Effect of Tolbutamide on Myocardial Metabolism and Mechanical Performance of the Diabetic Rat

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SUMMARY

Exposure of the isolated, glucose-perfused rat heart to buffer containing 0.4 mM tolbutamide resulted in significant changes in both energy metabolism and myocardial contractility. In the nondiabetic, tolbutamide mediated only small increases in mechanical function at low atrial filling pressure, but this effect increased with increasing preload. By contrast, the stimulation of mechanical function resulting from exposure of the diabetic heart to tolbutamide was independent of preload. As a result, the tolbutamide-mediated, positive inotropic effect in the diabetic heart was greater at lower, but not higher, preload values than the effect in the nondiabetic. Moreover, the changes in energy metabolism initiated by tolbutamide were considerably larger in the diabetic. The most prominent effect was the mobilization of glycogen by tolbutamide in the diabetic, which was considerably greater than that observed in the nondiabetic. The drug also enhanced glucose utilization. The net effect of sulfonylurea exposure was to shift from preferential use of fatty acids as an energy source for contraction to use of glucose. Since the most prominent effect of the drug in the diabetic was the stimulation of glycogenolysis, it is concluded that tolbutamide can dramatically alter the metabolism of a tissue without acting through insulin.

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The sulfonylureas are oral hypoglycemic agents that are used in the treatment of type II or non-insulin-dependent diabetes mellitus. Acute administration of these hypoglycemic agents stimulates the release of insulin from the pancreas. However, their antidiabetic activity is only partially associated with their effects on the pancreas. Chronic oral therapy of many sulfonylureas ameliorates hyperglycemia even though insulin plasma levels are normal,[1] suggesting that extrapancreatic actions are involved in the chronic antidiabetic effects of these drugs.

In 1969, Feldman and Lebovitz[2] reported that the sulfonylurea tolbutamide potentiated the effects of insulin on 2-deoxyglucose transport in mouse diaphragm. It was subsequently shown that several sulfonylureas increased the number of insulin receptors of tissue taken from normal and diabetic patients and animals.[3–5] Sulfonylurea-mediated changes in the affinity of insulin for its receptor have also been reported.[5] These observations have led to the hypothesis that the hypoglycemic effects of chronic sulfonylurea treatment are probably due to potentiation of insulin action on glucose metabolism of extrapancreatic tissue.[3–6] However, tolbutamide was recently reported to enhance glucose uptake in perfused rat hindlimb in either the presence or absence of insulin.[7] Similarly, Kramer et al.[8] found stimulation of both glucose utilization and glycogenolysis in perfused rat heart exposed to only tolbutamide. Although these results imply that certain actions of tolbutamide are completely independent of insulin, Daniels and Lewis[7] pointed out that the stimulation seen with tolbutamide alone could be caused by potentiation of residual tissue insulin. In this study, the effect of tolbutamide on hearts isolated from insulinopenic, severely wasting diabetic rats are examined. These results take on added importance in light of the University Group Diabetes Program report suggesting an association of tolbutamide therapy with increased incidence of cardiovascular deaths.[9]

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts), weighing between 250 and 300 g, were used for these studies. They were fed Purina Formulab Chow ad libitum and given free access to water. Diabetes was induced by i.p. injection of 70 mg/kg body wt of streptozocin (kindly provided by Dr. W. E. Dulin, Upjohn Co., Kalamazoo, Michigan) in sodium citrate buffer (pH 4.5). Animals were maintained in the diabetic state

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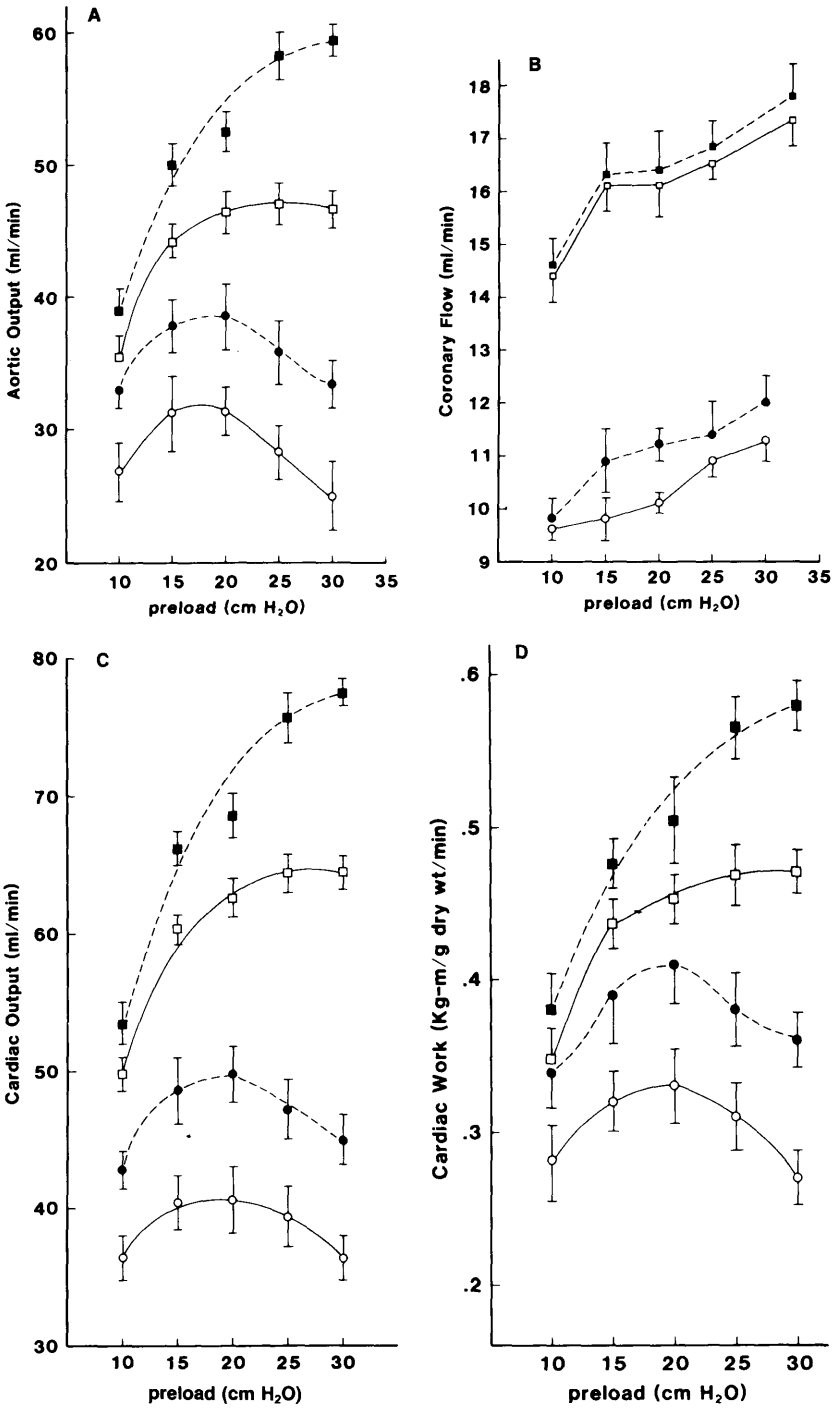

FIGURE 1. Effect of tolbutamide on mechanical performance of the diabetic and nondiabetic heart. Hearts from streptozocin-induced diabetic and nondiabetic rats were perfused with Krebs-Henseleit buffer containing 11 mM glucose. Preload was varied stepwise over the range of 10–30 cm H_2O, first in the absence of tolbutamide and then in its presence. With each change in preload, the heart was allowed to stabilize for approximately 5 min and aortic output (A), coronary flow (B), cardiac output (C), and cardiac work (D) were determined. Diabetic, tolbutamide-free (○—○); diabetic, 0.4 mM tolbutamide (●--●); nondiabetic, tolbutamide-free (□—□); nondiabetic, 0.4 mM tolbutamide (■--■). Each value represents the mean ± SEM of five hearts.

for 8 wk. At this juncture, only rats in which plasma glucose levels were >300 mg/dl (mean glucose 426 ± 16) were used for these studies. The mean plasma insulin of the diabetic rats was 0.198 ± 0.029 ng/ml. The mean body weight of the diabetics at the end of the study was 294 ± 11 g compared with 475 ± 11 g for age-matched controls. Glucose analyses were performed using a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, California).

Radioimmunoassays. Radioimmunoassays for insulin were performed by Dr. Ron Gingrich at Washington University in St. Louis, Missouri, using a double-antibody technique.

Perfusion techniques. Hearts from diabetic and nondiabetic, male Wistar rats were perfused on a standard working heart apparatus.[10,11] Afterload was fixed at 110 cm H_2O while preload was varied as described in the text. All hearts were paced at 300 beats/min. To focus on glucose metabolism, standard Krebs-Henseleit buffer containing 11 mM glucose with or without 0.4 mM tolbutamide was used. The concentration of tolbutamide employed is at the higher end of the therapeutic range and has been found to mediate maximal contractile and metabolic effects.[8] The apparatus contained two parallel lines designed to permit a rapid transition from the drug-free buffer to the one containing tolbutamide.

Two separate protocols were employed. In one study,

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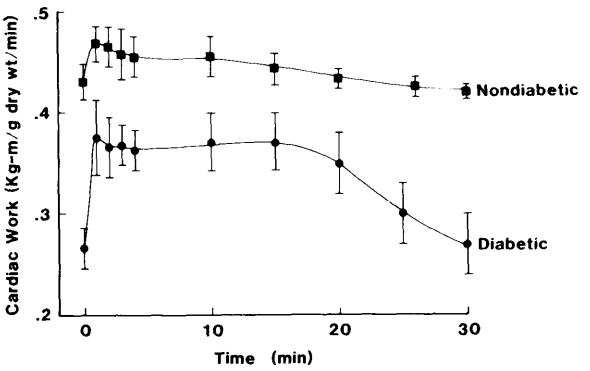

FIGURE 2. Kinetics of tolbutamide-mediated change in cardiac work. Effects of nondiabetic (■—■) and streptozocin-induced diabetic rats (●—●) were perfused at a fixed preload of 13 cm H_2O with Krebs-Henseleit buffer containing 11 mM glucose. After a 15-min stabilization period, the hearts were abruptly exposed to an identical buffer supplemented with 0.4 mM tolbutamide. At appropriate times, aortic output, coronary flow, and aortic pressure were measured and used to calculate cardiac work. Values represent means ± SEM of five hearts.

hearts were perfused with tolbutamide-free buffer for a stabilization period of 15 min at a preload of 13 cm H_2O. The preload was subsequently increased in a stepwise manner from 10 cm to 30 cm H_2O, according to the procedures used previously.[12,13] The identical method was repeated with the same isolated heart exposed to tolbutamide-containing buffer. After each change in preload the heart was allowed to stabilize for about 5 min and the appropriate parameters were measured. Contractile response to changes in preload was similar to that observed by other investigators.[10-13]

In the second protocol, hearts were abruptly exposed to tolbutamide-containing buffer after stabilization with drug-free buffer. At appropriate time intervals, the desired biochemical and mechanical function data were obtained.

Left ventricular systolic pressure was measured with a Statham P23Gb pressure transducer by inserting a 22-gauge needle through the ventricular wall, while aortic pressure was monitored with a Statham P23Gb pressure transducer placed above the aortic cannula. Cardiac output was estimated from the sum of left ventricular output measured with a flowmeter and the coronary flow rate. Pressure work was calculated according to Neely et al.[11]

Analytic procedures. The rate of glucose utilization was determined by measuring the rate of tritium release from (3-^{3}H)-glucose into water.[8] Coronary effluent samples used for the glucose utilization studies were also assayed spectrophotometrically by standard enzyme techniques to determine the rate of lactate production.[8] Oxygen content of the coronary effluent was continuously monitored with a Clark oxygen electrode.[8]

For analysis of tissue glycogen content, hearts were rapidly frozen with a Wollenberger clamp precooled in liquid nitrogen. A known weight of lyophilized ventricular tissue was extracted and assayed according to the procedure described by Kramer et al.[8]

RESULTS

Several investigators[12-14] have observed a decrease in cardiac performance of the overt diabetic animal. This is generally characterized by a significant fall in aortic output, cardiac output, and cardiac work (Figure 1). In this study, these changes were particularly apparent at preload conditions >20 cm H_2O. There was a progressive increase in cardiac contractility of both the nondiabetic and diabetic hearts associated with a rise in preload from 10 to 20 cm H_2O. However, further increases in left atrial filling pressure led to a progressive decrease in cardiac performance of the diabetic, while contractility continued to rise in the nondiabetic. As a result, cardiac work of the diabetic heart was 42% less than the nondiabetic at a preload of 30 cm H_2O but only 27% less at a preload of 20 cm H_2O.

Figure 1 also reveals the effect of tolbutamide on cardiac performance of the normal and diabetic heart. As reported previously, inclusion of 0.4 mM tolbutamide in the perfusion buffer resulted in only a small, positive inotropic effect in the nondiabetic subjected to low preload.[8] However, as the preload was increased in the nondiabetic, the percent increase in cardiac work mediated by tolbutamide rose significantly; the drug stimulated cardiac work by only 10% at 10 cm H_2O but by 23% at 30 cm H_2O. By contrast, preload had very little influence on tolbutamide-mediated changes in the diabetic; cardiac work rose 20% after exposing hearts to 0.4 mM tolbutamide at a preload of either 10 or 30 cm H_2O.

The time course of the tolbutamide-mediated positive inotropic effect is shown in Figure 2. In the diabetic, exposure of the heart to tolbutamide led to an initial 40% increase in cardiac work at a preload of 13 cm H_2O. The elevated contractile state was maintained for 15 min and then slowly decayed to pretolbutamide levels by 30-min exposure to the drug. By comparison, the initial rise in cardiac work of the nondiabetic was quite small (10%), but, like the diabetic, the mechanical performance of these hearts also declined to predrug levels by 30-min exposure to the sulfonylurea. Nondiabetic hearts perfused for 30 min without exposure to tolbutamide maintained a constant cardiac work of 0.43 ± 0.02

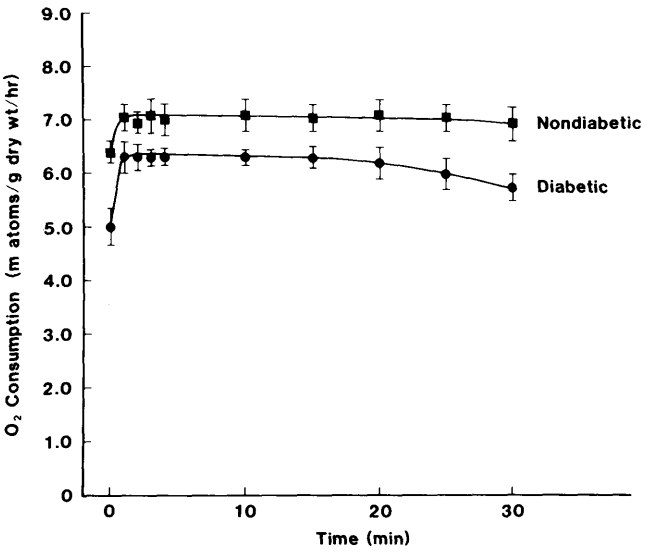

FIGURE 3. Effect of tolbutamide on oxygen consumption. Hearts of nondiabetic (■—■) and diabetic rats (●—●) were perfused as described in Figure 2. Oxygen consumption was calculated from the coronary flow and oxygen content of the coronary effluent, as measured with a Clark oxygen electrode. Values represent means ± SEM of five hearts.

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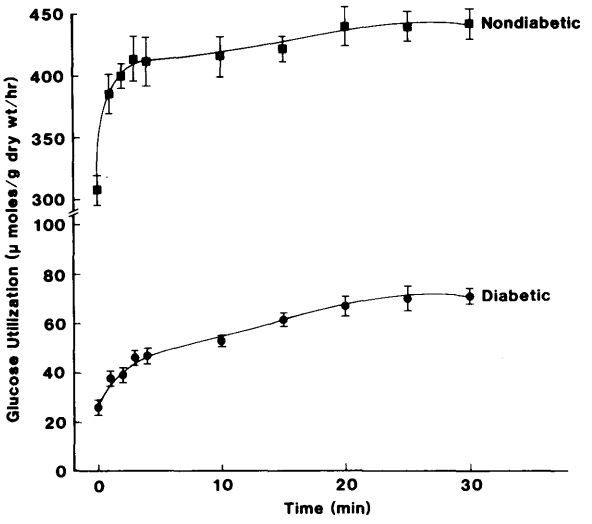

FIGURE 4. Effect of tolbutamide on glucose utilization. Hearts of nondiabetic (■—■) and diabetic rats (●—●) were perfused as described in Figure 2. Coronary effluent was collected and the rate of tritium release from (3-^{3}H)-glucose into water determined. Glucose utilization was calculated according to standard methods.[8] Values represent means ± SEM of five hearts.

kg-m/g dry wt/min throughout the perfusion. On the other hand, the nontreated, diabetic hearts maintained a steady-state cardiac work of 0.26 ± 0.02 kg-m/g dry wt/min for the first 20 min of perfusion; by 30 min of perfusion, cardiac work had slowly fallen to a value of 0.22 ± 0.02 kg-m/g dry wt/min.

Consistent with the mechanical function data, the oxygen consumption of the nondiabetic heart was significantly greater than that of the diabetic. This difference narrowed after tolbutamide exposure, since oxygen consumption increased nearly 30% in the diabetic while rising only 10% in the nondiabetic (Figure 3).

The most dramatic effect of tolbutamide in both diabetic and nondiabetic hearts was the stimulation of anaerobic metabolism. In agreement with numerous investigators,[12,13,15] glucose utilization by the diabetic heart was found to be exceedingly low (26 ± 2 μmol/g dry wt/h for the diabetic versus 310 ± 27 μmol/g dry wt/h for the nondiabetic). Tolbutamide accelerated the rate of glucose utilization by a factor of three in the diabetic, while increasing glucose utilization from 310 ± 27 to 450 ± 32 μmol/g dry wt/h in the nondiabetic heart (Figure 4). Also associated with tolbutamide exposure was a significant stimulation in glycogen mobilization (Table 1); tissue glycogen levels fell 30% and 10% after 5-min exposure to the sulfonylurea in the diabetic and nondiabetic, respectively. Glycogen was also mobilized from the untreated, diabetic heart but at a slower rate than in the sulfonylurea-treated myocardium; tissue glycogen content of the diabetic was 285.6 ± 35.5 and 183.0 ± 21.1 μmol glucose equivalents/g dry wt after 5 min and 30 min of sulfonylurea-free perfusion, respectively. Since the rate of glycogenolysis is one factor determining the rate of glycolytic flux, these results indicate that glycolysis is dramatically accelerated by tolbutamide. This effect is reflected in the rapid 2–2.5-fold rise in lactic acid production in the diabetic and nondiabetic hearts. After the initial increase in lactate production, triose output steadily declined in both the diabetic and nondiabetic hearts (Figure 5). This fall was associated with a similar decrease in the rate of glycogen breakdown, indicating a direct link between the two parameters.

TABLE 1
Effect of tolbutamide on myocardial glycogen content

Minutes of tolbutamide exposure	Glycogen (μmol/g dry wt)*	
	Nondiabetic	Diabetic
0	92.5 ± 4.3	310.6 ± 29.9
2	86.1 ± 3.7	254.9 ± 22.4
5	82.3 ± 3.6	219.2 ± 30.0
30	66.5 ± 5.1	135.6 ± 34.4

*Glycogen content is expressed in glucose equivalents.

DISCUSSION

The sulfonylureas have been shown to serve as regulators of fuel homeostasis in type II diabetes, an effect thought to largely involve an extrapancreatic mechanism. One of the most widely studied extrapancreatic actions of the sulfonylureas is the modification of cyclic nucleotide metabolism. In vitro work has shown that they activate adenylate cyclase and inhibit phosphodiesterase activity, causing an elevation in tissue cAMP levels.[16] This effect was postulated to play an important role in the regulation of carbohydrate and lipid homeostasis by these drugs. However, this idea is no longer widely accepted, since the sulfonylureas inhibit cAMP-associated, hormone-sensitive lipase activity in liver and adipose tissue,[17,18] regulate hepatic gluconeogenesis and glycogenesis in the absence of tissue cAMP changes,[18,19] and stimulate cardiac glucose utilization and glycogenolysis without altering myocardial cAMP content.[8]

Recently, several studies have focused on the effects of the sulfonylureas on glucose transport and metabolism. This

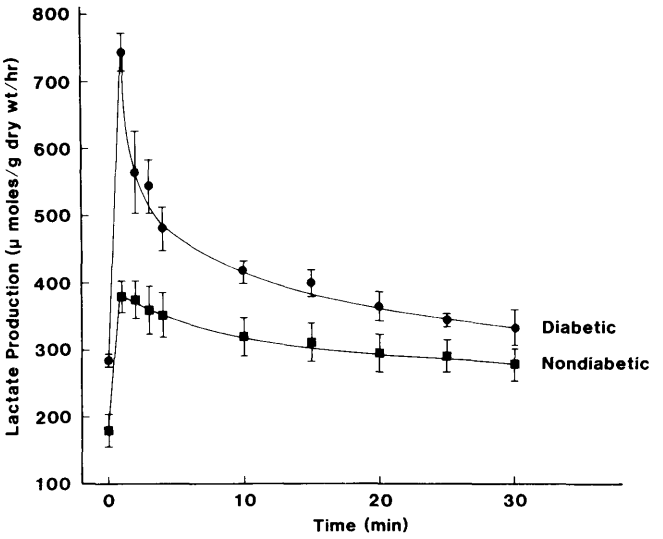

FIGURE 5. Effect of tolbutamide on lactate production. Perfusions were performed as described in Figure 2. Lactate content of the coronary effluent was measured spectrophotometrically and used to calculate the rate of lactate production.[8] Diabetic (●—●), nondiabetic (■—■). Values represent means ± SEM of five hearts.

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work was prompted by the 1967 observation of Reaven and Dray[1] that improved glucose tolerance in diabetic patients receiving chlorpropamide was not accompanied by a change or even a decrease in serum insulin. To explain this phenomenon, it has been argued that the chronic actions of the sulfonylureas are associated with their ability to increase insulin sensitivity in diabetic patients.[6] That the sulfonylureas potentiate insulin-stimulated glucose uptake by liver, muscle, and adipose tissue is without dispute.[2,6,7,20] However, the basis for these effects remains unclear. Olefsky and Reaven[3] have proposed that the hypoglycemic drugs function to increase the number of insulin receptors and/or the affinity of insulin for its receptors in the periphery. On the other hand, this interpretation is inconsistent with observations by Maloff and Lockwood,[2] who showed that the sulfonylurea tolazamide enhances insulin-stimulated hexose transport by rat adipocytes without influencing the insulin receptor.

The possibility that the sulfonylureas may stimulate hexose transport by skeletal muscle via an insulin-independent mechanism was suggested by the work of Gibson et al.[21] They found that exogenous insulin was not required to demonstrate tolbutamide-mediated potentiation of xylose uptake by chicken skeletal muscle. Recently, Daniels and Lewis[7] and Kramer et al.[8] have observed similar effects of the sulfonylureas using perfused rat hindlimb and rat heart. Yet, all three studies left open the possibility that tolbutamide merely potentiated the effects of residual insulin present in the respective tissues. In this study, the effect of tolbutamide on hearts from insulinopenic, severely wasting diabetic rats was examined. Table 2 reveals that the major action of the drug in both the diabetic and nondiabetic is the promotion of glucose and glycogen metabolism at the expense of endogenous fatty acid oxidation. In the nondiabetic, the 75% increase in glycolytic flux after exposure to tolbutamide is caused by a rise in both glucose utilization and glycogenolysis. On the other hand, the doubling of glycolytic flux in the sulfonylurea-treated diabetic heart is primarily mediated by a dramatic increase in the rate of glycogenolysis. Although tolbutamide nearly doubles the rate of glucose utilization in the diabetic heart, this has little influence on the overall metabolic pattern, since only 7% of the carbon entering the glycolytic pathway is derived from this source.

The tolbutamide-mediated increase in glycolytic flux is associated with a dramatic stimulation in glucose and glycolytic NADH oxidation, revealing that the drug also accelerates flux through the malate-aspartate shuttle and pyruvate dehydrogenase. This is particularly true in the diabetic heart, where the contribution of pyruvate entry to total citric acid cycle flux rises from 38 to 99% after addition of tolbutamide. The shift is less dramatic in the nondiabetic. Nevertheless, it is of sufficient magnitude to cause the rate of ATP synthesis from anaerobic glycolysis and glucose oxidation to increase 75%, while the contribution from endogenous fatty acid oxidation declines 25%.

The other major point to emerge from Table 2 is that tolbutamide stimulates ATP synthesis in both the diabetic and nondiabetic heart. This is most likely secondary to the observed increase in contractility rather than a response to high-energy phosphate deficiency. Unlike some investigators,[12,22] we detected no decrease in tissue ATP or creatine

TABLE 2
Effect of tolbutamide on metabolism rates of nondiabetic and diabetic heart

	Nondiabetic		Diabetic	
	Without tolbutamide	With tolbutamide	Without tolbutamide	With tolbutamide
Rate, μmol glucose equivalents · g dry wt^{-1} · h^{-1}				
Glycolysis	308	532	324	768
Glucose utilization	308	412	26	54
Glycogenolysis	0	120	298	714
Lactate output	30	172	141	235
Pyruvate output	30	28	28	22
Glucose oxidation	189	332	159	511
Rate, μmol O_2 · g dry wt^{-1} · h^{-1}				
O_2 consumption	3160	3520	2500	3130
Oxidation of pyruvate + glycolytic NADH	1164	2020	982	3088
Palmitate oxidation	1996	1500	1518	42
Tricarboxylic acid cycle	2145	2371	1675	2057
Pyruvate dehydrogenase	189	332	159	511
Glycolytic NADH	219	360	187	533
Palmitate β-oxidation	607	457	462	13
Rate, μmol ATP · g dry wt^{-1} · h^{-1}				
ATP synthesis	18,788	21,497	15,055	20,319
ATP from glucose	7610	13,197	6554	20,084
ATP from palmitate	11,178	8400	8501	235
Percentage ATP from				
Glucose	41	61	44	99
Palmitate	59	39	56	1

Hearts were perfused as described in Figure 2. Values were taken after 5-min exposure to the drug. Theoretical O_2 equivalents (mol/mol): glucose 6 (4 in tricarboxylic acid cycle + 1 at pyruvate dehydrogenase + 1 in oxidation of glycolytic NADH). Palmitate oxidation refers to that portion of O_2 consumption not due to glucose oxidation and represents endogenous fatty acid oxidation. The ratios of ATP produced to O_2 consumed (P/O ratios) utilized in calculating ATP synthesis are: glucose 3.17 and palmitate 2.8. The net yield of ATP from lactate output was assumed to be 2 μmol of ATP/μmol of glucose, whereas pyruvate output was assumed to be 8 μmol of ATP/μmol of glucose (2 for glycolysis and 6 for oxidation of cytoplasmic NADH).

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phosphate content in the diabetic heart perfused for 15 min with glucose (data not shown). Moreover, tolbutamide was found to have no influence on tissue ATP content.

The results discussed here clearly indicate that tolbutamide mediates insulin-independent actions in the diabetic heart. Perhaps the most dramatic effect of the drug is the stimulation of glycogenolysis. Since insulin promotes glycogen synthesis and not its mobilization, this action is clearly independent of insulin. Recent evidence suggests that the drug promotes glycogen breakdown by increasing calcium influx, thereby activating phosphorylase kinase.[23] Alterations in calcium transport would also account for the observed increase in myocardial contraction. However, the ability of the drug to stimulate glycolysis is apparently independent of tissue calcium content, since glucose utilization remains elevated after 30 min of drug exposure even though contractility and the rate of glycogenolysis have returned to predrug levels. Kramer et al.[8] have proposed that the sulfonylurea directly activates phosphofructokinase. There is also some evidence that it may accelerate glycolytic flux by increasing the entry of pyruvate into the citric acid cycle.[8]

The observation in this study that tolbutamide directly promotes glucose utilization in the absence of insulin is in apparent conflict with the results of Feldman and Lebovitz,[2] who found the sulfonylurea tolbutamide to be effective in stimulating deoxyglucose uptake by mouse diaphragm only in the presence of insulin. The basis for this apparent difference is not clear. Since putative insulin-independent effects of the sulfonylureas have been observed in both mammalian and avian species, the difference does not appear to be species specific, as suggested by Gibson et al.[21] It is also unlikely that the preparation can adequately account for the variation, since both insulin-independent and -dependent effects have been described in a wide range of tissue preparations.[2,7,8,20,21,24] A more likely explanation is the sensitivity and nature of the methods employed to detect hexose transport. Maloff and Lockwood[20] observed an increase in tolazamide-stimulated 2-deoxyglucose accumulation by rat adipocytes in the absence of insulin, although the 19% increase was found to be not significantly different from the control. Daniels and Lewis[7] also reported that tolbutamide-mediated acceleration in glucose uptake in the absence of insulin was small, but nevertheless significant. The work carried out by Kramer et al.[8] employed techniques that focused on the utilization of glucose rather than its transport.

In agreement with Penpargkul et al.,[13] the coronary flow of the diabetic heart was lower than the age-matched control. This is largely caused by the size of the heart, which was smaller in the diabetic rat. However, other factors also appear to influence coronary flow in the diabetic. Recent studies by Downing et al.[25] suggest that insulin regulates coronary flow. Depressed contractility, as seen in the diabetic heart, would be expected to also reduce flow rates. Finally, the possibility that changes in the vasculature occur that would alter coronary flow provides another explanation for the observation.[26]

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