Insulin Treatment and Myocardial Function in Isolated, Perfused Heart from Diabetic Rat

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Difficulty of some patients to maintain adequate cardiac output following the termination of cardiopulmonary bypass remains a significant problem in cardiac surgery. The patients with diabetes mellitus frequently fail to respond to therapy after the cardiopulmonary bypass. However, little is known about the relationship between the control of diabetes mellitus and myocardial performance. The purpose of the present study was to look at the effect of diabetes and insulin treatment upon ventricular function and myocardial microcirculation in isolated perfused rat heart.

Experimental diabetes was induced by injecting streptozotocin and some of them were treated by insulin injection. Severe form of ischemia was induced in heart from acute form of diabetes and functional recovery was compared among the control, diabetic and insulin treated groups. In chronic form of diabetes, myocardial function and microcirculation which was measured by local H2 generation method were studied during aerobic perfusion and mild form of ischemic perfusion.

The hearts from experimental diabetes were more susceptible to ischemia and insulin pretreatment protected the functional alterations. This beneficial effect of insulin was associated with improved glucose and fatty acid metabolism. Myocardial microcirculation in hearts from diabetes was significantly less than in control, however, this was not correctable by the insulin treatment.

Since the advent of elective cardiac arrest, many investigators have studied the biochemical, structural and functional alterations to the heart that occur with prolonged exposure to ischemia. Reperfusion or reoxygenation itself have also been reported to cause tissue damage. Many studies have subsequently been made to prevent or reduce the tissue damage caused by ischemia or reperfusion. However, the difficulty of some patients to maintain adequate cardiac output following the termination of cardiopulmonary bypass remains a significant problem in cardiac surgery, despite advances in methods of myocardial preservation. Among these patients, diabetes mellitus is one of the major factors for the hearts to fail to respond to therapy after the cardiopulmonary bypass.

There are many evidences that the frequency and severity of coronary artery disease resulted from atherosclerotic process is increased in diabetic patients. Diabetes may also induce myocardial dysfunction independent of involvement of large vessel disease. Therefore, it turns out to be more important and difficult to control the patients with diabetes mellitus when they undergo an open heart surgery.

Insulin has been implicated as a beneficial therapeutic agent in ischemic hearts by a number

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Key Words:
Insulin
Streptozotocin-induced diabetes
Working heart preparation
Ischemia
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TABLE 1  EFFECT OF DIABETES AND INSULIN TREATMENT ON BODY WEIGHT, HEART WEIGHT, BLOOD GLUCOSE, TRIGLYCERIDE AND TOTAL CHOLESTEROL. HEBRTS WERE QUICKLY FROZEN BY WOLLENBERGER CLAMP AT THE END OF PERFUSION AND LYOPHILIZED. BLOOD WAS TAKEN JUST AFTER THE ISOLATION OF THE HEART.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Treated diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1.</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Body weight (BW)</td>
<td>g</td>
<td>292.0 ± 36.8</td>
<td>322.0 ± 17.6*</td>
</tr>
<tr>
<td>Dry heart weight (HW)</td>
<td>g</td>
<td>0.211 ± 0.029</td>
<td>0.231 ± 0.009***</td>
</tr>
<tr>
<td>HW/BW × 10³</td>
<td>mg/dl</td>
<td>0.72 ± 0.04</td>
<td>0.76 ± 0.02*</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>mg/dl</td>
<td>138.8 ± 4.5</td>
<td>324.5 ± 22.0****</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dl</td>
<td>44.5 ± 11.1</td>
<td>81.2 ± 13.2****</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>mg/dl</td>
<td>78.5 ± 8.1</td>
<td>38.0 ± 5.0***</td>
</tr>
<tr>
<td><strong>Experiment 2.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (BW)</td>
<td>g</td>
<td>437.1 ± 14.1</td>
<td>276.0 ± 43.9****</td>
</tr>
<tr>
<td>Dry heart weight (HW)</td>
<td>g</td>
<td>0.245 ± 0.028</td>
<td>0.173 ± 0.017**</td>
</tr>
<tr>
<td>HW/BW × 10³</td>
<td>mg/dl</td>
<td>0.561 ± 0.022</td>
<td>0.650 ± 0.043**</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>mg/dl</td>
<td>124.0 ± 16.4</td>
<td>306.8 ± 8.7****</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dl</td>
<td>64.5 ± 6.6</td>
<td>363.0 ± 100.4****</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>mg/dl</td>
<td>41.8 ± 1.9</td>
<td>74.7 ± 4.5***</td>
</tr>
</tbody>
</table>

* = Not significant vs Control; ** = p < 0.1 vs Control; *** = p < 0.05 vs Control; **** = p < 0.001 vs Control; † = Not significant vs Diabetic; †† = p < 0.1 vs Diabetic; † † † † = p < 0.01 vs Diabetic; † † † † † = p < 0.001 vs Diabetic.

of studies. It has also been found that insulin secretion is depressed during cardiac surgery.\(^5\) Recently, Muller et al reported two malignant form of depressed cardiac output following cardiopulmonary bypass in diabetics and bypass was successfully terminated shortly after the insulin injection.\(^9\) In these respect, it is of interest to investigate the role of insulin administration in experimental diabetic animals.

The purpose of the present study was to compare the effect of insulin treatment upon ventricular function in isolated, perfused heart from normal and diabetic rats. Myocardial microcirculation was also studied to determine if there were some abnormalities in myocardial small vessels.

The experiments were performed in the following sequence:

Experiment 1: Effects of short-term diabetes and insulin treatment upon ventricular function.

METHODS

1. Experimental groups

Three experimental groups were made as follows:

1) Control group: Male Sprague-Dawley rats, initially weighing between 250–300g were used as a control group.

2) Diabetic group: Experimental diabetes was induced by injecting streptozotocin (STZ, 50 mg/kg) given intravenously and sacrificed 3–7 days after injection.

3) Treated diabetic group: Some of the experimental diabetes were receiving insulin daily (Novo lente insulin, 4 u/rat) subcutaneously and sacrificed after 1 week.

2. Heart perfusion

The hearts from each group were removed and perfused as working heart preparation previously described techniques.\(^10,11\) In this preparation, the aorta and left atrial appendage

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were cannulated. The perfusate was modified Krebs-Henseleit bicarbonate buffer containing in molar: NaCl 118; KCl 47; NaHCO₃ 25; CaCl₂ 25; MgSO₄·H₂O 1.2; EDTA 0.5; KH₂PO₄ 1.2, glucose 11.1. The perfusate was gassed with 95% O₂: 5% CO₂ mixture throughout the perfusion.

The hearts were perfused by Langendorff preparation for 5 min and switched to working heart preparation with a left atrial filling pressure of 7 mmHg and a ventricular hydrostatic pressure afterload of 60 mmHg and perfused for 3 min. Then, the hearts were electrically paced at 333 beats/min for 2 min and whole heart ischemia was induced by use of a one-way aortic valve described by Neely et al. which prevented retrograde perfusion of the coronary arteries during diastole. Following 10 min of severe ischemia, the electrical pacer was turned off and perfusion was continued for an additional 20 min. Coronary effluent was collected from the heart chamber. Aortic pressures were recorded.

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RESULTS

1. Body weight, heart weight and blood chemistry. (Table I: upper panel)

   There was no significant difference among the three groups in body weight, however, heart weight in diabetic group was significantly higher than either control or treated diabetic group. The heart-body weight ratio in diabetic group was significantly higher than in treated diabetic group.

   The levels of blood glucose and serum triglyceride in diabetic group were significantly higher than in either control or treated diabetic group.

2. Ventricular function estimated by the rate-pressure product.

Figure 1 illustrates the ventricular function as estimated by the product of peak systolic pressure and heart rate as a function of time. During preischemic period, there was no significant difference among the three groups of hearts. With induction of ischemia they decreased rapidly to 10–30% of the preischemic level at the end of ischemia which was not statistically significant.

   During aerobic reperfusion, however, ventricular function recovered to almost 100% of the preischemic level in both control and treated diabetic group, whereas the recovery of ventricular function in diabetic group was 45% of the preischemic level, which was significantly less than control group.

3. Rate of coronary flow

   Figure 2 illustrates the rate of coronary flow as a function of time. There was no significant difference among the three groups of hearts either during preischemic period or at the end of ischemia. During aerobic reperfusion, the recovery of coronary flow in diabetic group was
not statistically significant compared with control group, however, the recovery of coronary flow in treated diabetic group was significantly higher than in diabetic group.

4. Recovery time
The mean time for hearts to resume spontaneous beating without electrical pacing are $3.3 \pm 1.6$, $10.2 \pm 4.4$ and $4.7 \pm 1.7$ min from the beginning of reperfusion in control, diabetic and treated diabetic groups respectively.

Experiment 2: Effect of long-term diabetes and insulin treatment upon ventricular function and myocardial microcirculation.

METHODS
1. Experimental groups
Three experimental groups were made as follows;

1) Control group: Male Spraque-Dawly rats initially weighing approximately 250g were used as an age-matched control.

2) Diabetic group: Experimental diabetes was induced by injecting STZ (50 mg/kg) given intravenously and sacrificed 10 weeks later.

3) Treated diabetic group: Some of the diabetic rats, whose diabetes was confirmed by high blood glucose (more than 280 mg/dl) and positive glucose urea at 5 weeks after STZ injection, were receiving insulin daily for another 5 weeks until they were studied.

2. Heart perfusion
The hearts were perfused by Langendorff preparation for 5 min and switched to working heart preparation with a preload of 7 mmHg and an afterload of 60 mmHg and perfused for 20 min (aerobic perfusion). Then, whole heart ischemia was induced by the same manner as
experiment 1 except no electrical pacing and perfusion was continued for an additional 20 min (ischemic perfusion).

Aortic output was measured by the magnetic flowmeter (Nihon Kohden MFV-2100) which was set at the aortic outflow tract. Cardiac output was calculated by the sum of the aortic output and coronary flow. Myocardial microcirculation was measured by local H₂ generation method (Biochemical Science, Model RBF-1) in which electrode made by teflon coated platinum iridium wire was inserted into the myocardium.

RESULTS

1. Body weight, heart weight and blood chemistry (Table 1, lower panel).

Lower panel in Table 1 shows the levels of body weight, heart weight, blood glucose, serum triglyceride and total cholesterol. Both body weight and heart weight in diabetic group were significantly lower than in control group. They increased in treated diabetic group compared with diabetic group, however, they are not statistically significant. In contrast, heart-body weight ratio increased significantly in diabetic group compared with control and decreased significantly in treated diabetic group compared with diabetic group respectively.

Blood glucose in diabetic group was significantly elevated compared with either control or treated diabetic group. Triglyceride and total cholesterol in diabetic group were also increased significantly in diabetic group compared with either control or treated diabetic group.

2. Ventricular function estimated by the rate-pressure product.

Ventricular function as estimated by the product of peak systolic pressure and heart rate as a function of time is shown in Fig. 3. Ventricular function in diabetic group was lower than in control group but was maintained throughout the aerobic perfusion. In contrast, it was somewhat higher in treated diabetic group.
compared with diabetic group.

With induction of ischemia, it decreased to 65.8% of the preischemic level at 5 min from ischemia and recovered slightly to 64.5% of the preischemic level at the end of ischemia in control group. However, it decreased to 30.4% of the preischemic level in diabetic group at the end of ischemia without any recovery of the function during the ischemic perfusion. The decreased rate of ventricular function in treated diabetic group was similar to the fall in ventricular function observed in control group and it was significantly higher than in diabetic group.

3. Coronary flow measured by right heart outflow

Figure 4 illustrates the rate of coronary flow with respect to the perfusion time. There was no significant difference among three groups of hearts during aerobic perfusion, however, the decreased rate in treated diabetic group was significantly lower than in diabetic group.

4. Cardiac output

Figure 5 illustrates the cardiac output which was calculated by the sum of aortic output and coronary flow. During aerobic perfusion, cardiac output in diabetic group was significantly lower than either in control or treated diabetic group. With induction of ischemia, they decreased quickly to less than 50% of the preischemic level at 5 min from ischemia in three groups of hearts. However, it decreased to 25.4% of the preischemic level in diabetic group which was significantly lower than either in control (35.3%) or treated diabetic groups (48.1%).

5. Microcirculation

Figure 6 illustrates the rate of myocardial microcirculation (microflow) measured by the local H2 generation method. The microflow during 10–20 min of aerobic perfusion was 96.97 ± 10.52 ml/100g/min in control and 69.0 ± 7.22 in treated diabetic group which was statistically significant, whereas it was 73.11 ± 9.0 in treated diabetic group. There was no significant difference in microflow between diabetic and treated diabetic group. With
Fig. 6. Myocardial microcirculation measured by local H2 generation method.
Data obtained from hearts shown in Fig. 3.
Open bars represent control group. Striped bars represent diabetic group and dotted bars represent treated diabetic group.

induction of ischemia, they decreased to 56.53 ± 9.07 ml/100 g/min in control and 28.60 ± 6.97 in diabetic group at 0–10 min from ischemia which was also statistically significant. However, microflow in treated diabetic group (34.18 ± 15.56) was not significantly different from that in diabetic group.

DISCUSSION

Myocardial ischemia has been studied extensively in recent years and a great deal of descriptive and mechanistic work has been accomplished. The recovery of mechanical function during aerobic reperfusion of ischemic hearts depends on the extent of damage that occurred during ischemic insult and depends on new damage during reperfusion itself. As the number of cases in cardiac surgery has increased, need for protection of the ischemic and reperfused myocardium would be urgent problem in open heart surgery. Use of cardioplegic solutions and hypothermia during ischemia and temporary use of various cardiotonic agents during reperfusion improve recovery of myocardial function.15,16 However, some patients fail to respond to therapy with inotropic agents following cardiopulmonary bypass. This depressed myocardial function is frequently observed in patients with diabetes mellitus.

A higher incidence of cardiac mortality in patients with diabetes mellitus has been attributed to coronary atherosclerosis. Partamian et al reported the high incidence of immediate mortality and poor prognosis among 205 diabetic patients after acute myocardial infarction.17 Kannel et al reported the higher incidence of congestive heart failure in diabetic patients than in non-diabetic controls.18 Recent clinical and experimental studies also reported the new concept with diabetic cardiomyopathy that developed independent of large vessel disease.5,6 Regan et al performed cardiac catheterization to diabetic patients and biopsied a sample of left ventricle.6 They concluded that a diffuse extracellular abnormality might be a basis for

cardiomyopathic features in diabetes. Factor and Sonnenblick proposed a possibility of microvascular hyperreactivity (spasm) in hypertensive-diabetic rat heart. In experimental field, the defect in cardiac performance with increased workload in isolated perfused hearts and altered mechanics in left ventricular papillary muscle were observed in diabetic rats. Feuvray et al reported that the severe form of ischemia resulted in a faster rate of ventricular failure in hearts from diabetic rat. These abnormalities which have been observed in both clinical and experimental field principally seem to be attributable to insulin deficiency.

In normal heart muscle, insulin is known to accelerates glucose transport into the cell and have a positive inotropic effect, and this effect is independent of its effect on glucose uptake. Øye and Sinclair observed an increased left ventricular systolic pressure after adding insulin without glucose in the isolated perfused rat heart. Lucchesi et al reported that insulin increased maximum tension development in the canine heart using pyruvate as a substrate. Insulin is also known to have an effect on ion fluxes that are independent of glucose.

These effects of insulin were conducted initially by Sodi-Parales et al to the therapeutic use for the patient with myocardial ischemia as a polarizing solution (GIK solution). Maroko and Brounwald et al reported the effect of GIK infusions on cardiac metabolism and morphologic changes following coronary artery ligation of the dog heart. They indicated that GIK treatment had a protective effect against myocardial necrosis. They pointed out that preservation of tissue glycogen stores which was mainly due to the action of insulin which stimulated the activity of the more active form of glycogen synthetase, could be responsible for maintenance of normal histology following GIK treatment. Opie et al reported the effect of GIK infusion on the cardiac metabolism after coronary artery ligation using adult baboons. They concluded that GIK infusion had a beneficial effect when compared with ligated group in lessening of severity of tissue metabolic damage in the infarcted areas.

Moreover, it was reported that insulin secretion was diminished in patient with cardiogenic shock complicating acute myocardial infarction and in patient with severe chronic congestive heart failure. It was also been found that insulin secretion is depressed during cardiac surgery. Moffitt et al reported the usefulness of insulin in open-heart surgery. Muller et al succeeded in terminate cardiopulmonary bypass shortly after the insulin injection in diabetic patient with diminished myocardial contractility. This clinical observation led to experiment and insulin treatment of heart during aerobic reperfusion following transient ischemia significantly improved the recovery of myocardial function.

Little is known, however, about the relation between the insulin treatment and myocardial function in diabetic patients. It is difficult to investigate it clinically since diabetes mellitus is a long-standing metabolic disorder where various complications exist. It is, therefore, of interest to study the effect of diabetes and insulin treatment upon ventricular function in experiment under the controlled conditions provided by the isolated, working rat heart preparation.

The data presented in “acute” diabetes suggest that the hearts from experimental diabetes were more susceptible to global ischemia and functional recovery was significantly depressed during aerobic reperfusion. However, the heart from insulin treated diabetic rat improved the recovery of myocardial function during reperfusion. This improvement was reflected both by a shorter time required for the hearts to resume spontaneous beating and by increased heart rate and peak systolic pressure after resumption of beating.

In “chronic” diabetes, the myocardial function was lower than control but maintained during aerobic perfusion. However, mild form of ischemia resulted in a faster rate of ventricular failure which was reflected by the lower rate of both cardiac output and rate-pressure product. Insulin treatment in this case, however, kept the myocardial function higher than in diabetic group during aerobic perfusion and protected the failure of myocardial function during ischemic perfusion.

The beneficial effect of pretreatment of diabetic rat, by either acute or chronic insulin administration, is much more likely to be related to improved metabolic factors, such as maintained blood glucose and reduced serum triglyceride. This assumption was supported by another experiment in which the hearts from diabetes were perfused with insulin added buffer. According to this, the effect of insulin administration was less effective than the heart from pretreated diabetic rat. In diabetic heart,
glycolysis is severely restricted, resulted in poor supply for the glucose substrate and high concentration of FFA has been reported to depress myocardial contractility or oxidative respiration. Thus these detrimental effects of diabetes appeared to be corrected by the insulin administration, which lead to the restoration of normal myocardial function.

Heart to body weight ratios in treated diabetic groups, either acute or chronic, were significantly decreased when compared with diabetic group. The mechanism of this alteration is unknown, however, it may be related to the functional recovery in treated diabetic group.

Myocardial microflow in "chronic" diabetes was significantly depressed compared with control heart during 20 min of aerobic perfusion. This suggest that the hearts from diabetic rat were exposed to relative myocardial ischemia even when they were supposed to be in "aerobic" condition. This result might be also consistent with diabetic cardiomyopathy. Insulin treatment, however, could not overcome the depressed microflow during either aerobic or ischemic perfusion, which suggest that alteration of myocardial microflow in heart from diabetes was irreversible change although depressed myocardial function was protected by insulin treatment.

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