Streptozotocin-Induced Non–Insulin-Dependent Diabetes Protects the Heart From Infarction

Yongge Liu, BS; Jon D. Thornton, PhD; Michael V. Cohen, MD; James M. Downey, PhD; Stephen W. Schaffer, PhD

**Background.** The vulnerability of the myocardium of a diabetic animal to an ischemic insult is controversial. To address this issue, streptozotocin-induced non–insulin-dependent diabetes (NIDD) was induced in rats, and the effects of regional myocardial ischemia were assessed by measuring infarct size.

**Methods and Results.** Open-chest rats with NIDD and age-matched control rats underwent 30 or 45 minutes of regional ischemia and 2-hour reperfusion. Infarct size was measured by tetrazolium. Control rats had 32.0±3.3% infarction of the risk zone after a 30-minute coronary occlusion, whereas NIDD rats had significantly smaller infarcts (11.5±3.1% of the risk area, P<.005). When ischemic time was extended to 45 minutes, infarct size in control animals averaged 57.9±6.2%, whereas only 37.3±5.6% of ischemic myocardium was infarcted in NIDD rats (P<.05). In a subset NIDD group, rats experienced a period of ischemic preconditioning (three cycles of 5-minute ischemia/5-minute reperfusion) before 45-minute ischemia. Infarct size in these rats averaged only 6.9±3.0% (P<.01 vs nonpreconditioned NIDD rats with 45-minute coronary occlusions). Collateral flow was measured in NIDD rat hearts with radioactive microspheres. Collateral flow was <1% of normal myocardial blood flow.

**Conclusions.** We conclude that NIDD protects the heart from infarction and that this protection is not related to the development of coronary collaterals. Furthermore, preconditioning can further protect the NIDD heart. *(Circulation. 1993;88:1273-1278.)*

**Key Words** • myocardial infarction • ischemia • preconditioning • coronary collaterals

Diabetes mellitus causes atherosclerosis of peripheral as well as coronary arteries and is a major risk factor for the development of coronary artery disease. Because of this association, it is not surprising that myocardial infarction is common in diabetes. In addition, studies in rats have shown a diabetes-induced cardiomyopathy in the absence of coexisting coronary atherosclerosis.1 However, it has not been possible to determine clinically whether this metabolic disorder has independent direct effects on the ability of the myocardium to tolerate ischemia.

To more carefully evaluate the possible cardiac consequences of the metabolic abnormalities of diabetes, animal models of chemically-induced diabetes have been developed. Previous studies with these models have shown that diabetic myocardium exhibits a variety of abnormalities that may influence ischemic injury, including depressed Na⁺-Ca²⁺ and Na⁺-H⁺ exchange activities,2,3 decreased sarcoplasmic reticular Ca²⁺ pump activity,4,5 and elevated antioxidant defenses.6 Furthermore, whereas diabetic myocardium is less responsive to β-adrenergic stimulation,5,7 it may be more responsive to α-adrenergic stimulation.8,9

The tolerance of diabetic myocardium to ischemia also has been examined. But the differing protocols and variety of end points of ischemic injury have yielded confusing data. Earlier work using glucose-perfused diabetic rat hearts and evaluating the degree of ischemic injury by either recovery of mechanical function or changes in energy metabolism concluded that diabetes had an adverse effect on the ischemic heart.10,11 Others have reported that ischemia-reperfusion injury is not significantly different in normal and diabetic animals,12 whereas the most recent studies suggest that diabetic hearts are more resistant to ischemia. Tani and Neely13 noted that diabetic rat hearts had better functional recovery after global ischemia than hearts from normal animals,13 and Kusama and his colleagues14 demonstrated that diabetic rat hearts are less susceptible to reperfusion-induced arrhythmias.

Diabetes can be subdivided into insulin-dependent (IDD) and non–insulin-dependent (NIDD) types, and the response of each variety to ischemia is not necessarily the same. Previous studies evaluating susceptibility of the diabetic heart to ischemia used a chemically induced IDD model and were performed in vitro.10-14 The end point of these studies was functional recovery or arrhythmias instead of infarction. The present study was designed to directly assess the ability of the diabetic heart to tolerate an ischemic insult in situ by measuring myocardial infarct size after regional ischemia in NIDD and age-matched control rats. Our data in this study indicate that hearts of NIDD animals are more resistant.

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to infarction than normal hearts and that ischemic preconditioning can further protect the myocardium of NIDD rats.

**Methods**

**Animals**

The rat model of chemically induced NIDD was produced as described previously. Briefly, male Wistar rats at 2 days of age were injected intraperitoneally with 90 mg/kg of streptozotocin dissolved in 0.1 mL of citrated buffer, pH 4.5. Control littermates received sham injections of the citrate buffer. These animals were maintained ad libitum on water and Purina rat chow. All NIDD rats and the age-matched control rats were studied at 11 to 12 months of age. Methods to measure plasma glucose and insulin have been previously reported.

**Surgical Preparation of Animals**

The rat was anesthetized with sodium pentobarbital (60 mg/kg IP), and the trachea was intubated through a tracheotomy. Mechanical ventilation was achieved with a positive-pressure respirator (MD Industries, Mobile, Ala) using 100% oxygen, a tidal volume of 8 mL, and a rate of approximately 50 breaths per minute. The respiratory rate was adjusted if necessary to keep the arterial blood pH within the normal range. The left carotid artery and jugular vein were cannulated for blood pressure monitoring and administration of additional anesthetic as required, respectively. The heart was exposed through a left thoracotomy in the third intercostal space, and the pericardium was opened. A 2-0 silk thread was passed around the left coronary artery close to its origin with a taper needle, and the ends of the suture were passed through a small vinyl tube to form a snare. In several NIDD rats, a catheter was inserted into the left atrium for injection of radioactive microspheres. The coronary artery was occluded by pulling the snare. Myocardial ischemia was confirmed by the appearance of regional cyanosis. Reperfusion was achieved by releasing the snare and confirmed by observation of visible hyperemia over the surface.

**Measurement of Infarct and Risk Zone**

At the end of each experiment, the heart was quickly removed and mounted on a Langendorff apparatus, where it was flushed with room-temperature saline for 1 minute. The snare was tightened, and a 0.5% suspension of 1-10 μm zinc-cadmium fluorescent particles (Duke Scientific, Palo Alto, Calif) was infused into the perfusate to mark the ischemic (risk) zone as the nonfluorescent tissue. The heart was weighed and frozen, then sliced into 2-mm thick sections. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in pH 7.4 buffer for 15 minutes. The areas of infarcted TTC-negative myocardium under white light and risk zone, nonfluorescent under UV light, were measured by planimetry for each slice. Infarct and risk zone volumes were then calculated by multiplying each area by the slice thickness and summing the products. Infarct size was expressed as the percentage of the risk zone which had infarcted.

**Experimental Protocols**

This study evaluated six groups of animals; the protocol is shown in Fig 1. The animals were not fasted and were randomly assigned to groups. Groups 1 and 2 consisted of age-matched normal and diabetic rats subjected to a 30-minute coronary artery occlusion followed by 120-minute reperfusion. Groups 3 and 4 were the same as groups 1 and 2 except that the ischemic time was extended to 45 minutes. In the fifth group, diabetic rats underwent ischemic preconditioning before the 45-minute ischemia with three cycles of 5-minute ischemia/5-minute reperfusion. In the sixth group, myocardial collateral blood flow was measured in diabetic rats.

**Statistics**

All results are expressed as group mean±SEM. The significance of differences was determined by a one-way ANOVA and Scheffe’s post hoc test. Significance was accepted at a value of P < .05.
Results

Heart and body weights were not different in NIDD rats and age-matched control rats when studied at 11 to 12 months of age (Table 1). Data in these animals demonstrate that the diabetic rats have normal fasting serum insulin (0.09±0.01 ng/dL in control rats and 0.07±0.01 ng/dL in NIDD rats) and glucose (108±7 mg/dL in control rats and 131±8 mg/dL in NIDD rats) levels but that NIDD rats were markedly intolerant of glucose loads. After an intraperitoneal load of 2 g glucose/kg, plasma glucose (sampled at 15, 30, 60, and 120 minutes after the challenge) peaked at 1 hour and was 521±18 mg/dL in diabetic rats, which was significantly higher than in control rats (221±21 mg/dL, P<.01). Table 2 shows that hemodynamic data were not different between groups. Table 1 also shows risk and infarct size data as well as percentage of the risk zone infarcted in each group. The risk area is not significantly different in any of the groups. As shown in Fig 2, 30 minutes of ischemia produced 32.0±3.3% infarction of the risk zone in five control rats but caused only 11.5±3.1% (P<.005) infarction in six NIDD rats. Because these infarcts in NIDD rats were so small, it would be exceedingly difficult to demonstrate potential salvage of ischemic tissue by ischemic preconditioning. Consequently, the time of ischemia was extended to 45 minutes. Infarct size was increased to 57.9±6.2% in five control rats. Although infarcts after 45 minutes of ischemia in seven diabetic rats (37.3±5.6%) were significantly larger than those in diabetic rats with only 30-minute coronary occlusions (P<.05), they were still significantly smaller than those in control animals with 45-minute coronary occlusions (P<.05). Three cycles of 5-minute ischemia/5-minute reperfusion before 45 minutes of ischemia further reduced infarction in five NIDD rats to 6.9±3.0% (P<.01 vs nonpreconditioned NIDD rats with 45-minute ischemia).

Coronary collateral flow was measured in three rats with NIDD. Collateral blood flow was 0.2%, 0.7%, and 0.9% of blood flow to normal myocardial tissue in the three animals and averaged 0.6±2.2%.

Discussion

Streptozotocin treatment of newborn rats results in a condition that has been widely accepted as a good model of non–insulin-dependent diabetes. 15,16 After streptozotocin administration, there is an initial decline in pancreatic insulin levels. However, unlike adult rats, the pancreas of the neonate is able to partially regenerate its β-cells. As a result, plasma insulin levels quickly return to the normal range. Yet, at this early stage in the development of the disease, glucose-mediated insulin secretion is impaired. Since the release of insulin by many other secretagogues is normal, 16 the animal at this age resembles the early stages of non–insulin-dependent diabetes. As the animal ages, it becomes increasingly glucose intolerant and insulin resistant. By 6 months of age, the animal exhibits hyperinsulinemia and hyperglycemia in response to a glucose challenge. 17 Like humans with non–insulin-dependent diabetes, the pancreas eventually loses its ability to maintain elevated levels of insulin secretion. 18 At some time between the ages of 6 and 12 months of age, a transition takes place and the animal becomes relatively insulinopenic compared with the nondiabetic. Nevertheless, as is characteristic of non–insulin-dependent diabetes, nonfasting plasma glucose levels remain <200 mg/dL, and there is no significant evidence of muscle wasting.

The present data demonstrate that NIDD protects the heart against myocardial necrosis and that this protection is not related to the development of collat-

Table 1. Risk Zone and Infarct Size Data in Non–Insulin-Dependent Diabetic Rats and Age-Matched Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Body wt (g)</th>
<th>Heart wt (g)</th>
<th>Risk zone (cm²)</th>
<th>Infarct size (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-Minute control</td>
<td>688±13</td>
<td>2.9±0.2</td>
<td>0.39±0.06</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>30-Minute NIDD</td>
<td>683±33</td>
<td>2.6±0.2</td>
<td>0.38±0.05</td>
<td>0.05±0.02*</td>
</tr>
<tr>
<td>45-Minute control</td>
<td>684±22</td>
<td>2.3±0.1</td>
<td>0.33±0.07</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td>45-Minute NIDD</td>
<td>628±34</td>
<td>2.3±0.1</td>
<td>0.43±0.02</td>
<td>0.16±0.03†</td>
</tr>
<tr>
<td>PC3+45-minute NIDD</td>
<td>610±23</td>
<td>2.1±0.1</td>
<td>0.38±0.06</td>
<td>0.02±0.01‡</td>
</tr>
</tbody>
</table>

NIDD indicates non–insulin-dependent diabetes; PC3, three cycles of preconditioning (5-minute ischemia/5-minute reperfusion).

*P<.005 vs 30-minute control; †P<.05 vs 45-minute control; ‡P<.01 vs 45-minute NIDD.

Table 2. Hemodynamic Data in Non–Insulin-Dependent Diabetic Rats and Age-Matched Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Preischemia</th>
<th>Ischemia*</th>
<th>Reperfusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBP (mm Hg)</td>
<td>HR (bpm)</td>
<td>MBP (mm Hg)</td>
</tr>
<tr>
<td>30-Minute control</td>
<td>74±13</td>
<td>336±23</td>
<td>74±10</td>
</tr>
<tr>
<td>30-Minute NIDD</td>
<td>73±13</td>
<td>334±20</td>
<td>80±10</td>
</tr>
<tr>
<td>45-Minute control</td>
<td>84±10</td>
<td>388±16</td>
<td>71±8</td>
</tr>
<tr>
<td>45-Minute NIDD</td>
<td>79±11</td>
<td>310±19</td>
<td>89±12</td>
</tr>
<tr>
<td>PC3+45-minute NIDD</td>
<td>81±9</td>
<td>334±15</td>
<td>78±12</td>
</tr>
</tbody>
</table>

MBP indicates mean arterial blood pressure; HR, heart rate; bpm, beats per minute; NIDD, non–insulin-dependent diabetes; PC3, three cycles of preconditioning (5-minute ischemia/5-minute reperfusion).

*Hemodynamic data during ischemia and reperfusion were measured at the end of ischemia and 60-minute reperfusion, respectively.
Animal studies on the tolerance of myocardium to ischemia in IDD models have provided contradictory data. In previous investigations, hyperglycemia characteristic of IDD was not a factor affecting outcome, since all of those studies were performed in vitro and therefore normal and diabetic hearts were perfused with the same buffer. In the present study, we used an NIDD rat model. Although this study was done in situ, we do not believe that hyperglycemia contributed to the outcome in these experiments either. Diabetes in these animals resembles NIDD in humans in that fasting insulin and blood glucose levels are normal and that nonfasting insulin and blood glucose levels are only slightly elevated. Moreover, because the ischemia is a no-flow type, myocardial glucose utilization in these animals will be negligible irrespective of blood glucose levels.

What then are the possible mechanisms of the cardioprotection observed in NIDD rats? Previous studies have shown that there are several factors that can influence myocardial infarction. Coronary collateral flow is an important determinant of the size of infarcts. From early studies of experimental myocardial infarction in dogs, it was found that the size of infarcts is inversely related to blood flow to the ischemic tissue. Since our study involved animals in which diabetes had been induced 11 to 12 months previously by streptozotocin injection, it was considered possible that sufficient coronary collaterals could have developed in our diabetic rats to affect infarct size despite the fact that normal rat hearts are poorly collateralized. However, our results indicate that collateral blood flow in NIDD rats is <1% of normal myocardial flow, a level that would be expected to have minimal or no influence on infarct size.

Oxygen free radicals can cause reperfusion-induced dysfunction and arrhythmias. It has been reported that IDD rats have higher catalase and glutathione reductase contents and are more resistant to oxidant stresses than control animals, perhaps accounting for the better mechanical functional recovery and lower incidence of arrhythmias after ischemia. However, because it has been difficult to show a role for free radicals in infarction, it seems unlikely that oxygen free radicals and changes in antioxidant enzyme activities have affected the present observation.

Another postulated mechanism of myocardial protection is production of stress proteins. This family of proteins can be synthesized in response to a variety of stimuli including heat stress and has been detected in heart tissue of several species. Elevated levels of stress proteins have been shown to provide protection against reperfusion-induced myocardial dysfunction. Whether the level of stress protein might be chronically elevated in NIDD rat heart and thus provide protection in this model needs further investigation.

It has been reported that sarcolemmal Na+/Ca^2+ exchange activity is suppressed in both IDD and NIDD rats. Depressed Na+/H+ exchange has also been shown in IDD rats. Drugs inhibiting Na+/H+ exchange activity appear to possess antiarrhythmic effects. Inhibition of Na+/H+ and Na+/Ca^2+ exchange activities may limit cellular Ca^2+ overloading. However, total calcium levels are elevated in rats with NIDD although variable in IDD rats. Thus the effect of calcium and depressed Na+/H+ and Na+/Ca^2+ exchange activities in this model remains to be determined.

Noradrenaline release during ischemia has been proposed to contribute to myocardial necrosis. Several studies have demonstrated that the response of diabetic hearts to β-adrenergic stimulation is reduced in both IDD and NIDD hearts. Therefore, it is possible that the diminished response of diabetic hearts to adrenergic stimuli could decrease energy consumption during ischemia and protect the myocardium. In a study by El-Hage et al., it was demonstrated that diabetic mice had a significant decrease in the severity of cardiac necrosis induced by the administration of isoproterenol. However, evidence that β-blockers or sympathectomy offer little protection against myocardial infarction does not support the hypothesis that attenuated β-adrenergic function might have accounted for the anti-infarct effect seen in the NIDD rats.

Hearts from IDD rats exhibit enhanced sensitivity to α-adrenoceptor stimulation. α-Adrenoceptor stimulation in ischemic myocardium is thought to promote adenosine release, which has cardioprotective effects. Endogenous adenosine release or exogenous adenosine administration can significantly reduce myocardial necrosis in some models. Several studies have demonstrated that activation of α-adrenergic receptors ameliorates the effects of ischemia by release of adenosine from myocardium. Hence, it is possible that increased sensitivity to α-adrenergic stimulation during ischemia may induce protection through increased adenosine release. Future studies can easily address this possibility.

The accumulation of lactate during ischemia has been associated with the development of cellular damage. Glycolysis and lactate production in these NIDD rats are lower than those in age-matched control rats under...
normoxia. Lac
tate accumulation in these NIDD animals might be expected to be lower during ischemia and possibly account for the protection observed. However, lactate accumulation during no-flow ischemia is dramatically affected by the rate of glycogenolysis and amount of glycogen stores, which are approximately the same in NIDD and nondiabetic hearts. Furthermore, Tani and Neely reported that at comparable levels of lactate and pH, hearts from IDD rats demonstrated greater func
tional recovery after ischemia than normal hearts. It is not clear whether this latter observation can be extrapolated to NIDD rats.

Our data show that preconditioning can reduce infarct size in NIDD rats. Preconditioning is a dramatic phenomenon that limits myocardial infarction as in normal hearts. Brief episodes of myocardial ischemia before a prolonged ischemic period actually cause less infarction than if only the prolonged ischemia had occurred. The mechanism of ischemic preconditioning is currently unknown. Although NIDD limits myocardial infarction by an amount comparable to that seen in preconditioned normal rats, ischemic preconditioning provided an added increment of protection. It is not clear whether the cellular changes resulting in protection in NIDD rats are amplified by superimposed ischemic preconditioning or whether the latter causes a further effect by an unrelated mechanism. Future investigations will help to distinguish between these two possibilities.

Conclusions

We have found that hearts from rats with streptozot
cin-induced non–insulin-dependent diabetes had less necrosis after coronary occlusion than age-matched control rat hearts. The mechanism of this protection is not yet apparent (and is not related to development of collaterals) but could offer important insights into the biochemical reactions that determine irreversibility of cellular damage. We can further reduce necrosis by preconditioning diabetic hearts.

Acknowledgments

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