Hearts From Diabetic Rats Are More Resistant to In Vitro Ischemia: Possible Role of Altered \( \text{Ca}^{2+} \) Metabolism

Masato Tani and James R. Neely

The effects of whole heart ischemia were studied in isolated perfused rat hearts from control and diabetic animals. When whole heart ischemia was maintained for 30 minutes at 37°C, diabetic hearts recovered 100% whereas hearts from normal animals recovered 30% of their preischemic function. Reperfusion \( \text{Ca}^{2+} \) uptake was about 2.5 \( \mu \text{M/g dry wt} \) in diabetic hearts compared with 10 \( \mu \text{M/g dry wt} \) in control hearts. When the ischemic period was extended to 40, 50, and 60 minutes, diabetic hearts had depressed recovery of ventricular function, and greater \( \text{Ca}^{2+} \) overload but reperfusion function was still significantly higher and \( \text{Ca}^{2+} \) overload significantly less than in control hearts. Depressed function and increased \( \text{Ca}^{2+} \) uptake were both linearly related to low tissue levels of residual high energy phosphates and inversely related to the amount of lactate that accumulated in the tissue during ischemia. However, regression lines relating these metabolic changes to depressed function and increased \( \text{Ca}^{2+} \) uptake showed that for any level of residual high energy phosphate or ischemic lactate, diabetic hearts performed much better and had less \( \text{Ca}^{2+} \) uptake than control hearts. These effects of diabetes were due to the diabetogenic action of the drugs used since both streptozotocin and alloxan had the same effect and in vivo insulin treatment reversed the effect. Diabetic hearts had a reduced maximum inotropic effect to increased extracellular \( \text{Ca}^{2+} \) under control aerobic perfusion conditions. The improved recovery of ventricular function during reperfusion of ischemic hearts from diabetic animals was highly correlated with reduced \( \text{Ca}^{2+} \) uptake, and regression lines relating depressed ventricular function to \( \text{Ca}^{2+} \) overload showed that data from control and diabetic hearts fell on the same line; that is, when depressed function occurred it was related to increased \( \text{Ca}^{2+} \) uptake to the same extent in both control and diabetic hearts. 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. The observations suggest a role of increased reperfusion \( \text{Ca}^{2+} \) influx in ischemic damage and that alterations of sarcolemmal \( \text{Ca}^{2+} \) transport systems in diabetic myocardium may account for the greater resistance of these hearts to ischemia. (Circulation Research 1988;62:931-940)

Many biochemical alterations contribute to irreversible cell injury during myocardial ischemia and reperfusion. Most, if not all, of these events are interrelated, making it difficult to distinguish between cause and effect. The most apparent alterations that precede ultrastructural evidence of damage are depletion of high-energy phosphates, loss of adenine nucleotides, accumulation of glycolytic and other metabolic products, and changes in tissue electrolytes.1-4. Shen and Jennings5,8 showed that massive amounts of \( \text{Ca}^{2+} \) accumulate in ischemic reperfused myocardium, and they, as well as many others, have suggested an association between \( \text{Ca}^{2+} \) accumulation and irreversible cell damage. As pointed out by Hearse et al,2 cell damage as a consequence of the calcium paradox, oxygen paradox, and reperfusion of ischemic myocardium have in common accumulation of cellular \( \text{Ca}^{2+} \). Death of cells that resulted from exposure to a variety of toxic compounds was associated with accelerated \( \text{Ca}^{2+} \) influx in each case.10 However, it is not clear if the alterations in cells that allow \( \text{Ca}^{2+} \) overload are simply associated with other changes that cause cell death or are themselves responsible for cell death by allowing \( \text{Ca}^{2+} \) overload. It has been proposed that increased cellular \( \text{Ca}^{2+} \) may be the essential common pathway that results in irreversible cell damage. Whether \( \text{Ca}^{2+} \) overload results from or is the cause of myocardial cellular injury due to ischemia may not be known, but calcium overload during reperfusion of ischemic hearts certainly is an indication of the presence of cell damage.

Hearts from diabetic animals are known to have a decreased inotropic response to increased extracellular \( \text{Ca}^{2+} \),11 decreased \( \text{Ca}^{2+} \) uptake in response to isoproterenol,12 and altered sarcolemmal \( \text{Na}^{+}, \text{K}^{+} \) ATPase or \( \text{Ca}^{2+} \) pump activities.13,14 With these modifications in \( \text{Ca}^{2+} \) metabolism by hearts of diabetic animals it might be expected that these hearts would respond differently to ischemia if \( \text{Ca}^{2+} \) is important in causing ischemic cell damage. Therefore, hearts from streptozotocin- and alloxan-induced diabetic rats were exposed to whole heart ischemia in vitro, and recovery of ventricular function, \( \text{Ca}^{2+} \) uptake, and tissue
metabolites were followed during 30 minutes of reperfusion.

Materials and Methods

Animals and Treatment

Male Sprague-Dawley rats weighing between 330–380 g were randomly separated into four groups. In one group, diabetes was induced by a single tail vein injection of streptozotocin (60 mg/kg; Sigma Chemical, St. Louis, Missouri) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). The second group of rats received a subcutaneous injection of protamine zinc insulin (9 units/kg; Eli Lilly, Indianapolis, Indiana) immediately after injection of streptozotocin and was maintained on the same dose of insulin daily for 2 days. The third group of rats received an injection of alloxan (60 mg/kg; Sigma) dissolved in citrate buffer. Control animals received a single tail vein injection of an equal volume of citrate buffer. All rats were maintained ad libitum on tap water and rat chow for 48 hours. Serum glucose was measured by a standard enzymatic method by the time the animals were killed between 8 A.M. and 5 P.M. on the day of use. Serum glucose levels were 10.1 ± 0.2, 32.0 ± 2.1, 59.6 ± 4.4, and 7.4 ± 0.8 mM for control, streptozotocin-induced diabetic, alloxan-induced diabetic, and insulin-treated diabetic rats, respectively. Diabetic rats with serum glucose levels more than 15 mM were excluded.

Heart Perfusion

Hearts were perfused by the Langendorff procedure as described earlier, except that ventilatory pressure was monitored by placing a plastic catheter with a small perforated ball tip into the left ventricle via the mitral valve. The catheter was filled with perfusate and connected to a Statham P23Db pressure transducer and the pressure was recorded on a polygraph recording system (model 79D, Grass Instruments, Quincy, Massachusetts). The perfusate was Krebs-Henseleit bicarbonate buffer containing 11 mM glucose and 5 mM pyruvate to support energy metabolism. The free Ca²⁺ concentration was generally 1.25 mM. In some experiments, the Ca²⁺ concentration was changed by adding increments of 12.5 μmol Ca²⁺ to 50 ml of circulating perfusate at a location distant to the heart to determine the inotropic response to increased extracellular Ca²⁺. The hearts received a 15-minute washout perfusion with buffer gassed with a 95:5 mixture of O₂:CO₂ at 60 mm Hg aortic perfusion pressure. Coronary flow rate was about 15 ml/min in each case during this washout period. The hearts then were switched to a recirculating perfusion with oxygenated buffer for 10 minutes at a coronary flow of 15 ml/min before inducing global ischemia by cross-clamping the aortic perfusion tube and reducing coronary flow to zero. Preischemic ventricular function was determined during this time by measuring ventricular pressure and heart rate. The hearts were maintained at 37°C at all times. Heart rate was maintained at 360 beats/min by electrical pacing for 5 minutes before and during ischemia. Preischemic function was determined without electrical pacing. After various periods of ischemia, the hearts were reperfused at a constant aortic pressure of 60 mm Hg by recirculating 50 ml of oxygenated buffer containing about 6 μCi of ⁴⁵Ca²⁺ (ICN Radiochemicals, Costa Mesa, California). Recovery of ventricular function was followed for 30 minutes. A sample of coronary effluent containing ⁴⁵Ca²⁺ was collected for 20 seconds at the end of the recovery period for estimation of extracellular ⁴⁵Ca²⁺ specific activities. After reperfusion, the hearts were switched to a perfusate free of ⁴⁵Ca²⁺ for 3 minutes to wash out any extracellular radioactivity and were then quick frozen with Wollenberger clamps cooled in liquid nitrogen. Other hearts were frozen at the end of the ischemic period for measurement of tissue metabolites.

Analytical Methods

Tissue levels of ATP, ADP, AMP, creatine phosphate (CP), and lactate were determined using neutralized perchloric acid extracts as described earlier by standard enzymatic procedures. For estimates of ⁴⁵Ca²⁺ uptake, aliquots of tissue extracts and coronary effluent were counted in a liquid scintillation counter (model LS3801, Beckman Instruments, Fullerton, California). Ventricular function was assessed by measuring systolic and diastolic pressures and heart rate. The percentage of recovery of ventricular function was calculated from the product of developed pressure (systolic minus diastolic pressures) and heart rate measured after 30 minutes of reperfusion and during the preischemic perfusion.

Statistical Analysis

The results are presented as mean ± SEM. Statistical analysis was carried out by unpaired Student’s t tests to assess the data in each group. Analysis of variance was also used to assess statistical significance between groups. The probability was considered significant if less than 0.05. To assess the relation between two variables, linear or logarithmic regression lines were obtained by the least-squares method.

Results

Several previous studies have suggested that hearts removed from diabetic animals may have altered mechanical function and Ca²⁺ metabolism. However, the exact response may depend on the duration and severity of diabetes. Thus, we characterized the effects of 48-hour drug-induced diabetes on the functional response to extracellular Ca²⁺ of well-oxygenated hearts. Although chronic exposure to diabetes reduces heart rate, after 48 hours of diabetes heart rate was not significantly reduced (Figure 1A) in agreement with earlier studies. However, the developed pressure increased less in diabetic hearts as extracellular Ca²⁺ was elevated (Figure 1B), and the maximum inotropic response to Ca²⁺ was significantly lower in well-oxygenated hearts from diabetic animals.
Since developed ventricular pressure and heart rate were essentially the same in control and diabetic hearts exposed to lower Ca^{2+} concentrations, we chose the physiological concentration of 1.25 mM for determining the response of diabetic hearts to ischemia to avoid any change in preischemic function. It is well known that agents that reduce cardiac function prior to ischemia (high K^+, Ca^{2+} antagonist, etc.) provide some protection during a subsequent exposure to ischemia.

Effects of Various Periods of Ischemia on Reperfusion Recovery of Ventricular Function in Hearts From Normal and Diabetic Animals

Hearts from normal and diabetic rats were exposed to various periods of zero flow ischemia and then reperfused for 30 minutes with oxygenated buffer containing Ca^{45}. Recovery of ventricular function was significantly better in hearts from diabetic animals (Figures 2A and 2B). After exposure to 30 minutes of global ischemia, hearts from control animals had severely depressed developed ventricular pressure and heart rate. In comparison, hearts from diabetic animals exposed to 30 minutes of ischemia developed a much better ventricular pressure during recovery and heart rate was significantly higher. After exposure to longer periods of ischemia, function was more severely depressed in both groups but recovery of ventricular function was still significantly higher in the hearts from diabetic animals even after 50 minutes of ischemia. The percentage of recovery of the product of heart rate and developed pressure was higher in the diabetic hearts at all times (Figure 2C). These data indicate that the hearts from diabetic animals are more resistant to global ischemia as shown by their ability to recover mechanical function upon reperfusion. In addition, cellular uptake of Ca^{45} during reperfusion of diabetic hearts was only 20% of the control after 30 minutes of ischemia and was greatly reduced after all periods of ischemia (Figure 2D).

From a metabolic standpoint, it is difficult to explain the improved resistance of diabetic hearts to ischemia (Table 1). Tissue levels of high energy phosphates and lactate were the same as control hearts prior to ischemia and after exposure to ischemia levels of adenine nucleotides, CP and lactate were not significantly different. During reperfusion after 30 minutes of ischemia, the diabetic hearts recovered somewhat higher levels of ATP and CP than did the control hearts but at all other ischemic times, recovery of high energy phosphates were about the same.

To rule out any direct effects of drugs, one group of animals were made diabetic with alloxan rather than streptozotocin (Table 2). When exposed to 30 minutes of global ischemia, these hearts recovered 100% of their preischemic ventricular function similar to the hearts from streptozotocin-treated animals. Recovery of metabolites was similar, and reperfusion Ca^{45} uptake was as low as in the hearts from streptozotocin-treated animals. Also, when the streptozotocin diabetic animals were treated with insulin for 2 days prior to removal of the heart, the response to exposure to 30 minutes of ischemia was similar to that of hearts from untreated, control animals (Table 3). Recovery of ventricular function was only 40% of the preischemic function and Ca^{45} uptake was greatly increased in comparison to the non-insulin-treated diabetic and was similar to control hearts. These data indicate that the protective effect of diabetes is not related to the drug used to induce diabetes but rather to the diabetic state itself.
To assess the relation between residual metabolites after reperfusion and the recovery of mechanical function, the percentage of recovery of ventricular function was plotted against levels of ATP and CP (Figures 3A and 3B). Residual ATP or CP levels at the end of 30 minutes reperfusion correlated fairly well with the percentage of recovery of ventricular function in each group, but very different regression lines were obtained for control and diabetic hearts; that is, for the relation between ATP and the percentage of recovery, regression lines were $Y=4.87X-9.07$ ($r=0.76$) in control hearts and $Y=11.61X-36.84$ ($r=0.88$) in diabetic hearts, and for the relations between CP and the percentage of recovery, regression lines were $Y=2.31X-11.47$ ($r=0.71$) in control hearts and $Y=4.68X-29.89$ ($r=0.90$) in diabetic hearts. These results suggest that the greater percentage of recovery of ventricular function in diabetic hearts was not due to better preservation of high energy phosphates during ischemia since function was greater in hearts from diabetic animals than that in hearts from control animals at any level of ATP or CP.

Accumulation of glycolytic products during ischemia (lactate, H+) has been implicated as a condition
TABLE 1. Effects of Global Ischemia and Reperfusion on Metabolites of Control and Streptozotocin-Induced Diabetic Hearts

<table>
<thead>
<tr>
<th>Time of ischemia (min)</th>
<th>Reperfused</th>
<th>ATP (μmol/g dry wt)</th>
<th>ADP (μmol/g dry wt)</th>
<th>AMP (μmol/g dry wt)</th>
<th>CP (μmol/g dry wt)</th>
<th>Lactate (μmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+ Control</td>
<td>20.5 ±0.8</td>
<td>34.1 ±0.8</td>
<td>2.5 ±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Diabetic</td>
<td>21.2 ±0.4</td>
<td>30.1 ±1.5*</td>
<td>3.6 ±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>- Control</td>
<td>5.7 ±0.5</td>
<td>8.2 ±0.06</td>
<td>5.6 ±0.1†</td>
<td>4.5 ±0.5</td>
<td>181 ±10</td>
</tr>
<tr>
<td></td>
<td>- Diabetic</td>
<td>5.5 ±0.6</td>
<td>8.0 ±0.2</td>
<td>4.2 ±0.05</td>
<td>5.0 ±0.3</td>
<td>189 ±4</td>
</tr>
<tr>
<td>40</td>
<td>+ Control</td>
<td>7.6 ±0.5</td>
<td>19.3 ±1.3</td>
<td>6.8 ±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Diabetic</td>
<td>13.4 ±0.3†</td>
<td>32.8 ±1.6†</td>
<td>4.0 ±0.3†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>- Control</td>
<td>4.9 ±0.6</td>
<td>8.1 ±0.8</td>
<td>9.2 ±0.5</td>
<td>4.9 ±0.4</td>
<td>227 ±16</td>
</tr>
<tr>
<td></td>
<td>- Diabetic</td>
<td>3.6 ±0.2</td>
<td>6.0 ±0.3*</td>
<td>7.1 ±0.5*</td>
<td>3.1 ±0.3†</td>
<td>244 ±6</td>
</tr>
<tr>
<td>60</td>
<td>+ Control</td>
<td>6.4 ±0.1</td>
<td>15.2 ±0.5</td>
<td>9.6 ±1.1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>+ Diabetic</td>
<td>7.9 ±0.2†</td>
<td>17.6 ±0.6*</td>
<td>5.5 ±1.9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>- Control</td>
<td>2.9 ±0.3</td>
<td>5.1 ±0.2</td>
<td>8.5 ±0.2</td>
<td>3.2 ±0.3</td>
<td>235 ±11</td>
</tr>
<tr>
<td></td>
<td>- Diabetic</td>
<td>3.2 ±0.1</td>
<td>5.5 ±0.1</td>
<td>7.1 ±0.5*</td>
<td>3.3 ±0.3</td>
<td>253 ±12</td>
</tr>
<tr>
<td>80</td>
<td>+ Control</td>
<td>4.5 ±0.2</td>
<td>9.8 ±0.7</td>
<td>20 ±2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Diabetic</td>
<td>5.5 ±0.4</td>
<td>13.7 ±0.9†</td>
<td>17 ±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>- Diabetic</td>
<td>2.3 ±0.3</td>
<td>5.0 ±0.1</td>
<td>7.5 ±0.1</td>
<td>2.4 ±0.6</td>
<td>267 ±5.4</td>
</tr>
<tr>
<td></td>
<td>+ Diabetic</td>
<td>4.5 ±0.4</td>
<td>9.3 ±0.8</td>
<td>20 ±4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CP, creatine phosphate. All data are expressed in μmol/g dry wt of tissue ± SEM for 4–12 hearts. Statistical analysis was made between control and diabetic heart using a one-way analysis of variance.

*P<0.05; †P<0.001; ‡P<0.025.

that accelerates development of irreversible damage. This effect of metabolic products was independent of residual adenine nucleotide levels.5 Thus, the percentage of recovery of ventricular function during reperfusion was plotted against the lactate that accumulated during ischemia (Figure 4). Mean values of lactate and function for each group were used to calculate regression lines because the data for lactate were obtained on hearts frozen at the end of ischemia and function was assessed on different hearts during reperfusion. As reported previously, there was a good negative correlation between tissue lactate during ischemia and recovery of function during reperfusion in control5 and also in diabetic hearts. However, the regression line for diabetic hearts was different than the line for control or insulin-treated diabetic hearts. (In diabetic hearts: Y=0.96X+276.57, r=0.97; in control or insulin-treated hearts: Y=0.52X+133.29, r=0.90.) These data confirm the previous report(s) that high levels of lactate during ischemia are associated with the time-dependent damage that occurs, but since diabetic hearts had better functional recovery at all levels of lactate, lactate may not be the primary cause of cellular damage. On the other hand, the greater recovery of function in diabetic hearts at any level of lactate may simply mean that the time-dependent alterations that occur in response to high lactate in control hearts are not occurring in diabetic hearts at the same rate.

Since damage to contractile function is closely associated with reperfusion Ca2+ uptake in hearts

TABLE 2. Effects of Global Ischemia and Reperfusion on Metabolites and Hemodynamics of Alloxan-Induced Diabetic Heart

<table>
<thead>
<tr>
<th>A. Metabolites and Ca2+ uptake (μmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>12.8 ±0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Ventricular function</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP (mm Hg)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Before ischemia</td>
</tr>
<tr>
<td>After reperfusion</td>
</tr>
</tbody>
</table>

LVSP, left ventricular systemic pressure; LVEDP, left ventricular end-diastolic pressure; DP, developed pressure (i.e., the difference between systolic and end-diastolic pressure); HR, heart rate.

Hearts were exposed to ischemia for 30 minutes and reperfused for 30 minutes. Metabolic data are expressed in μmol/g dry wt of tissue. These data were compared with the data of streptozotocin-induced diabetic hearts presented in Table 1 and the figures using a one-way analysis of variance. Differences between streptozotocin- and alloxan-induced diabetic hearts were not significant in all parameters. All data are mean ± SEM for six hearts.
from normal animals, the percentage of recovery of ventricular function was plotted against reperfusion $^{45}$Ca$^{2+}$ uptake for all hearts in the present study (Figure 5). Hearts from both normal and diabetic animals took up 0.8 ± 0.1 µmol/g dry wt of $^{45}$Ca$^{2+}$ during 30 minutes of control, aerobic perfusion. With 30 minutes of reperfusion of ischemic hearts, on the other hand, $^{45}$Ca$^{2+}$ uptake was accelerated and the rate depended on the time of exposure to ischemia (Figure 2D) and on whether the animals had been normal or diabetic. However, there was a good correlation between Ca$^{2+}$ uptake and the degree of reduction in reperfusion ventricular function, whether or not the animals had been diabetic, diabetic treated with insulin, or normal (Figure 5, $Y = 434.18 - 296.48 \ln X$, $r = 0.92$). Although it took a longer time of exposure to ischemia to produce the same degree of damage to contractile function in the hearts from diabetic animals, when...
functional damage was equivalent to that in control hearts so was "Ca" uptake. These data suggest that the resistance of diabetic hearts to damage by ischemia is associated with a lower rate of Ca" influx into the cells during reperfusion.

The percentage of recovery of ventricular function was calculated from the preischemic and postischemic product of developed pressure and heart rate. Recovery of both of these parameters decreased with the duration of ischemia but less so in hearts from diabetic rats (Figure 2). Since developed pressure was calculated as the difference between end-diastolic and peak-systolic pressures, recovery of these individual pressures were plotted against "Ca" uptake during reperfusion for all hearts.

Regression lines were calculated using the mean values for lactate and recovery rather than values for individual hearts.
FIGURE 6. Relations between left ventricular systolic or end-diastolic pressure and reperfusion "Ca 2+ uptake. Although left ventricular systolic pressure stayed about 60-70 mm Hg, end-diastolic pressure increased progressively in relation to an increase in reperfusion "Ca 2+ uptake. The data are mean values for groups of control and diabetic hearts.

groups of hearts (Figure 6). It is clear that the major effect of ischemia on reperfusion ventricular function is an increase in the diastolic pressure and that diastolic pressure is related to the rate of reperfusion Ca 2+ uptake.

Discussion

Diabetes is known to have several effects on cardiac function and metabolism. Myocardial contractility is reduced in association with a shift in myosin isozymes from predominantly V1 to V2.19,20,22 Muscle relaxation as estimated from a decrease in negative dP/dt is slower22 in association with a decrease in sarcoplasmic reticular Ca 2+, ATPase.23,25 Metabolically, the decrease in glucose transport is probably the best known effect.37 However, many other changes occur such as decreased phosphofructokinase activity,28,29 decreased glycogen synthetase,30 inactivation of pyruvate dehydrogenase,31 decreased transport of pantothenic acid,31 etc. Metabolism of Ca 2+ is modified in chronic diabetes. A decrease in extracellular Ca 2+ resulted in a greater reduction in ventricular function in isolated perfused hearts from diabetic animals.11 These data indicate that there was a decrease in the K act for influx of the Ca 2+ responsible for contractile force and that higher saturating concentrations of Ca 2+ were needed for maximum inotropic effects. The results presented in the present study show a decrease in V max that resulted in lower contractile force at high Ca 2+ concentrations. The Ca 2+ pump activity of sarclemmal membrane vesicles is depressed,4,32 and the Na+ -Ca 2+ exchange in sarclemmal vesicles was reduced.35 Thus, diabetes results in alterations of several apparently diverse biochemical processes in cardiac muscle. The exact mechanisms of these changes are not known but most cannot be reversed in vitro by acute treatment with insulin, although many are reversed by long-term insulin treatment in vivo.

An equally complex, but different, series of alterations occurs in hearts exposed to ischemia. A reduction in coronary flow has the immediate effect of reduced oxygen supply and decreased oxidative metabolism. A rapid reduction in CP with a slower decline in ATP results. Myocardial contractility is rapidly depressed. Many metabolic products accumulate in the tissue secondary to slow washout of vascular spaces and decreased oxidative metabolism. Some of these, such as CO 2, H +, and inorganic phosphate, accumulate rapidly while others, such as lactate and degradation products of ATP, increase more slowly. The ischemic tissue then goes through a graded, time-dependent transition from reversal to irreversible damage.19 The biochemical mechanisms of this transition are not known, and because of the complexity of the changes that occur, it is difficult to determine the exact early events that initiate the time-dependent alterations that eventually result in irreversible damage. In addition, the cellular structures and processes that these initial events act upon to cause cellular damage is even more difficult to determine because several processes are modified simultaneously.

From the present study, the perplexing question is how one very complex disease, diabetes, can result in the protection of the heart from a second very complex pathological condition, ischemia. A common process must be affected in both conditions but in opposite directions. One might think that this common pathway relates to carbohydrate metabolism since it is greatly modified in both conditions. During ischemia, the change from aerobic to anaerobic conditions results in the conversion of glycolytically produced pyruvate to lactate, which accumulates in the cells.38 The slow buildup of lactate increases cytosolic reduced nicotinamide dinucleotide and H +, which eventually inhibits glycolysis28 but not until lactate concentrations reach
40 mM or higher. The accumulation of lactate has been associated with development of cellular damage, and reducing lactate accumulation by prior glycogen depletion, inhibition of glycolysis, or maintenance of low washout coronary flow prolonged the onset of irreversible ischemic damage. Since glucose transport and phosphofructokinase are known to be inhibited in diabetic hearts, lower production of lactate during ischemia might provide protection. However, lactate accumulated to levels as high or higher in diabetic as in normal hearts during ischemia. This is probably due to higher intracellular levels of glycogen in the diabetic hearts. At any rate, hearts from diabetic animals were more resistant to the damaging effects of ischemia at equally high levels of lactate and presumably equally low pH.

Calcium metabolism is another common process affected by both diabetes and ischemia and, to some extent at least, in opposite directions. Calcium influx via the slow channels and Na^+-Ca^2+ exchange may be reduced in diabetic myocardium. In the present study, the protection afforded the heart during ischemia by diabetes was certainly associated with a reduced rate of Ca^2+ uptake during reperfusion following ischemia. Ischemic damage did occur in the hearts from diabetic animals to the same extent as in hearts from normal animals but was delayed several minutes. When contractile function did deteriorate, Ca^2+ influx increased in proportion to depressed function in both control and diabetic hearts. These observations point to the interesting possibility that modifications of Ca^2+ channels or antiport systems in the sarcolemmal membrane during diabetes may make these processes more resistant to alterations that occur in ischemic myocardium that result in Ca^2+ overload with reperfusion. A diabetic-induced reduction in slow channel Ca^2+ influx may not account for this protection since treatment of the heart with Ca^2+-channel blockers at the time of reperfusion does not lower Ca^2+ overload or improve recovery of function. However, a decrease in the Na^+-Ca^2+ exchange might well account for the observed effect of diabetes. If myocytes are overloaded with Na^+ during ischemia, the presence of high intracellular Na^+ may promote extra Ca^2+ uptake during reperfusion. A reduced activity of this exchange might require a higher concentration of intracellular Na^+ and, therefore, longer exposure to ischemia to promote damaging rates of Ca^2+ influx with reperfusion. Although this hypothesis is based primarily on speculation, it provides some direction for future investigations using the heart of diabetic animals as a tool that may help identify the primary causes of irreversible damage to ischemic myocardium.

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