHg/s)</th>
<th>LV-dP/dt (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (n=4)</td>
<td>254±19</td>
<td>92±9</td>
<td>4.3±0.8</td>
<td>3200±200</td>
<td>2650±290</td>
</tr>
<tr>
<td>1wDM (n=11)</td>
<td>225±18</td>
<td>100±4</td>
<td>5.5±1.0</td>
<td>3000±240</td>
<td>2650±250</td>
</tr>
<tr>
<td>4wDM (n=8)</td>
<td>246±8</td>
<td>98±4</td>
<td>4.4±0.5</td>
<td>2960±180</td>
<td>2810±240</td>
</tr>
<tr>
<td></td>
<td>235±8</td>
<td>93±4</td>
<td>4.8±0.5</td>
<td>2920±210</td>
<td>2530±80</td>
</tr>
<tr>
<td></td>
<td>241±10</td>
<td>101±10</td>
<td>5.0±0.7</td>
<td>2480±210</td>
<td>2060±160</td>
</tr>
<tr>
<td></td>
<td>211±8</td>
<td>98±9</td>
<td>6.4±0.8</td>
<td>2760±180</td>
<td>2080±220</td>
</tr>
</tbody>
</table>

HR, heart rate; LVDP, left ventricular developed pressure (ie, the difference between systolic and end-diastolic pressure); LVEDP, left ventricular end-diastolic pressure. Hearts were exposed to global ischemia for 20 min and reperfusion for 30 min. Pre- and post-ischemic values were measured at 10 min before ischemia and after 30 min of reperfusion, respectively. In hearts without reperfusion-induced ventricular fibrillation, there were no significant differences between pre- and post-ischemic values of hemodynamics in the three groups. All data are mean±SE.

of diabetes Similar cardiac functional alterations have been reported in hearts obtained from 8-week rats rendered diabetic by streptozotocin injection. The number of β-adrenergic receptors was reduced in cardiac membranes from diabetic rats. Atkins et al reported that after 2 weeks of diabetes, the response of adenylate cyclase to isoproterenol stimulation was not altered. However, after 4 weeks of diabetes, the sensitivity of adenylate cyclase to isoproterenol stimulation was depressed and abnormalities in cardiac contractility were noted, including a depressed response of LV dP/dt to graded isoproterenol infusion. Furthermore, the defect in calcium transport in microsomal vehicles of diabetic animals was not evident until 28 days after streptozotocin injection. These observation supported the conclusion that streptozotocin-induced diabetes produced cardiac dysfunction at 4 weeks after the induction, whereas the alterations were not noticed at 1 week.

The influence of heart rate on reperfusion arrhythmias is a more complicated issue. This study was performed under spontaneous beating which we believe to be more physiologic. Some authors have shown that lowering the heart rate during regional ischemia in the dog reduces the incidence of reperfusion arrhythmias. Zuanetti et al prevented ventricular fibrillation in the cat by lowering the heart rate via vagal stimulation a few seconds before reperfusion. Furthermore, their study suggested that the heart rate influenced reperfusion-induced ventricular fibrillation as a consequence of some effects on the rate of evolution of ischemic injury. Other investigators found that pacing at a high frequency increased the incidence of ischemia-induced arrhythmias, with lower rates being protective.

In the present study, while there was no significant difference in heart rate between the diabetic and control groups, the incidence of ventricular fibrillation in the diabetics was significantly lower than that in the controls. Therefore, we conclude that diabetic rat hearts are resistant to ischemia/reperfusion injury as shown by the occurrence of reperfusion-induced arrhythmias. Furthermore, cardiac function was no different between the control and diabetic groups without ventricular fibrillation. We would like to emphasize that diabetic rat hearts may be not sensitive to ischemia/reperfusion injury with regard to cardiac performance.

Previous studies have reported that diabetic hearts are sensitive to ischemia and ischemia/reperfusion injury. Hearse et al showed that diabetic hearts appear to be far more vulnerable to anoxia than normal hearts in the isolated perfused working rat heart model. The explanation was that, in diabetes, post-anoxic recovery of the hearts was supported in part by endogenous supplies of pre-formed high energy phosphates and in part from energy derived from the glycolysis of endogenous glycogen. In-
gebretsen et al have also suggested that the increased susceptibility to anoxia in diabetic hearts might be related to the limited glucose available for anaerobic ATP production in the diabetic tissue. Feuvray et al reported the mechanical failure of the diabetic heart in response to ischemia was associated with a more rapid rise in myocardial long-chain acyl-CoA and acyl carnitine esters.

On the other hand, a few investigators observed that the severity of injury following ischemia with reperfusion was not increased in diabetic hearts. Tani et al proposed that the resistance to ischemia in diabetic hearts was not related to higher tissue levels of high energy phosphates during reperfusion nor to lactate accumulation during ischemia, and that alteration of sarcolemmal Ca2+ transport systems in the diabetic myocardium may account for the greater resistance of these hearts to ischemia.

In conclusion, we found that ischemia/reperfusion injury was not increased in diabetic hearts. Furthermore, the incidence of reperfusion-induced arrhythmias was significantly lower in the diabetic hearts than in the controls, indicating that diabetic hearts exhibit greater resistance to ischemia/reperfusion.

REFERENCES


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