Effects of Ischemia on Rat Myocardial Function and Metabolism in Diabetes

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SUMMARY   The function and metabolism of hearts removed from diabetic rats and subsequently perfused in vitro were investigated. Diabetic hearts perfused under aerobic conditions had higher tissue levels of total CoA and long-chain acyl-CoA, lower levels of total carnitine, but higher levels of long-chain acyl carnitine esters, and used glucose at slower rates than did normal hearts. At low levels of cardiac work, mechanical function of the diabetic hearts was not significantly different than normal hearts for perfusion periods of up to 65 minutes. A mild form of whole heart ischemia (i.e., a 50% reduction in coronary flow) was tolerated just as well by hearts from diabetic rats as by those from normal rats. This degree of ischemia accelerated glucose use in normal but not in diabetic hearts. Mild ischemia resulted in increased tissue levels of acyl esters of CoA and carnitine in both normal and diabetic hearts, but the rise was greater in the diabetic tissue. A more severe form of ischemia resulted in a faster rate of ventricular failure in hearts from both normal and diabetic rats, but the rate of failure was fastest in the diabetic hearts. This earlier mechanical failure in diabetic tissue was associated with a more rapid rise in tissue long-chain acyl-CoA and acyl carnitine esters. Tissue K^+ was lost during ischemic perfusion to about the same extent in both normal and diabetic hearts. Thus, early pump failure of diabetic hearts in response to ischemia was not associated with a greater loss of cellular K^+. Circ Res 44: 322-329, 1979

DIABETES results in a number of diverse alterations in cardiac metabolism. The most noted change is decreased glucose transport into muscle cells (Park et al., 1968; Morgan et al., 1961). Transport of other compounds such as amino acids (Morgan and Neely, 1972) and ions (Zierler, 1972) may also be affected. Alterations of intracellular metabolism include increased synthesis and tissue levels of glycogen (Neely and Morgan, 1974), increased tissue levels of lipids (Denton and Randle, 1967), and net degradation of proteins as a result of altered rates of both synthesis and degradation of proteins as a result of altered rates of both synthesis and degradation (Rannels et al., 1975; Rannels, et al., 1977). Some of these effects have been attributed to the direct action of insulin on membrane function (i.e., glucose transport), whereas others may result indirectly from altered myocardial and whole body metabolism. For example, changes in glycogen, fatty acid, and lipid metabolism may occur in large part as a consequence of increased plasma levels of fatty acids and ketone bodies (Randle et al., 1966).

In hearts removed from diabetic rats, rates of glucose utilization are low, and oxidation of fatty

acids supports a greater portion of total energy metabolism (Randle et al., 1966). These hearts also have higher tissue levels of long-chain acyl-CoA which persist through several minutes of in vitro perfusion in the absence of exogenous fatty acid (Garland and Randle, 1964). Presumably, this higher level of acyl-CoA represents only a greater fractional conversion of the total CoA to the acyl ester due to increased supply of plasma fatty acids in vivo and to increased release of fatty acids from triglycerides in vitro (Randle et al., 1966). However, changes in tissue levels of CoA have not been ruled out. Both CoA and carnitine are essential cofactors for fatty acid metabolism, and carnitine levels have been shown to increase in livers of diabetic animals (McGarry and Foster, 1976, 1977). The rise in liver carnitine is associated with increased oxidation of fatty acid to ketone bodies and decreased conversion of fatty acids to triglycerides. Altered tissue levels of total CoA and carnitine in hearts of diabetic animals may be associated with the altered rates of fatty acid metabolism in this tissue.

Whether alterations in myocardial metabolism in diabetics have any direct effects on cardiac function is not clear. Hearts from diabetic rats could not resist anoxic perfusion in vitro as well as normal hearts (Hearse et al., 1975). The decreased ability to recover function after anoxic perfusion was associated with the inability to use glucose. Long-term diabetes in dogs has been reported to increase ventricular stiffness (Regan, 1975), and these hearts showed greater reductions in work output in response to regional ischemia (Haider et al., 1977).

The purpose of the present study was to deter-

mine the functional and metabolic response of hearts from diabetic rats to global ischemia in vitro.

Methods

Male Sprague-Dawley rats weighing between 200 and 250 g were used. Severe diabetes was induced by iv injection of alloxan (60 mg/kg body weight) in the femoral vein. Hearts were removed from these rats 48 hours after alloxan injection. To rule out direct toxic effects of alloxan, one group of rats was maintained on insulin for 4 days following alloxan injection, and the insulin treatment was terminated 48 hours before the animals were killed. Function and metabolism of the hearts from these rats were no different than those removed 48 hours after alloxan injection without insulin treatment. In another group of rats used to determine myocardial levels of total CoA and carnitine, a less severe form of diabetes was induced by injection of alloxan, 37.5 mg/kg, and these rats were killed at the times indicated in the tables. Diabetes was confirmed in each rat by measuring serum glucose at the time it was killed. Serum glucose was 8 ± 0.3 and 36 ± 4 mM for control and diabetic rats, respectively, in the fed state. Diabetic rats with serum glucose less than 20 mM were not used.

Heart Perfusion

Hearts were removed from the rats and perfused by the working heart technique as described earlier (Neely et al., 1967). The perfusate was Krebs bicarbonate buffer gassed with a 95% O_2–5% CO_2 mixture and containing 11 mM glucose. In each case, the perfusate passed through the heart only once. Global ischemia was induced by use of a one-way ball valve in the aortic outflow tract as described earlier (Neely et al., 1973). Ventricular function was assessed by measuring aortic pressure and heart rate. The product of peak systolic pressure and heart rate was calculated as an estimate of total cardiac pressure developed per minute.

Analytical Methods

Rates of glucose utilization were determined by measuring ^3H_2O production from [2-^{3}H]glucose (Neely et al., 1972). Tissue levels of CoA and carnitine and their long-chain acyl derivatives were determined as described in *Methods of Enzymology* (Lowenstein, 1969). Tissue K^+ was determined on 3% wt/vol NaCl extracts using a Perkin-Elmer model 360 atomic absorption spectrophotometer.

Results

Effects of Diabetes on Ventricular Function and Glucose Utilization

It is well known that hearts isolated from diabetic rats have decreased rates of glucose transport and glycolysis and that they use endogenous stores of triglycerides to support mechanical function during

short-term perfusion (15 minutes). In the present study, ventricular function and glucose utilization were compared in normal and diabetic hearts (Fig. 1). Hearts from both groups maintained low levels of mechanical function equally well. Mechanical function was maintained in spite of a greatly reduced rate of glucose utilization in the diabetic hearts, indicating that oxidation of endogenous lipids was at least adequate for energy needs at low levels of work output.

Effects of Ischemia on Ventricular Function and Glucose Use in Normal and Diabetic Hearts

It was of interest to determine if alterations in metabolism made the diabetic heart more susceptible to global ischemia. In the working heart preparation, it is possible to reduce coronary flow with a one-way aortic valve which reduces coronary diastolic perfusion (Neely et al., 1973). Peak systolic pressure is unaltered initially. If the hearts are not electrically paced, coronary flow decreases by 50–60% and O_2 supply is initially decreased by about 30%. In the normal heart, peak pressure is maintained for up to 1 hour, but heart rate decreases. The lower heart rate results in a reduction in energy consumption to match the reduced supply of oxygen. This condition is referred to below as mild ischemia. If the hearts are electrically paced at 300 beats/min, energy utilization is not allowed to de-
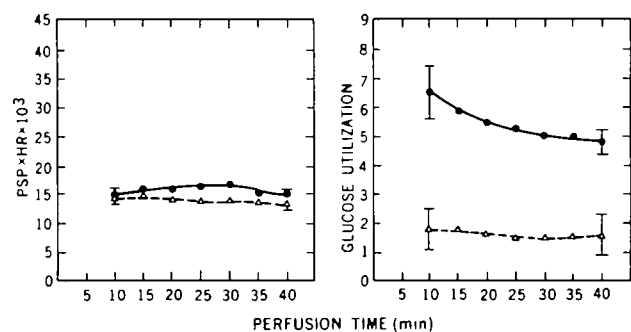

FIGURE 1   *Effects of mild diabetes on ventricular function and glucose use at low levels of cardiac work. Hearts were removed from rats that had received alloxan, 37.5 mg/kg, 7 days prior to the experiment. At the time the rats were killed, serum glucose averaged 8 ± 0.5 and 26 ± 3 mM in control and diabetic rats, respectively, in the fed state. The perfusate contained glucose (11 mM). Each heart received a 10-minute washout perfusion prior to beginning the experiment. Ventricular pressure development was adjusted to 60 mm Hg at zero time. Total ventricular function was estimated as the product of peak systolic pressure (PS) and heart rate (HR) and is expressed as mm Hg/min × 10^3. Glucose utilization is expressed as μmoles glucose/g dry per min. Data for control hearts are shown by solid lines and for diabetic hearts by dashed lines. Values are mean ± SEM for six hearts in each group.*

crease by a slower heart rate after ischemia is induced, and ventricular failure is reflected by a decrease in pressure development. The decrease in pressure reduces coronary flow further and function continues to deteriorate. This condition is referred to below as severe ischemia. The response of normal and diabetic hearts to these two types of ischemia was investigated.

When coronary flow was reduced after 5 minutes of control perfusion in unpaced hearts, decreased rate in the normal heart resulted in a decrease in the product of peak pressure and rate (Fig. 2A). Function was well maintained at this reduced level for an additional 35 minutes. The decrease in function between 35 and 65 minutes was due to a decrease in both heart rate and peak pressure. The diabetic heart, in contrast, had a somewhat higher rate of coronary flow and a lower level of work during the control perfusion (0–5 minutes). Ventric-

ular function was less affected by the reduction in coronary flow. The difference in flow between diabetic and normal hearts was evident throughout 60 minutes of ischemic perfusion. Thus the initial reduction in O_2 supply in relation to work output was less in the diabetic heart, which may account for the lack of a decrease in function. As reported earlier (Neely et al., 1975), a reduction in coronary flow of this magnitude resulted in accelerated rates of glycolysis similar to those shown in Figure 2A. The rate of glucose use in diabetic hearts was low during control perfusion and was increased only slightly over the 60 minutes of ischemic perfusion.

Ventricular function and glycolytic rates of normal and diabetic hearts during 65 minutes of perfusion with normal levels of coronary flow are shown in Figure 2B for comparison. Ventricular function and coronary flow were well maintained in both groups of hearts. Glycolytic rate was depressed
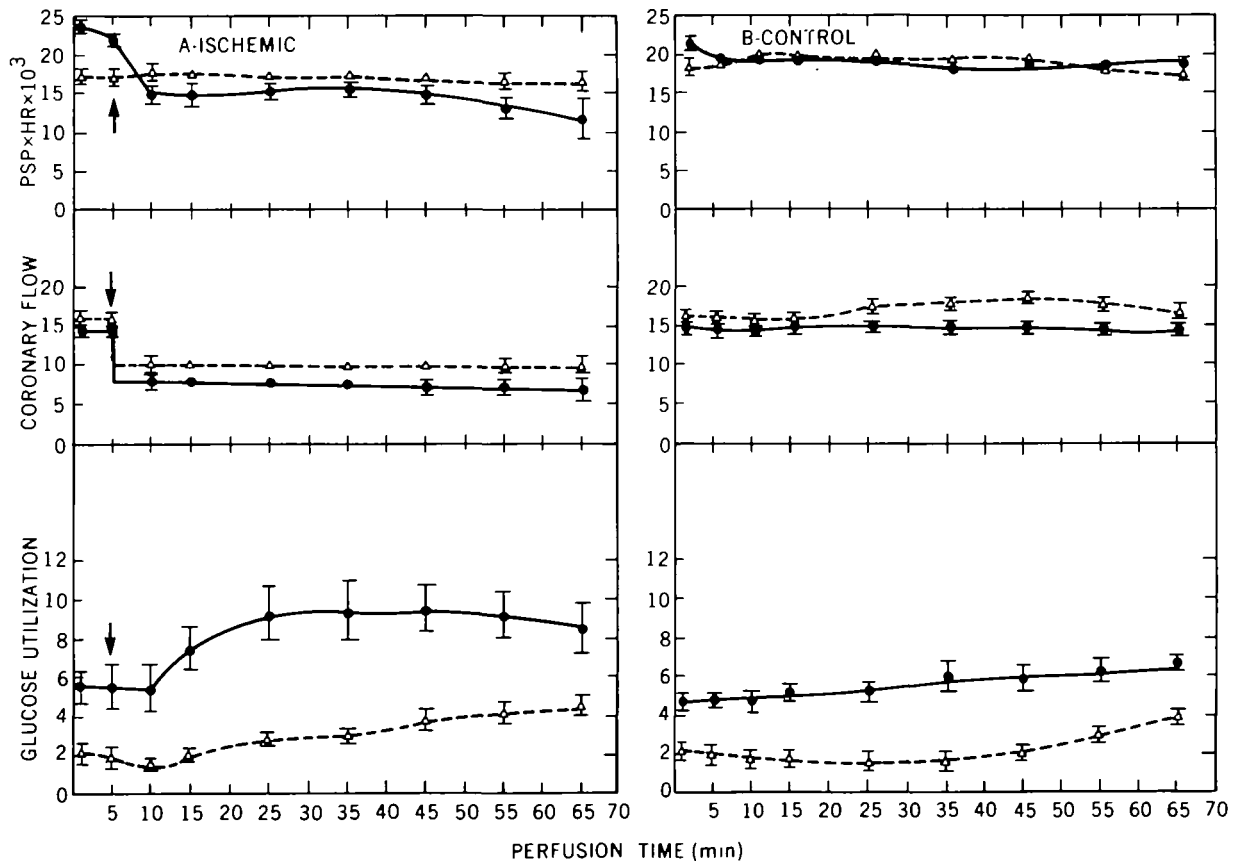

FIGURE 2 Effects of mild ischemia on ventricular function and glucose use in normal and diabetic hearts. Diabetes was induced by injection of 60 mg of alloxan per kg body weight and the hearts were used 48 hours later. The hearts received a 10-minute washout perfusion prior to zero time and a 5-minute control working perfusion with left atrial pressures of 7 mm Hg and peak systolic pressures of about 75 mm Hg. The perfusate contained 11 mM glucose. Ischemia was induced at 5 minutes (vertical arrow in panel A) by reducing coronary flow an average of 53% in the normal (solid lines) and diabetic (broken lines) hearts. In panel B, perfusion was continued for 65 minutes with control rates of coronary flow. Peak systolic pressure (PSP) × heart rate (HR) is expressed as mm Hg/min × 10^3; coronary flow is in ml/min, and glucose utilization is in μmoles/g dry per min. The hearts in each group were frozen at 65 minutes, and tissue levels of K^+, CoA, carnitine and acyl esters of CoA and carnitine were determined. There were seven or eight hearts in each group, and the values are mean ± SEM.

in the diabetic hearts during the first 40 minutes of perfusion and then began to rise. This late rise in glycolysis probably resulted from a progressive decrease in tissue lipids due to their use for oxidative metabolism (Denton et al., 1967; Randle et al., 1966). Likewise, the slow steady increase in glycolytic rate in the normal hearts probably resulted from depletion of tissue lipid stores. These data indicate that the diabetic heart is capable of maintaining low levels of cardiac work for extended periods of in vitro perfusion even though the rate of glucose utilization is low. In addition, the inability to utilize glucose did not appear to compromise function under mildly ischemic conditions.

Normal or diabetic hearts that receive no reduction in coronary flow can maintain performance for at least 1 hour of perfusion when paced at 300 beats/min (data not shown). However, when coronary flow to electrically paced hearts was reduced, developed ventricular pressure decreased following the initial reduction in coronary flow (Fig. 3). The rate of decline in pressure development was faster in the diabetic hearts. A decline in peak pressure to 30 mm Hg (9×10^3 for peak systolic pressure × heart rate) was arbitrarily chosen to represent ventricular failure. The average time required for diabetic hearts to reach this low level of performance was 2.8 ± 0.4 minutes following the initial reduction in coronary flow (indicated by horizontal lines in Figure 3). In contrast, normal hearts required 13 ± 2.4 minutes to reach the same state of failure. As a result of the more rapid failure of pressure development in the diabetic hearts, coronary flow continued to declined at a fast rate following the initial reduction. Glucose utilization (lower panel, Fig. 3) was inhibited in normal hearts by this type of severe ischemia as reported earlier (Neely et al., 1975), and the already low glycolytic rate in diabetic hearts remained low.

Effects of Diabetes and Ischemia on Tissue Levels of CoA, Carnitine, and K⁺

The effects of diabetes on tissue levels of total CoA and carnitine are shown in Tables 1 and 2. Tissue levels of total carnitine decreased in hearts removed from mildly diabetic animals and assayed immediately (Table 1). Carnitine levels were lower by 7 days and continued to decline through 34 days. The level of total CoA, on the other hand, had increased by 7 days and remained high through 34 days. Also, the level of CoA was higher and carnitine was lower in hearts removed from rats 48 hours after injection of 60 mg of alloxan (Table 2). These changes in diabetic hearts represent a dramatic shift in the whole tissue content of the cofactors involved in fatty acid metabolism and could be expected to have major effects on the intracellular fate of fatty acids.

The levels of acyl-CoA and acyl carnitine esters in hearts perfused under control or mildly ischemic

TABLE 1 *Effects of Mild Diabetes on Tissue Levels of CoA and Carnitine*

Condition		Days	Tissue levels (nmoles/g dry)	
			CoA	Carnitine
Control	(11)	0	498 ± 17	5785 ± 78
Diabetic	(3)	7	743 ± 30	5476 ± 100
	(4)	19	589 ± 26	4958 ± 239
	(4)	34	607 ± 27	4246 ± 109

Diabetes was induced by injection of alloxan (37.5 mg/kg body weight) and the rats were maintained without insulin treatment for the times indicated before they were killed The hearts were removed from the rats, trimmed, and blotted, and the ventricles were quickly frozen by clamping between blocks of aluminum cooled in liquid nitrogen. Total tissue CoA and carnitine were determined after alkaline hydrolysis of the acyl esters. Values are mean ± SEM. The number of hearts in each group is shown in parentheses.

TABLE 2 *Effects of Diabetes on Tissue Levels of CoA and Carnitine in Isolated Perfused Hearts*

Condition	Total tissue levels (nmoles/g dry)	
	CoA	Carnitine
Control (25)	462 ± 14	5769 ± 112
Diabetic (22)	570 ± 19	5114 ± 96

Diabetes was induced by injection of 60 mg of alloxan/kg body weight, and the hearts were removed 48 hours later. Groups of hearts were perfused for 10, 30, and 60 minutes with buffer containing glucose (11 mM). The hearts were quick-frozen by clamping with aluminum blocks cooled in liquid nitrogen, homogenized, hydrolyzed under alkaline conditions, and analyzed for total CoA and carnitine. There were no noticeable differences in the levels at various perfusion times and the data have been combined Values are means ± SEM for the number of hearts shown in parentheses.

conditions with buffer containing glucose as the only substrate are shown in Table 3. Ischemia resulted in increased levels of acyl-CoA and acyl carnitine in both normal and diabetic hearts, but the increase was greater in the diabetic tissue. This increase was still apparent after 60 minutes of perfusion with glucose as the only exogenous substrate. Since diabetic hearts had low rates of glucose usage (Fig. 2), it might be expected that tissue stores of lipids would be used up during 60 minutes of perfusion, and it is perhaps surprising that acyl esters of CoA and carnitine remained elevated for this time.

TABLE 3 *Effects of Long-Term Ischemia in Vitro on Acyl Esters of CoA and Carnitine in Isolated Hearts*

Condition	Tissue levels long-chain acyl esters (nmoles/g dry)	
	Acyl-CoA	Acyl carnitine
Normal control	26 ± 8	239 ± 35
Normal ischemic	104 ± 9	532 ± 58
Diabetic control	111 ± 28	204 ± 55
Diabetic ischemic	243 ± 33	830 ± 109

The hearts used for these analyses were perfused as described for Figure 2 and were frozen at 65 minutes. Long-chain acyl-CoA and acyl carnitine levels were determined on the perchloric acid precipitate as free CoA and carnitine released after alkaline hydrolysis. The values represent mean ± SEM for six to eight determinations

Changes in the levels of acyl-CoA and acyl carnitine were also measured in severely ischemic hearts at early stages of failure (i.e., at the time ventricular failure began, as indicated by the diagonal arrows in Figure 3). The levels of acyl esters were higher in diabetic than in normal hearts after 10 minutes of control perfusion (Table 4). With short-term ischemia, normal hearts showed no significant rise in long-chain acyl-CoA, but acyl carnitine levels had increased almost 2-fold. In the diabetic hearts, there was a 46% increase in long-chain acyl-CoA and a 3-fold increase in long-chain acyl carnitine levels after only 2-3 minutes of ischemic perfusion. A comparison of the acyl esters between normal ischemic and diabetic ischemic hearts indicated that the levels were about 3-fold higher in the diabetic tissue. These data indicate that the earlier onset of ventricular failure in diabetic hearts was associated with increased tissue levels of acyl-CoA and acyl carnitine esters.

It is well known that the ischemic myocardium loses cellular K^+ and, since both acyl carnitine and insulin may influence K^+ flux, it was of interest to determine whether tissue K^+ decreased more rapidly in diabetic hearts exposed to ischemia. After 60 minutes of perfusion under control conditions, diabetic hearts had only slightly less K^+ than normal controls ($P < 0.05$) (Fig. 4). Long-term (60-minute) exposure to mildly ischemic conditions resulted in
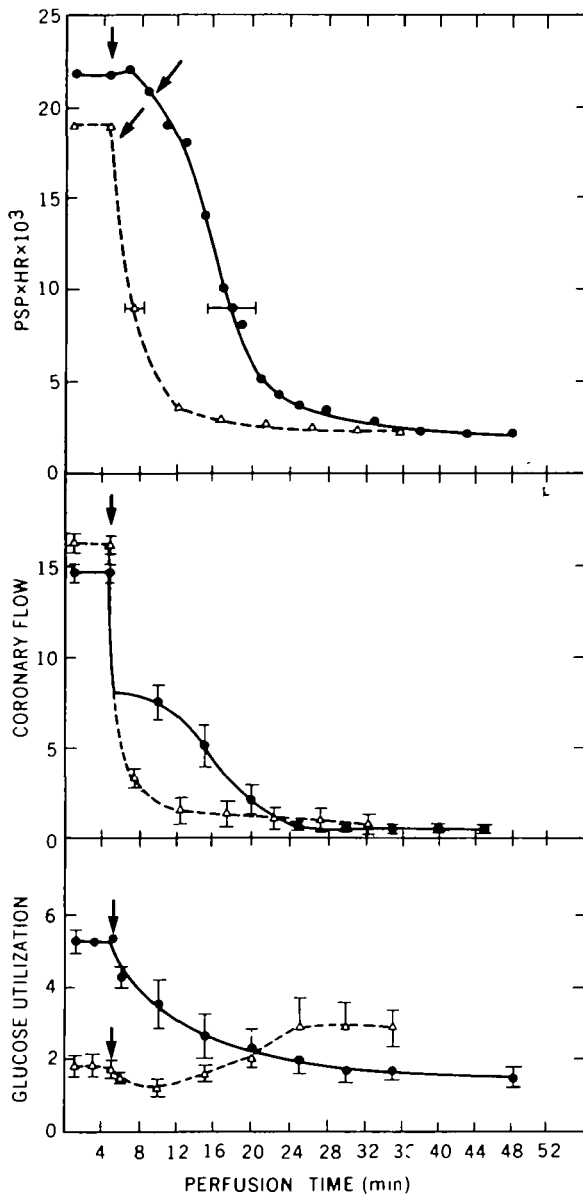
FIGURE 3 Effects of severe ischemia on ventricular function and glycolytic rate in normal and diabetic hearts. These perfusions were similar to those in Figure 2, except that the hearts were electrically paced at 300 beats/min starting at zero time. The decline in the product of peak systolic pressure (PSP) and heart rate (HR) thus represents decreased pressure development. Ischemia was induced at 5 minutes (vertical arrow) by reducing coronary flow to about 50% of the preischemic rate. Ventricular pressure development decreased rapidly in both groups, but the decline was faster in diabetic hearts. Since coronary flow depends on peak systolic pressure in this ischemic preparation using a one-way aortic valve, coronary flow also decreased at a faster rate in the diabetic hearts along with developed pressure. The average time required for PSP × HR to decrease to 9 × 10³ is shown by horizontal bars. This level of function was chosen to represent ventricular failure and corresponds to a PSP of 30 mm Hg. Perfusion of
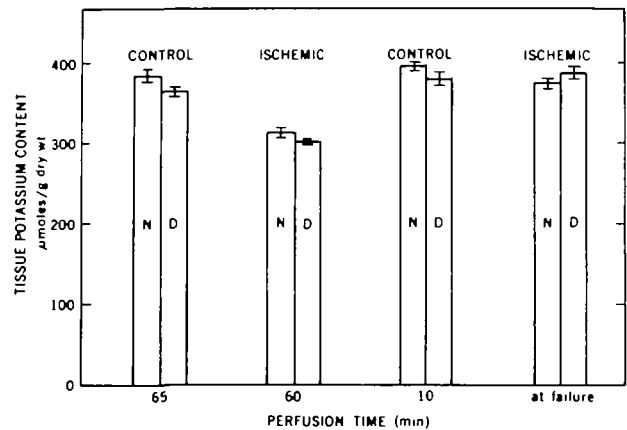
FIGURE 4 Effects of diabetes and ischemia on cellular K^+. The control (65 minutes) and ischemic hearts (60 minutes) are those perfused for Figure 2. The 10-minute control and ischemic hearts at failure are the same as those used to obtain the data for Table 4, and were perfused as described in Figure 3.

these hearts was continued for an additional 30 minutes following failure. In another group of hearts, the tissue was frozen at the beginning of ventricular failure (times indicated by diagonal arrows) and were used for analysis of CoA, carnitine, and K^+. In both cases, the perfusate contained 11 mM glucose. Each point represents mean values from six hearts.

TABLE 4 *Effects of Short-Term Ischemia on CoA and Carnitine Esters in Isolated Perfused Hearts*

Condition	Tissue levels (nmoles/g dry)			
	Acid-soluble CoA	Long-chain acyl-CoA	Acid-soluble carnitine	Long-chain acyl carnitine
Normal control (6)	361 ± 7	92 ± 2	5927 ± 122	130 ± 13
Normal ischemic (9)	342 ± 15	99 ± 8	5593 ± 132	240 ± 32
Diabetic control (8)	441 ± 19	167 ± 10	4947 ± 186	277 ± 79
Diabetic ischemic (7)	291 ± 16	244 ± 15	4218 ± 264	1087 ± 272

Hearts used for these studies were perfused as described for Figure 3 with electrical pacing, but were frozen just at the beginning of ventricular failure (diagonal arrows in Fig. 3). The average time of ischemic perfusion was 3 minutes in the diabetic and 7 minutes in the normal. The perfusate contained glucose as the only exogenous substrate. Control hearts from both normal and diabetic rats were perfused for 10 minutes. Acid-soluble CoA and carnitine, which includes both free and acetyl esters, were determined on the perchloric acid extract after alkaline hydrolysis, and the long-chain acyl esters were determined on the perchloric acid precipitate. Values are means ± SEM for the number of hearts shown in parentheses.

about a 25% decrease in cellular K⁺ in both normal and diabetic hearts, but there was no significant difference between the two groups. Also, K⁺ levels were not significantly different in normal and diabetic hearts at the onset of ventricular failure. The levels in ischemic tissue at failure were not significantly different from the 10-minute controls. These data indicate that loss of cellular K⁺ during ischemia does not correlate with increased cellular levels of acyl carnitine or failure of ventricular function.

Discussion

The direct effect of diabetes on myocardial function is poorly understood. Evidence has been presented which indicates that chronic diabetes in the dog results in decreased diastolic ventricular compliance (Regan, 1975) and that the heart of chronically diabetic animals shows greater reductions of function in response to regional ischemia (Haider et al., 1977). In the present study, diabetic hearts tolerated perfusion under mildly ischemic conditions in vitro just as well as did normal hearts. However, a more severe reduction in the oxygen supply-to-demand ratio did result in a faster rate of ventricular failure in diabetic than in normal hearts. The cause of this effect of diabetes is not known, but changes in glucose and fatty acid metabolism in the diabetic heart may contribute to depressed function under ischemic conditions.

The low rate of glucose use in hearts from diabetic animals results from both insulin deficiency (Park et al., 1968; Morgan et al., 1961) and accelerated use of fatty acid (Morgan et al., 1961; Randle et al., 1966; Kerbey et al., 1976). The inhibition of glycolysis that is due to fatty acid oxidation cannot be fully corrected by insulin in vitro. This apparently results from higher tissue levels and faster rates of in vitro utilization of triglycerides (Denton and Randle, 1967). Since oxidation of fatty acids by normal hearts inhibits glucose transport (Neely et al., 1969), glucose phosphorylation (Randle et al., 1966), and pyruvate dehydrogenase (Kerbey et al., 1976), it is reasonable to conclude that faster oxi-

dation of endogenous lipids in diabetic hearts may account for their in vitro resistance to insulin stimulation of glucose utilization. However, this inability of diabetic hearts to use glucose does not appear to interfere with their ability to develop ventricular pressure. In the present study, the rate of use of glucose by diabetic hearts was only about 30% of the rate in normal hearts, but low levels of mechanical function were maintained just as well in normal hearts. Also, rates of oxygen consumption and mechanical function of isolated working rat hearts were not altered by diabetes when the hearts were developing peak pressures of about 135 mm Hg (Opie et al., 1971). These studies indicate that energy production from oxidation of lipids compensates for reduced glucose use, which allows the diabetic heart to maintain normal function. However, the above studies were conducted in hearts performing less than maximum mechanical work, and it is possible that intrinsic defects in the ability of diabetic hearts to produce ATP and maintain function may not be detected unless the myocardium is stressed to near maximum functional capacity. In the normal isolated rat heart, the maximum pressure development that can be achieved is about 200 mm Hg (Kobayashi and Neely, 1977). Therefore, the present study with hearts performing low levels of mechanical work, and that reported earlier (Opie et al., 1971), did not test the maximum work capacity of diabetic hearts. In addition, hearts of diabetic animals may be depressed in vivo due to altered environment, i.e., high plasma fatty acids, ketone bodies, glucose, and H⁺, and may quickly regain normal function when placed in the in vitro perfusion system with buffer maintained at normal pH and substrate concentrations. A true test of altered ventricular function should include maximum work output in hearts perfused with concentrations of substrates and products simulating the in vivo environment of a diabetic heart.

Another aspect of cardiac metabolism that is greatly altered by diabetes is the tissue levels of metabolites associated with fatty acid oxidation. In addition to higher tissue levels of triglycerides and

free fatty acids (Denton and Randle, 1967), levels of long-chain acyl-CoA, acetyl-CoA, and citrate are also elevated (Randle et al., 1966). In the present study, tissue levels of both long-chain acyl-CoA and acyl carnitine were higher in diabetic hearts perfused under aerobic conditions. This difference was still evident even after 60 minutes of perfusion with glucose as the only substrate available, suggesting that tissue lipid stores were adequate to maintain oxidative metabolism even for prolonged periods of in vitro perfusion.

Ischemia greatly elevates tissue levels of both acyl-CoA and acyl carnitine, particularly when exogenous fatty acids are available (Whitmer et al., 1978). High tissue levels of these long-chain acyl esters have been shown to inhibit several enzyme systems, and their accumulation in ischemic myocardium has been implicated in the deterioration of energy metabolism and ventricular function. Long-chain acyl-CoA inhibits adenine nucleotide transport in isolated mitocnondria (Chua and Shrago, 1977), but there is no evidence that this inhibition occurs in the intact tissue. Long-chain acyl carnitine inhibits Na^+,K^+-ATPase (Wood et al., 1977) and sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} uptake (Cohen et al., 1978). Again, it is not known if these effects are expressed in the intact cell. However, levels of acyl carnitine do increase in the ischemic myocardium to concentrations in excess of that needed for inhibition in vitro. High levels of tissue free fatty acids may also contribute to decreased function of ischemic myocardium (Lamers and Hülsmann, 1977), but in the present study, no exogenous fatty acid was present, and tissue levels of free fatty acids would be expected to be low.

The diabetic heart may be more affected by accumulation of free fatty acids and acyl esters of CoA and carnitine during ischemia. These hearts not only had increased levels of acyl esters under aerobic conditions, but also showed a larger increase under ischemic conditions. The faster rate of ventricular failure in severely ischemic diabetic hearts compared to normal hearts was associated with a more rapid rise in acyl-CoA and acyl carnitine. Tissue free fatty acids were not measured, but the levels of these products of lipolysis may also have increased laong with their CoA and carnitine esters. At the time of failure, levels of acyl esters were 3 times higher in diabetic than in normal ischemic hearts at comparable perfusion times. Since tissue levels of K+ were not altered by diabetes, higher levels of acyl carnitine did not appear to interfere with Na^+,K^+ pump activity. Although measurement of tissue levels is a gross estimate of K^+ movement out of the cells, it does suggest that large changes in cellular K^+ did not occur, and it is unlikely that acyl carnitine inhibition of the Na^+,K^+ pump was a significant factor in ischemic tissue. Whether acyl carnitine interferes with Ca^{2+} metabolism under these conditions remains to be demonstrated, but altered metabolism of Ca^{2+} provides

an attractive explanation for the rapid failure of the diabetic heart under ischemic conditions.

Changes in the total tissue pool of CoA or carnitine in diabetic hearts could greatly alter cellular metabolism of fatty acids. About 95% of the CoA in cardiac muscle is located in the mitochondrial matrix and 95% of the carnitine is cytosolic (Idell-Wenger et al., 1978). It has been proposed that a high carnitine-to-CoA ratio in the cytosol compared to the mitochondrial matrix directs fatty acids toward mitochondrial oxidation in heart. Liver, on the other hand, normally has a lower cytosolic carnitine-to-CoA ratio and synthesizes large amounts of triglycerides. In diabetic animals, levels of carnitine increase in liver, and fatty acids are directed away from lipid synthesis and toward oxidation to ketone bodies (McGarry and Foster, 1976, 1977). In the present study, levels of CoA increased and carnitine decreased in hearts from diabetic rats. The cellular localization of these changes in cofactor concentrations is not known, but if the changes are localized to specific cellular compartments, metabolism of fatty acids can be expected to be greatly altered.

References

Chua B, Shrago E: Reversible inhibition of adenine nucleotide translocation by long chain acyl-CoA esters in bovine heart mitochondria and inverted submitochondrial particles. Comparison with atractylate and bongkrekic acid. J Biol Chem 252: 6711–6714, 1977

Cohen D, Wang T, Sumida M, Adams R, Tsi LI, Grupp G, Schwartz A: Effect of palmityl carnitine on cardiac and skeletal sarcoplasmic reticulum (abstr). Fed Proc 37: 376, 1978

Denton RM, Randle PJ: Concentration of glycerides and phospholipids in rat heart and gastrocnemius muscles. Effects of alloxan-diabetes and perfusion. Biochem J 104: 416–422, 1967

Garland PB, Randle PJ: Regulation of glucose uptake by muscle. Biochem J 93: 678–687, 1964

Haider B, Ahmed SS, Moschos CB, Oldewurtel HA, Regan TJ: Myocardial function and coronary blood flow response to acute ischemia in chronic canine diabetes. Circ Res 40: 577–583, 1977

Hearse DJ, Stewart DA, Chain EB: Diabetes and the survival and recovery of the anoxic myocardium. J Mol Cell Cardiol 7: 397–415, 1975

Idell-Wenger, JA, Grotyohann LW, Neely JR: Coenzyme A and carnitine distribution in normal and ischemic hearts. J Biol Chem 253: 4310–4318, 1978

Kerbey AL, Randle PJ, Cooper RH, Whitehouse S, Pask HT, Denton RM: Regulation of pyruvate dehydrogenase in rat heart. Biochem J 154: 327–348, 1976

Kobayashi K, Neely JR: Maximum rates of energy production from glycolysis and glucose oxidation in cardiac muscle. Proceedings of the Third US-USSR Joint Symposium on Myocardial Metabolism. U.S. DHEW Publication No. (NIH) 78-1457, 1977, pp 361–376

Lamers JMJ, Hülsmann WC: Inhibition of $(Na^+ + K^+)$-stimulated ATPase of heart by fatty acids. J Mol Cell Cardiol 9: 343–346, 1977

Lowenstein JM, ed.: Methods in Enzymology, vol 13. New York, Academic Press, 1969

McGarry JD, Foster DW: Ketogenesis and its regulation. Am J Med 61: 9–13, 1976

McGarry JD, Foster DW: Hormonal control of ketogenosis. Arch Intern Med 137: 495–501, 1977

Morgan HE, Cadenas E, Regen DM, Park CR: Regulation of glucose uptake in muscle. III. Rate-limiting step and effects of

insulin and anoxia in heart muscle from diabetic rats. J Biol Chem **236**: 262–268, 1961

Morgan HE, Neely JR: Insulin and membrane transport. *In* Handbook of Physiology, sec 7, Endocrinology, vol 1, edited by DF Steiner, N Freinkel. Washington, D.C., American Physiological Society, 1972, pp 323–331

Neely JR, Liebermeister H, Battersby EJ, Morgan HE: Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol **212**: 804–814, 1967

Neely JR, Bowman RH, Morgan HE: Effects of ventricular pressure development and palmitate on glucose transport. Am J Physiol **216**: 804–811, 1969

Neely JR, Denton RM, England PJ, Randle PJ: The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in perfused rat heart. Biochem J **128**: 147–159, 1972

Neely JR, Rovetto MJ, Whitmer JT, Morgan HE: Effects of ischemia on ventricular function and metabolism in the isolated working rat heart. Am J Physiol **225**: 651–658, 1973

Neely JR, Morgan HE: Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Annu Rev Physiol **36**: 413–457, 1974

Neely JR, Whitmer JT, Rovetto MJ: Effects of coronary flow on glycolytic flux and intracellular pH in isolated rat hearts. Circ Res **37**: 733–741, 1975

Opie LH, Mansford KRL, Owen P: Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. Biochem J **124**: 475–490, 1971

Randle PJ, Garland PB, Hales CN, Newsholme EA, Denton RM, Pogson CI: I. Protein Hormones. Interactions of metabolism and the physiological role of insulin. Recent Prog Horm Res **22**: 1–48, 1966

Park CR, Crofford OB, Kono T: Mediated (non-active) transport of glucose in mammalian cells and its regulation. J Gen Physiol **52**: 2963–3185, 1968

Rannels DE, Kao R, Morgan HE: Effect of insulin on protein turnover in heart muscle. J Biol Chem **250**: 1694–1701, 1975

Rannels DE, McKee EE, Morgan HE: Regulation of protein synthesis and degradation in heart and skeletal muscle. Biochemical Action of hormones, vol 4, edited by G Litwack. New York, Academic Press, 1977, pp 135–195

Regan TJ: The heart and diabetes. *In* Report of the National Commission on Diabetes, U.S. Dept. of Health, Education, and Welfare, National Institutes of Health, DHEW Publication No. (NIH) 76–1023, vol III, 1975, pp 222–225

Whitmer JT, Wenger JI, Rovetto MJ, Neely JR: Control of fatty acid metabolism in hypoxic and ischemic hearts. J Biol Chem **253**: 4305–4309, 1978

Wood JM, Bush B, Pitts BJR, Schwartz A: Inhibition of bovine heart Na^+,K^+-ATPase by palmitylcarnitine and palmityl-CoA. Biochem Biophys Res Commun **74**: 677–684, 1977

Zierler KL: Isulin, ions, and membrane potentials. *In* Handbook of Physiology, sec 7, Endocrinology, vol 1, edited by DF Steiner, N Freinkel. Washington, D.C., American Physiological Society, 1972, pp 347–388

Supraventricular Pacemaker Underdrive in the Absence of Sinus Nodal Influences in the Conscious Dog

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SUMMARY   The ability to reduce and maintain heart rate by electrical stimulation (underdrive) was tested in three groups of chronically instrumented dogs: sinoatrial node intact, ectopic atrial pacemaker produced by injection of rapidly hardening latex into the sinoatrial nodal artery, and idioventricular pacemaker produced by injection of formalin into the atrioventricular node. In the conscious unsedated state, underdrive of sinoatrial or idioventricular pacemakers resulted in competition between driven and intrinsic foci. However, the cycle length of ectopic atrial pacemakers could be increased by 148.4 ± 30.7 msec ($P < 0.001$) and maintained at that value. Cessation of underdrive resulted in a period of pacemaker suppression similar to that produced following overdrive. Single premature stimuli produced marked cycle length prolongations in dogs with ectopic atrial foci. Total autonomic blockade had no significant effect on the production of underdrive. Thus, the results of the present experiments provide evidence for maintained capture of cardiac pacemakers at rates significantly below intrinsic control and indicate that underdrive varies as a function of pacemaker site. Underdrive may explain the failure of junctional escape in the presence of slower atrial rhythms. *Circ Res 44: 329–334, 1979*

IT IS generally recognized that cardiac pacemakers will follow electrical drive at rates in excess of their inherent rate and that the cessation of pacing normally is followed by a period of quiescence called

overdrive suppression (Vassalle, 1977). It has been demonstrated that the susceptibility to overdrive suppression varies such that the sinus node is least susceptible, whereas increasing susceptibility is shown by ectopic atrial, atrioventricular (AV) junctional, and ventricular pacemakers, respectively (Lange, 1965). Overdrive suppression has also been used to explain the hierarchial dominance of cardiac pacemakers (Vassalle, 1970). However, it is clear that prolonged drive is not a prerequisite in order to provoke pacemaker suppression. Thus, transitory