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EFFECT OF TOLBUTAMIDE ON MYOCARDIAL ENERGY METABOLISM OF THE ISCHEMIC HEART*

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Abstract—The oral hypoglycemic agent tolbutamide has been found to protect the ischemic myocardium against irreversible mechanical failure. The possibility that this salutary effect of tolbutamide was related to its ability to alter energy metabolism was examined in ischemic rat hearts perfused with 5 mM glucose, 5 mM acetate and 2.5 units/l insulin. In the presence of 0.6 mM tolbutamide, coronary flow and oxygen consumption were unaltered; however, glucose utilization was stimulated by 30%, glycogenolysis was enhanced by 23%, and the drop in ATP content was reduced by 17% after 30 min, of low-flow perfusion. This elevation in glycolytic flux occurred without a parallel rise in the production of inhibitory metabolites; lactate production was unaltered and tissue lactate/pyruvate ratio decreased. Pyruvate dehydrogenase flux measurements reveal that the mechanism by which tolbutamide increases glycolysis without increasing lactate production is by promoting the entry of pyruvate into the mitochondria. The basis for the observed stimulation of anaerobic metabolism and pyruvate oxidation and how this contributes to the increase in ATP content and benefits the ischemic heart is discussed.

The oral hypoglycemic agent tolbutamide is widely used in the treatment of type II or noninsulin-dependent diabetes mellitus. Interest in the cardiovascular effects of this drug was stimulated by the report of the University Group Diabetes Program (UGDP) in 1970, which concluded that long-term therapeutic regimens of tolbutamide in diabetic patients are associated with an increased risk of cardiovascular mortality [1]. However, several investigators have questioned the validity of the UGDP conclusions [2, 3]. A preliminary report of an ongoing study by Carlström *et al.* [4] has even suggested that tolbutamide reduces the incidence of angina pectoris, intermittent claudication, or myocardial infarction in diabetic patients. A cardiovascular protective effect of tolbutamide was also reported by Weiss *et al.* [5] who showed that pretreatment with tolbutamide reduces the incidence of cardiotoxicity in rats given large doses of isoproterenol. In addition, several investigators have reported that tolbutamide treatment relieves ischemic pain associated with coronary and peripheral vascular diseases [6–8].

Recently our group has shown that short-term treatment with tolbutamide protects the isolated working rat heart against ischemia-induced damage [9]. This dose-dependent protective effect was associated with an improved metabolic state of the ischemic heart, leading us to suggest that the drug meliorated the O_2 balance of the heart. Since it has been shown that tolbutamide increases uptake and oxidation of glucose in a variety of tissues [10, 11], we thought it possible that the drug may benefit the ischemic myocardium by stimulating anaerobic metabolism. Alternatively, the sulfonylurea could be improving the O_2 balance of the ischemic heart by increasing oxygen delivery to the cells. To distinguish between these possibilities we investigated the effects of tolbutamide on anaerobic metabolism and oxygen consumption in the isolated perfused rat heart subjected to ischemia.

MATERIALS AND METHODS

Hearts from 240 to 260 g male Wistar rats were perfused on a standard working-heart apparatus [12, 13]. Under nonischemic conditions, preload and afterload were fixed at 13 and 110 cm H_2O respectively. The standard perfusate consisted of Krebs–Henseleit buffer containing 5 mM glucose, 5 mM acetate and 2.5 units/l insulin. Buffers were maintained at a constant temperature of 37° and gassed with a 95% O_2–5% CO_2 mixture to maintain a pH of 7.4.

Rat hearts paced at 300 beats/min were allowed to stabilize for 20 min prior to the induction of ischemia. Ischemia was carried out by perfusing hearts under low-flow conditions for 30 min according to the method of Vary *et al.* [13]. Ischemic conditions were controlled so that the volume of coronary flow over a 1-min interval was adequate enough to perform metabolite assays. Tolbutamide-treated hearts were perfused under identical conditions to that of the untreated ischemic hearts, except that tolbutamide was either present in the perfusate throughout the experiment or 5 min after the induction of ischemia. A concentration of 0.6 mM tolbutamide was chosen for this study since it had been shown previously to provide a maximum amount of protection to the ischemic rat heart [9].

The rate of glucose utilization was determined by

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measuring the rate of tritium release from [3-^{3}H] glucose into water [14]. Coronary effluent samples used for the glucose utilization studies were also assayed spectrophotometrically by standard enzyme techniques to determine the rates of lactate and pyruvate production [14]. Oxygen content of the coronary effluent was continuously monitored in a flow-through lucite chamber fitted with a standard Yellow Springs oxygen electrode as described by Schaffer *et al.* [14]. In another series of experiments, the rate of pyruvate utilization was determined in hearts perfused with Krebs–Henseleit buffer supplemented with 5 mM [1-^{14}C]pyruvate, 5 mM acetate and 2.5 units/l insulin by measuring the production of $^{14}CO_2$ according to Olson *et al.* [15]. In a related study, the rate of acetate utilization was determined from the generation of $^{14}CO_2$ according to the method described by Kramer *et al.* [11].

For assay of tissue metabolic intermediates, hearts were rapidly frozen with a Wollenberger clamp precooled in liquid nitrogen. A known weight of lyophilized ventricular tissue was powdered and then extracted according to the procedure of Schaffer *et al.* [14]. Tissue intermediates were assayed fluorometrically and spectrophotometrically as previously described [14]. To determine tissue glycogen content, lyophilized ventricles were first extracted according to the procedure of Walaas and Walaas [16]. The extract was then hydrolyzed in 1 N H_2SO_4 for 3 hr at 100° and subsequently neutralized to pH 7.4. Glycogen content was measured in terms of glucose units with a Beckman glucose analyzer.

RESULTS

Previous studies [11, 17] have revealed that tolbutamide has little influence on coronary blood flow

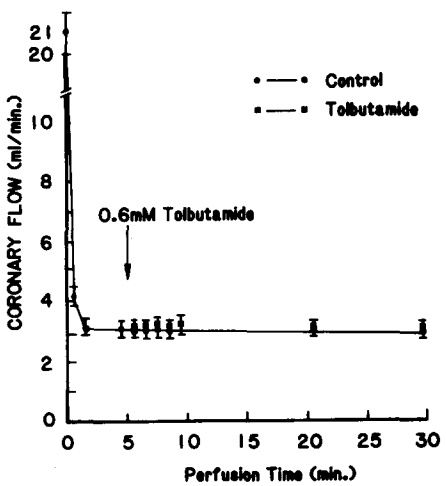

Fig. 1. Effect of tolbutamide on coronary flow during ischemia. All hearts were perfused with Krebs–Henseleit buffer supplemented with 5 mM glucose, 5 mM acetate and 2.5 units/l insulin. Following stabilization, ischemia was induced at time zero as described in Methods. For treated hearts, 0.6 mM tolbutamide was added 5 min after the induction of ischemia. Coronary flow was measured over 1-min intervals. Values represent means ± S.E.M. of four to six hearts.

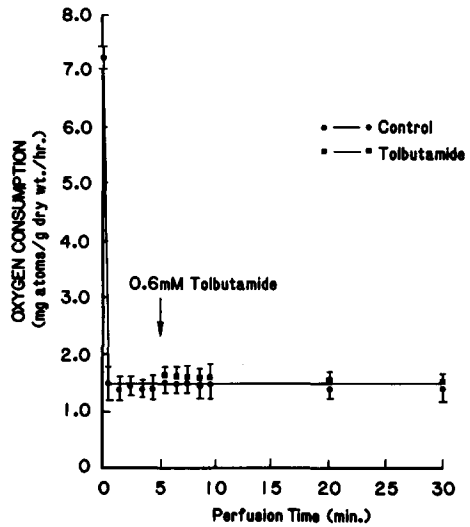

Fig. 2. Effect of tolbutamide on oxygen consumption in the ischemic heart. Hearts were perfused as described in the legend of Fig. 1. Oxygen consumption was monitored continously with an oxygen electrode. Values represent means ± S.E.M. of four to six hearts.

of either the isolated rat heart or intact dog. In apparent agreement with these results, it was found that 0.6 mM tolbutamide also had no appreciable effect on coronary flow of the mildly ischemic heart in which flow was reduced by 86% (Fig. 1). Moreover, the sulfonylurea had no influence on oxygen consumption of the ischemic heart, which was reduced 80% under conditions of restricted flow (Fig. 2).

In accordance with several other investigators [13, 18], high energy phosphate levels fell rapidly following introduction of ischemia. However, tolbutamide was found to attenuate the fall in ATP levels; after 30 min of ischemia, tissue ATP content was 8.1 ± 0.5 and 10.2 ± 0.4 μmoles/g dry wt in hearts untreated and treated with tolbutamide respectively (Table 1). The sulfonylurea-treated hearts also exhibited higher levels of creatine phosphate (CrP) and an elevated ATP/ADP ratio, indicating that the drug improves the metabolic state of the ischemic myocardium.

To test whether the improved energy state of the heart was related to elevations in anaerobic metabolism, the rates of glucose utilization and glycogenolysis were measured. Untreated hearts exhibited a rapid decline in glucose utilization during the first 5 min of ischemia followed by a progressive increase over the next 25 min to a value 34% higher than pre-ischemic. This pattern is consistent with other investigators who have shown that glucose utilization in the perfused rat heart increases during ischemia when coronary flow is reduced by values less than 90% [19]. Addition of tolbutamide prior to the onset of ischemia (Fig. 3A) or after 5 min of low-flow perfusion (Fig. 3B) resulted in an elevation in glucose utilization above the level in untreated ischemic hearts. After 30 min of ischemia the rate of glucose utilization was 30% higher in hearts treated with the sulfonylurea.

Table 1. Effect of tolbutamide on tissue levels of metabolic intermediates*

	ATP	CRP	ATP/ADP	Lactate/Pyruvate
	(μmoles/g dry wt)			
Pre-ischemia	20.3 ± 0.1	32.9 ± 0.6	4.20 ± 0.08	7.95 ± 1.10
Untreated ischemia	8.1 ± 0.5	6.13 ± 0.34	2.62 ± 0.23	54.8 ± 5.0
Tolbutamide-treated ischemia	10.2 ± 0.4†	7.84 ± 0.40‡	3.61 ± 0.29‡	28.7 ± 3.7§

* Values are means ± S.E.M. of four to six hearts. Hearts were perfused as described in the legend of Fig. 1. Following 20 min of stabilization (pre-ischemia values) or 30 min of ischemia, hearts were rapidly frozen with Wollenberger clamps precooled in liquid N_2. Samples were extracted and assayed using procedures mentioned in methods.

† Significantly different from untreated ischemia value ($P < 0.002$).

‡ Significantly different from untreated ischemia value ($P < 0.05$).

§ Significantly different from untreated ischemia value ($P < 0.005$).

Tolbutamide also promoted glycogen breakdown in the ischemic heart (Fig. 4). In agreement with Rovetto *et al.* [20], induction of ischemia was found to transiently accelerate glycogen breakdown, causing a drop in glycogen levels during the first 5 min of ischemia from 125 to 88 μmoles glucose/g dry wt. In the presence of tolbutamide, the fall in glycogen was even more extensive, reaching a new steady-state level of 68 μmoles glucose/g dry wt. The effect of tolbutamide on glycogenolysis significantly altered the rate of glycolysis. In the absence of the drug, ischemia led to a rapid 3-fold stimulation in glycolysis, followed by a decrease in flux to a steady-state value 34% greater than the initial rate (Table 2). When tolbutamide was added to the heart during the steady-state period, there was a further stimulation in glycolysis to levels approximately 2-fold greater than the control.

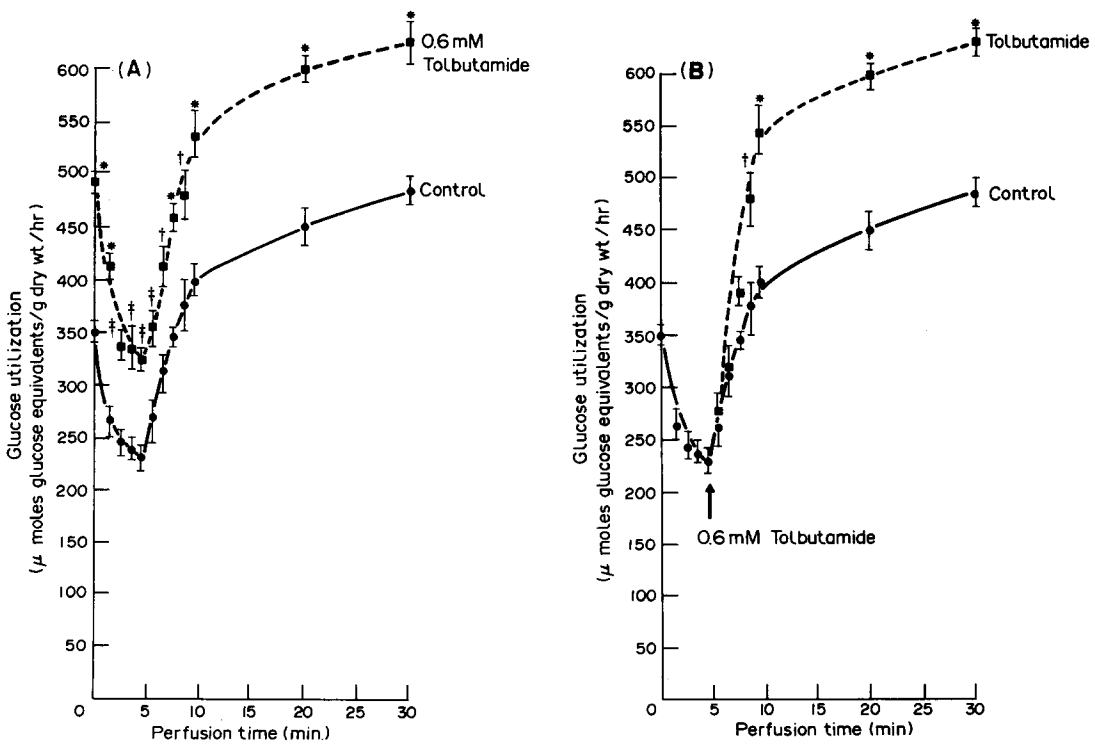

Fig. 3. Effect of tolbutamide on glucose utilization during ischemia. All hearts were perfused with Krebs–Henseleit buffer supplemented with 5 mM [3-^{3}H]glucose, 5 mM acetate and 2.5 units/l insulin. For treated hearts, 0.6 mM tolbutamide was present either at the beginning of the experiment (A) or 5 min after the induction of ischemia (B). The rate of glucose utilization was determined from the generation of ^3H_2O. Values represent means ± S.E.M. of four to six. Key: (*) significantly different from corresponding untreated value ($P < 0.001$); (†) significantly different from corresponding untreated value ($P < 0.002$); and (‡) significantly different from corresponding untreated value ($P < 0.005$).

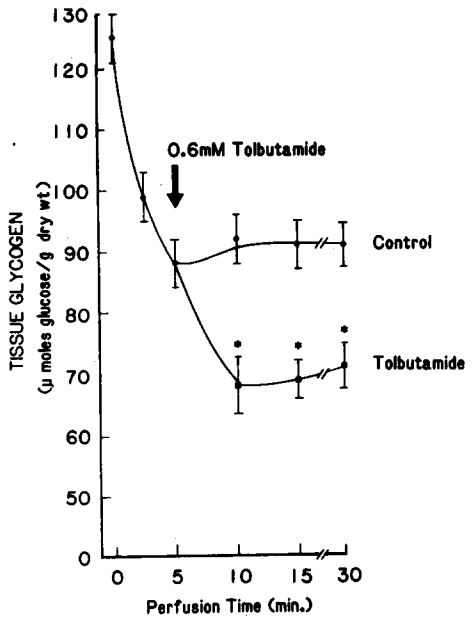

Fig. 4. Effect of tolbutamide on tissue glycogen content in the ischemic heart. Hearts were perfused as described in the legend of Fig. 1. At the designated time intervals, they were rapidly frozen and assayed for glycogen content as described in Methods. Values are means ± S.E.M. of four to six hearts. Key: (*) significantly different from corresponding untreated ischemia value ($P < 0.01$).

Since aerobic metabolism is severely reduced under the ischemic conditions employed, we anticipated that tolbutamide-mediated increases in glucose utilization and glycogen mobilization would be associated with parallel increases in lactate and pyruvate production. This proved not to be the case. As shown in Fig. 5A, the dramatic increase in lactate production observed under ischemic conditions was not altered significantly with tolbutamide treatment. Figure 5B also illustrates no influence of the sulfonylurea on pyruvate production, which fell 70% by 5 min of ischemia. Particularly noteworthy is the observation, that tolbutamide mediated a 48% decrease in the tissue lactate/pyruvate ratio (Table 1).

Calculations reveal that, of the 485 μmoles per g dry wt per hr of glucose consumed after 30 min of ischemia in the absence of tolbutamide, approximately 95% left the heart as lactate. On the other hand, only 74% of the 630 μmoles per g dry wt per hr of glucose consumed in the tolbutamide-treated hearts was converted to lactate, suggesting that a larger percentage of glucose is further metabolized in the tricarboxylic acid cycle in the presence of tolbutamide.

To provide further evidence for this conclusion, we examined the initial step in the oxidation of triose units by the tricarboxylic acid cycle; namely, the conversion of pyruvate to acetyl CoA by pyruvate dehydrogenase. Flux through pyruvate dehydrogenase was monitored by following the rate of $^{14}CO_2$ formation from [1-^{14}C]pyruvate. As illustrated in Fig. 6, the rate of pyruvate utilization decreased within 30 sec from 1517 ± 54 to 440 ± 18 μmoles per g dry wt per hr and then slowly declined to a rate of 240 ± 11 μmoles per dry wt per hr by 30 min of ischemia. When tolbutamide was introduced after 5 min of ischemia, the rate of pyruvate decarboxylation increased slightly for several minutes and then leveled off by 30 min to a rate 27% higher than that observed in untreated ischemic hearts. Since the drug failed to influence pyruvate utilization when acetate was not present in the buffer (data not shown), it was thought that tolbutamide promoted glucose and pyruvate oxidation at the expense of acetate oxidation. To further test this possibility, the effect of tolbutamide on acetate oxidation of the ischemic myocardium was examined. Figure 7 shows that the rate of acetate utilization fell abruptly in the ischemic myocardium. In the absence of the drug, the rate rebounded somewhat to establish a new rate of 310 ± 20 μmoles per g dry wt per hr. However, when tolbutamide was added to the buffer

Table 2. Effect of tolbutamide on glycolysis*

Condition	Glucose utilization	Glycogenolysis† (μmoles/g dry wt/hr)	Glycolytic flux
Control	350	0	350
Ischemia (without tolbutamide)			
3 min	240	760	1000
5 min	230	240	470
10 min	400	0	400
30 min	470	0	470
Ischemia (with 0.6 mM tolbutamide)‡			
10 min	550	240	790
30 min	630	0	630

* Hearts were perfused as described in the legend of Fig. 1. The glucose utilization and glycogenolysis values were derived from the data in Figs. 3 and 4 respectively.

† Values represent μmoles glucose metabolized/g dry wt/hr and are based on the rate at which tissue glycogen content decreases.

‡ When the tolbutamide was included in the buffer, it was added 5 min after ischemia. The 10- and 30-min periods represent the total time of pre-tolbutamide and post-tolbutamide treatment.

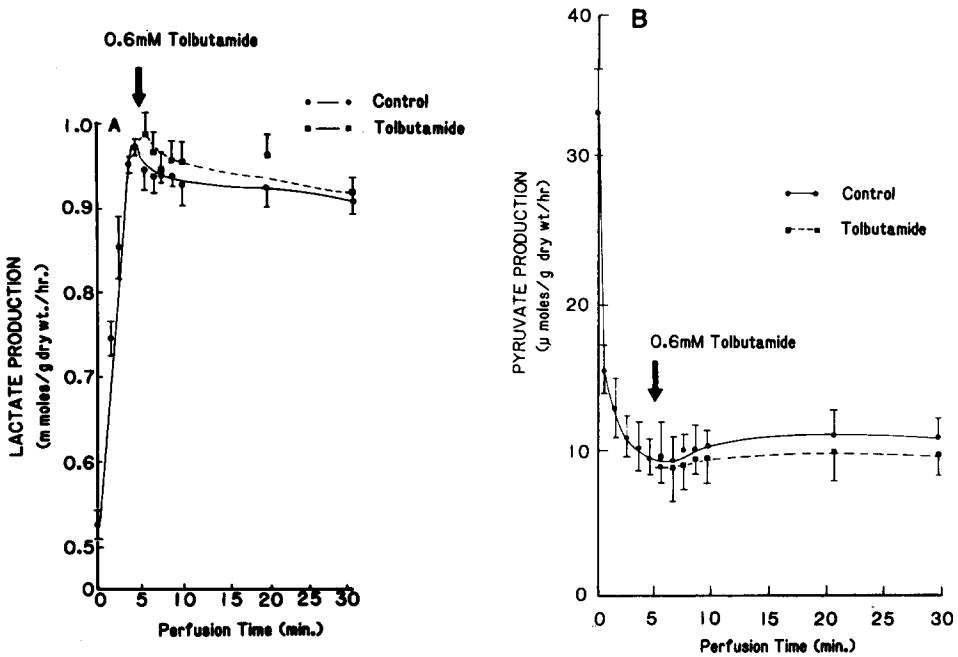

Fig. 5. Effect of tolbutamide on lactate (A) and pyruvate (B) production during ischemia. Hearts were perfused as described in the legend of Fig. 1. Perfusate was collected at 1-min intervals and assayed for lactate and pyruvate content. Values represent means ± S.E.M. of four to six hearts.

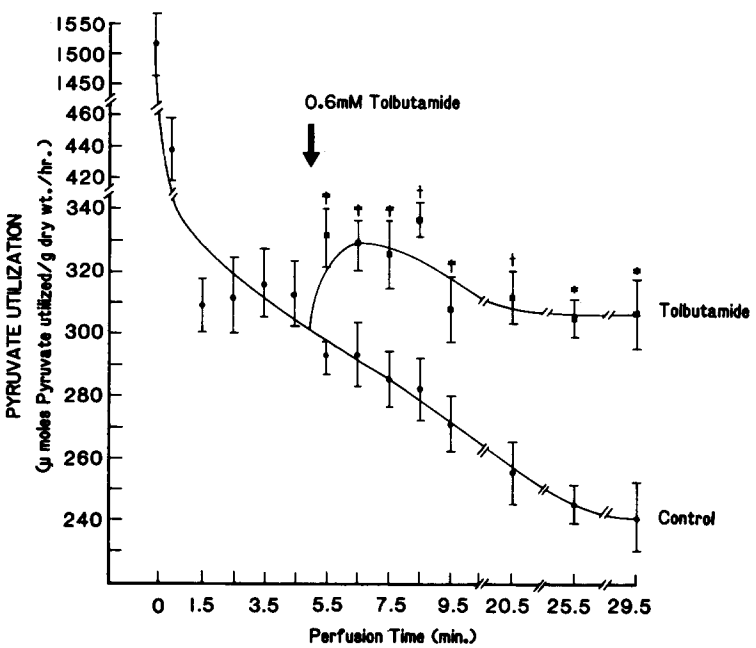

Fig. 6. Effect of tolbutamide on pyruvate utilization in the ischemic heart. All hearts were perfused with Krebs–Henseleit buffer supplemented with 5 mM [1-^{14}C]pyruvate, 5 mM acetate and 2.5 units/l insulin. For treated hearts, 0.6 mM tolbutamide was added 5 min after the induction of ischemia. The rate of pyruvate utilization was determined from the generation of $^{14}CO_2$. Values represent means ± S.E.M. of four to six hearts. Key: (*) significantly different from corresponding untreated ischemia value ($P < 0.001$); (†) significantly different from corresponding untreated ischemia value ($P < 0.005$); and (‡) significantly different from corresponding untreated ischemia value ($P < 0.05$).

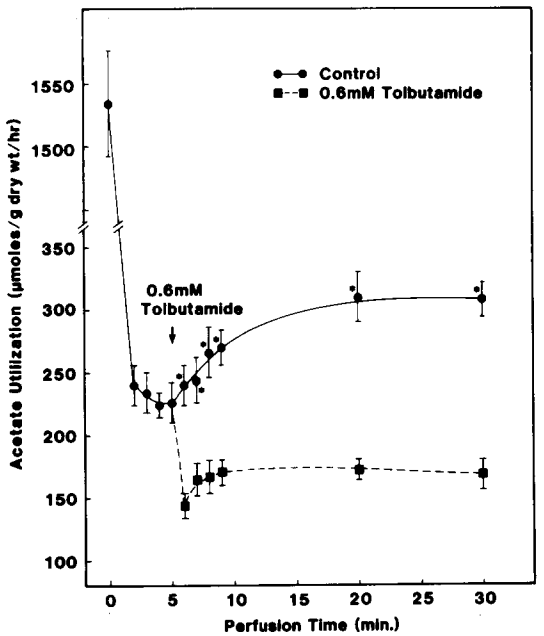

Fig. 7. Effect of tolbutamide on acetate utilization in the ischemic heart. All hearts were perfused with Krebs–Henseleit buffer supplemented with 5 mM glucose, 5 mM [^{14}C-2]acetate and 2.5 units/l insulin. For treated hearts, 0.6 mM tolbutamide was added 5 min after induction of ischemia. The rate of acetate utilization was determined from the generation of $^{14}CO_2$. Values represent means ± S.E.M. of four hearts. Key: (*) significantly different from untreated ischemic value ($P < 0.01$).

after 5 min of ischemia, acetate utilization plunged to a new steady state which was nearly half the rate of the untreated hearts.

DISCUSSION

In the well-oxygenated myocardium, a balance between energy production and utilization is maintained. By contrast, during ischemia an imbalance develops which ultimately leads to a fall in tissue ATP levels. Attempts have been made to reduce the loss of cellular ATP by altering energy metabolism. This work has centered mainly on the use of glucose–insulin–potassium therapy to promote glycolytic ATP production [21]. However, this approach is believed to be of limited value since under conditions of critically reduced metabolite washout the increase in tissue lactate content, the decrease in intracellular pH, and the rise in the cytosolic NADH/NAD ratio act synergistically to slow glycolytic flux [22, 23]. In contrast to glucose–insulin–potassium treatment, we observed in this study that tolbutamide stimulated glycolytic flux (and glycolytically produced ATP) during ischemia without increasing lactate production and actually causing a decrease in tissue lactate and lactate/pyruvate ratio (Table 1 and Ref. 9). Therefore, tolbutamide not only promotes the acceleration of glycolysis but also the removal of inhibitory end products which would act to restrict glycolytic flux.

The fact that glycolytic flux is increased without a rise in lactate production or the tissue lactate/pyruvate ratio suggests that tolbutamide is stimulating NADH oxidation by the malate-aspartate shuttle and promoting the entry of pyruvate into the mitochondria. This is supported by the pyruvate dehydrogenase flux measurements presented in Fig. 6. Kobayashi and Neely [24] reported that, although pyruvate dehydrogenase remains largely in the active form during ischemia, flux through this enzyme would be severely depressed because of feedback inhibition. We found this to be the case; the rate of pyruvate decarboxylation in control hearts was reduced 84% after 30 min of ischemia. With tolbutamide treatment this drop in pyruvate dehydrogenase flux attenuated significantly. This could be due to a direct effect of the drug on pyruvate oxidation as suggested by Gryglewski [25]. Alternatively, it could be caused by changes in calcium metabolism [26] or potentiation of the actions of insulin [10], both widely accepted actions of the drug. Another possibility is that the drug preserves even flow throughout the heart for longer periods and thereby promotes pyruvate uptake and metabolism [27, 28]. However, the most likely explanation is that the drug functions to reduce acetate utilization (Fig. 7), thereby decreasing the tissue levels of AcSCoA, a known inhibitor of pyruvate dehydrogenase [15].

The observation that tolbutamide promotes glycolytic flux would be one mechanism by which the drug would benefit the ischemic myocardium. Recent studies by Opie and coworkers [29, 30] and Higgins and coworkers [31, 32] suggest that glycolytic energy production plays a special role in the maintenance of sarcolemmal function. In 1978, Bricknell and Opie [29] reported that elevated rates of glycolysis in the ischemic myocardium attenuate enzyme release and the severity of arrhythmias upon reperfusion. Higgins *et al.* [31] extended this study, reporting that a graded reduction in glycolysis causes parallel increases in enzyme release from the anoxic heart. In a subsequent paper, Higgins and Bailey [32] showed that release of enzyme from cyanide or iodoacetate intoxicated hearts is more closely associated with the rate of glycolysis rather than total tissue ATP content. Similarly, Bricknell *et al.* [30] reported that severity of ischemic contracture was determined by the source of ATP rather than total tissue ATP content. Thus, the fact that tolbutamide can maintain elevated rates of glycolysis during ischemia would contribute to the improvement in the contractile and metabolic status of the heart.

One other way tolbutamide would benefit the ischemic heart is by promoting pyruvate oxidation at the expense of acetate oxidation. Glucose and pyruvate are much more efficient substrates than acetate and would produce more ATP per unit of oxygen consumed [33]. Since oxygen levels are restricted in ischemia, tolbutamide would increase ATP synthesis.

Therefore, tolbutamide treatment combines the advantages of glucose–insulin–potassium therapy, in that it stimulates glycolysis, and those of pyruvate or dichloroacetate therapy, because it promotes pyruvate utilization [34, 35]. This combination of effects acts synergistically to dramatically improve the status of the ischemic myocardium.

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