Regulation of Hepatic Glutaminase in the Streptozotocin-Induced Diabetic Rat

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The liver of diabetic animals removes increased quantities of glutamine. We therefore examined factors that affect hepatic glutaminase activity in hepatocytes and mitochondria. Glutamine use, through glutaminase, was measured in isolated rat hepatocytes by monitoring the production of $^{14}CO_2$ from [1-^{14}C]glutamine. Hepatocytes from streptozotocin-induced diabetic rats use glutamine more rapidly than do hepatocytes from normal or insulin-maintained diabetic rats. Glutamine use in all of these hepatocytes was stimulated by glucagon and epinephrine. Glutaminase activity, assayed in broken mitochondrial membranes, was increased ~2.5-fold in diabetic rats. The sensitivity of glutaminase, measured in intact liver mitochondria, to phosphate was markedly left-shifted in mitochondria from diabetic rats compared with those from controls. In fact, glutaminase was increased 10-fold at 2.5 mmol/l phosphate compared with controls. This increased sensitivity of glutaminase to physiological concentrations of phosphate is characteristic of its hormonal activation. Therefore, activation of glutaminase plays a major role in diabetes and is as important as increases in its total enzyme amount in determining the increased glutamine uptake in diabetes. *Diabetes* 46:1945–1949, 1997

Glutamine is the most abundant amino acid in mammalian plasma. It serves as a precursor in the biosynthesis of purines, pyrimidines, glucosamine, and NAD. In liver, it is a precursor of urea and glucose. Under normal physiological conditions, there is no significant uptake or release of glutamine by the liver (1). This changes to a net uptake of glutamine in diabetes (1,2). Such an increase in uptake suggests an increased hepatic metabolism of glutamine associated with diabetes.

Glutaminase, which catalyzes the hydrolysis of glutamine to glutamate and ammonia, is the major enzyme of glutamine catabolism in liver cells. This enzyme is located within the mitochondria (3) and is found in a population of periportal hepatocytes adjacent to the portal inflow (4). Glutaminase has been found to be loosely bound to the inner mitochondrial membrane (5). The enzyme is regulated by a number of effectors; in particular, it is activated by ammonia (6), one of its products, and by alkaline pH (7), bicarbonate ions (8), phosphate (5), and spermine (10). Glutamine is transported into hepatocytes via the system N transporter; however, glutaminase, rather than transport, exercises maximum flux control over glutamine utilization in hepatocytes from fed rats (11).

Previous studies using isolated perfused rat liver (12) and isolated hepatocytes (8,9) showed that flux through glutaminase can be stimulated by the glucogenic hormone glucagon. Liver mitochondria isolated from rats injected 30 min previously with glucagon have been shown to accelerate rates of glutamine catabolism compared with control mitochondria from saline-injected rats, even though the total amount of glutaminase remained unchanged (13). Glucagon's activation of glutaminase was particularly evident as an increased sensitivity to activation by phosphate within intact mitochondria.

IDDM involves a destruction of β-cells, resulting in a cessation of insulin secretion. Associated with this disorder is an increase in the level of the counterregulatory hormone glucagon (14,15). Therefore, we studied the effects of diabetes on glutaminase flux in isolated intact mitochondria from streptozotocin (STZ)-induced diabetic rats. If diabetes does result in an increase in glutaminase flux, then we wanted to determine if insulin treatment of diabetic rats would prevent or reduce such an increase. In addition, we wished to examine whether any stimulation associated with diabetes is due to an activation of glutaminase, an increase in total glutaminase, or both.

RESEARCH DESIGN AND METHODS

Materials. Collagenase A was obtained from Boehringer Mannheim (Montreal, Canada). Protamine zinc insulin (pork and beef) was provided by Eli Lilly (Toronto, Canada). [1-^{14}C]Glutamate and omnifluor were obtained from Dupont-New England Nuclear (Mississauga, Canada). QAE Sephadex A-25 was obtained from Pharmacia LKB (Baie D'Urfe, Canada). STZ was obtained from Sigma (St. Louis, MO). Other reagents were of analytical grade. [1-^{14}C]Glutamine was prepared from [1-^{14}C]glutamate using a crude preparation of glutamine synthetase from rat liver as described previously (12,16).

Animals and experimental procedure. Male Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 200–300 g were used for all studies. They were allowed water and Purina Rat Chow (Ralston-Purina, St. Louis, MO) ad libitum. Rats were made diabetic by a single injection of STZ (100 mg/kg body wt) dissolved in 0.01 mol/l citrate buffer (pH 4.5), administered into the tail vein under light ether anesthesia. STZ-induced diabetic rats were injected with Protamine Zinc Insulin, 3–5 U/day, for 5 days. The precise insulin dosages were adjusted to maintain blood glucose at 4–8 mmol/l and permit weight gain. Insulin was withheld from diabetic rats for 6 days preceding experimentation, which caused them to become frankly diabetic (1). Insulin-maintained diabetic rats were injected with insulin up to the day of the experimentation. Daily blood glucose levels were monitored with an Ames Glucometer GX (Miles Canada, Etobicoke, Canada) using blood obtained by tail prick of conscious rats. An hepatic portal blood sample was taken on the day of the experiment for determination

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Received for publication 20 February 1997 and accepted in revised form 7 August 1997.

BSA, bovine serum albumin; STZ, streptozotocin.

of glucose levels by the method of Bergmeyer et al. (17). All of these procedures were approved by Memorial University's Institutional Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

Preparation of hepatocytes. Hepatocytes were prepared as described previously (18). Cell viability was determined by 0.2% trypan blue exclusion and was greater than 98% in all cases. Hepatocytes were resuspended in Krebs-Henseleit medium containing 120 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l KH_2PO_4, 1 mmol/l $MgSO_4$, 25 mmol/l $NaHCO_3$, 2.5 mmol/l $CaCl_2$ with 2.5% (wt/vol) bovine serum albumin (BSA; fraction V, essentially fatty acid free), and equilibrated with O_2/CO_2 (95%/5%).

Measurement of flux through glutaminase in isolated hepatocytes. The procedure for determining flux through glutaminase involves the trapping and counting of $^{14}CO_2$ after incubation of cells with [1-^{14}C]glutamine (1 mmol/l) (19). Incubations were in triplicate in 25-ml Erlenmeyer flasks containing 4–6 mg dry weight of hepatocytes in a final volume of 1 ml. Cells were preincubated in Krebs-Henseleit medium for 20 min at 37°C before the addition of [1-^{14}C]glutamine, to give a final concentration of 1 mmol/l. Where indicated, ammonium chloride was added at the same time as the [1-^{14}C]glutamine. Each flask was gassed with 95:5 O_2:CO_2 for 20 s after the addition of the cells and also after addition of [1-^{14}C]glutamine. The incubation flasks were equipped with rubber septa in which plastic center wells were suspended. NCS tissue solubilizer was introduced into center wells through the septa just before termination of incubation with 0.15 ml of 30% (wt/vol) perchloric acid. $^{14}CO_2$ was collected for 1 h, after which the center wells were transferred to scintillation vials containing 15 ml of scintillation fluid (omnifluor); radioactivity was then determined. Glutamine was determined enzymatically by using glutaminase and glutamate dehydrogenase (20). Glutamate was determined using the glutamate dehydrogenase method (21).

Preparation of liver mitochondria. Liver mitochondria isolated from diabetic rats using standard procedures were found to have poor respiratory control ratios. This has been reported by other investigators for both alloxan- (22,23) and STZ-induced diabetes (24,25), and has been attributed to decreased state 3 respiration due to an inhibition of adenine nucleotide penetration (22,23). This may be a consequence of the increased hepatic lipid content, particularly long chain acyl-CoA esters, that occurs in diabetes and is known to inhibit the adenine nucleotide translocase (23). Similar results have been found during hibernation and exhaustive exercise (23–27). The sluggish response to ADP in liver mitochondria may be partially reversed by addition of high concentrations of BSA to the isolation medium (23). Similar procedures have been used to reverse the poor response in liver mitochondria isolated from rats previously exposed to exhaustive exercise (24,27).

We devised a procedure that permitted us to isolate well-coupled mitochondria from STZ-induced diabetic rats. The key point was to flush the liver with a mitochondrial homogenization medium that contained a high concentration of serum albumin. Thus the extracellular space was replaced with this medium so that, upon homogenization, lipids were efficiently complexed. To reduce the fatty acid content of the liver, it was first flushed through a portal vein cannula with 50 ml of mitochondrial isolation medium containing 50 mg/ml BSA (fraction V, essentially fatty acid–free) through a portal vein cannula. The liver was then removed and placed in ice-cold isolation medium containing 5 mg BSA/ml. The liver was homogenized in this medium. The centrifugation and resuspension steps were the same as for the standard mitochondrial isolation procedure, except that all media contained 5 mg BSA/ml. This procedure resulted in well-coupled mitochondria that responded well to the addition of ADP, using α-ketoglutarate as substrate. Livers from control, insulin-maintained diabetic, and diabetic rats were subjected to this procedure.

Preparation of mitochondrial membranes. The mitochondrial suspension was diluted 1:1 with distilled water, and disrupted by freezing in liquid nitrogen and thawing in water at 37°C. This freezing/thawing cycle was repeated three times (7). The final suspension was diluted 1:4 with mitochondrial isolation medium, followed by centrifugation at 40,000*g* for 20 min at 5°C to isolate the membrane fraction. The pellet formed was resuspended in isolation medium and adjusted to a final protein concentration of 20 mg/ml, as determined by the biuret procedure, with BSA as standard (28).

Glutaminase assay. Flux through glutaminase in isolated intact mitochondria was determined as described (13). For the assay of glutaminase in broken mitochondria, membranes isolated from frozen then thawed mitochondria were incubated at 37°C in 300 mmol/l mannitol, 10 mmol/l HEPES, 0.7 mmol/l NH_4Cl, 20 mmol/l glutamine, and 20 mmol/l phosphate (pH 7.4) (7). The reaction was started by adding 50 µl of 20 mg/ml of membranes, to give a final protein concentration of 1 mg/ml. The reaction was terminated after 10 min by adding 0.3 ml of 7% (wt/vol) perchloric acid. The supernatant was adjusted to pH 8.8 with K_3PO_4, and glutamate was determined using the glutamate dehydrogenase method (21).

Data analyses. All values are expressed as means ± SE. Statistical analysis was by Student's *t* test or, in the case of multiple comparisons, by Tukey's multiple comparison test.

RESULTS

Twenty-four hours following STZ injection, blood glucose levels were greater than 12 mmol/l, thus confirming the induction of diabetes. On the day of the experiment, the portal venous glucose levels were 9.1 ± 0.6 mmol/l for controls, 8.0 ± 1.5 mmol/l for insulin-maintained diabetic rats, and 29.2 ± 1.7 for diabetic rats. The body weight of the diabetic rats was also significantly lower (298 ± 9 g) compared with either controls (340 ± 13 g) or insulin-maintained diabetic rats (330 ± 6 g).

Glutamine disappearance and $^{14}CO_2$ release in isolated hepatocytes. There are two potential problems with using CO_2 release to measure flux through glutaminase. The first stems from the fact that there are two steps between glutaminase and the actual release of CO_2 by α-ketoglutarate dehydrogenase. Thus CO_2 release is somewhat removed from the actual enzyme being measured, and there is opportunity for isotope dilution to occur. The second complication arises because the isolated hepatocyte suspension contains periportal cells with glutaminase and perivenous cells with glutamine synthetase. Thus glutamate, produced by glutaminase, could be resynthesized into glutamine by glutamine synthetase without release of $^{14}CO_2$, resulting in an underestimation of the extent of glutamine utilization by glutaminase. Methionine sulfoximine, however, can be used as an inhibitor of glutamine synthetase (29). To test the validity of using $^{14}CO_2$ release from [1-^{14}C]glutamine to determine glutaminase flux, glutamine disappearance was determined chemically and $^{14}CO_2$ was simultaneously collected in both the presence and absence of 1 mmol/l methionine sulfoximine (19). In the absence of methionine sulfoximine, no glutamine disappearance was observed, but $^{14}CO_2$ release did occur (Table 1). This suggests that glutamine disappearance determined enzymatically in the absence of methionine sulfoximine underestimates glutaminase flux in isolated hepatocytes. This could be due to the production of glutamine by glutamine synthetase in the perivenous cells of the isolated hepatocyte suspension. This was confirmed by the results showing that, in the presence of methionine sulfoximine, glutamine disappearance was the same as $^{14}CO_2$ production (Table 1). It should also be noted that $^{14}CO_2$ release was unaffected by the presence of methionine sulfoximine. Thus $^{14}CO_2$ production from [1-^{14}C]glutamine does represent a valid method for determining flux through glutaminase in isolated hepatocytes (19).

The results in Table 1 also show the effects of added glucagon on hepatic glutamine and glutamate metabolism. There was a significant increase in glutamine disappearance and $^{14}CO_2$ release in the presence of glucagon. The results also suggest that glucagon causes an increase in glutamate oxidation, as indicated by a decrease in glutamate accumulation.

Flux through glutaminase in isolated hepatocytes. Flux through glutaminase in hepatocytes isolated from the three different groups of rats was examined (Table 2). At a near-physiological portal vein concentration of 0.5 mmol/l ammonium chloride, flux through glutaminase was significantly higher in hepatocytes isolated from diabetic rats than in those from nondiabetic controls or insulin-maintained diabetic rats (13.74 ± 2.09 vs. 6.98 ± 1.28 and 5.43 ± 1.58 nmol $CO_2 \cdot mg^{-1}$ dry wt cells $\cdot$ 30 min^{-1}). Thus STZ-induced diabetes was accompanied by increased flux through glutaminase in isolated hepatocytes. Daily insulin treatment of the diabetic rats prevented this increase. Glucagon and epinephrine are

TABLE 1
Glutamine disappearances and $^{14}CO_2$ release in isolated hepatocytes

Additions	Glutamine disappearance	$^{14}CO_2$ production	Glutamate production	Glutamine disappearance ($^{14}CO_2$ production)
None	–0.062 ± 0.09 (5)	0.19 ± 0.01 (5)	0.11 ± 0.01 (5)	—
MSO	0.18 ± 0.04 (5)	0.20 ± 0.01 (5)	0.15 ± 0.02 (5)	0.89
Glucagon	0.12 ± 0.05 (4)	0.35 ± 0.02 (4)*	0.05 ± 0.01 (4)*	0.34
Glucagon + MSO	0.25 ± 0.03 (3)	0.32 ± 0.01 (4)*	0.07 ± 0.01 (4)*	0.79

Data are means ± SE (n) and are expressed as nmol · mg^{-1} dry wt · min^{-1}. The concentrations used are 1 mmol/l [1-^{14}C]glutamine, 2 mmol/l ammonium chloride, 10^{-7} mol/l glucagon, and 1 mmol/l methionine sulfoximine (MSO). Normal nondiabetic rats were used. *P < 0.05 versus no added glucagon.

known to stimulate flux through glutaminase in perfused rat liver (12) and isolated hepatocytes (9). Table 2 also shows the effects of glucagon and epinephrine on glutaminase flux in isolated hepatocytes. In the absence of added ammonium chloride, there was no stimulation by either glucagon or epinephrine. In the presence of 0.5 mmol/l added ammonium chloride, glucagon and epinephrine stimulated flux in the isolated hepatocytes from the three groups.

Glutaminase flux in isolated intact mitochondria. Lacey et al. (13) established that glutaminase flux in intact mitochondria from glucagon-injected rats was more sensitive to activation by inorganic phosphate than mitochondria isolated from saline-injected controls. This activation was not evident when glutaminase was assayed in broken mitochondria. A similar phenomenon has been reported by Ewart and Brosnan (30) for glutaminase activated after high-protein feeding. Table 3 gives the glutaminase flux in isolated intact liver mitochondria from control, insulin-maintained diabetic, and diabetic rats. The flux was determined at three different phosphate concentrations. At all phosphate concentrations examined, glutaminase flux in the mitochondria from diabetic and insulin-maintained diabetic rats was significantly higher than in mitochondria from nondiabetic control rats (P < 0.05). The mean fluxes through glutaminase in the mitochondria from insulin-maintained diabetic rats were not as high as those from diabetic rats, but were not significantly different from those found in diabetic rats. Table 3 also gives the ratio of flux determined at 20 mmol/l phosphate compared with that determined at 2.5 mmol/l phosphate. This ratio is significantly higher (P < 0.05) in mitochondria from control, nondiabetic (9.88 ± 2.26) than diabetic rats (2.90 ± 0.87). Thus, as in mitochondria isolated from glucagon-injected rats (13) and high-protein–fed rats (30), glutaminase in intact mitochondria from diabetic rats is more sensitive to stimulation by phosphate. The increased glutaminase flux in mitochondria from diabetic rats could not be explained by inadvertent activation by increased concentrations of ammonia. When glutaminase flux was determined in the presence of 10 mmol/l Pi and a maximal stimulatory NH_4Cl concentration of 1 mmol/l, glutaminase flux was still observed to be higher in mitochondria isolated from diabetic rats (44.6 vs. 16.5 nmol · min^{-1} · mg^{-1} protein; average of two separate experiments).

Glutaminase activity in mitochondrial membranes. We assayed glutaminase in broken mitochondrial membranes isolated from diabetic, insulin-maintained diabetic, and nondiabetic control rats. The activities were 48 ± 3 (control), 127 ± 14 (diabetic), and 98 ± 3 (insulin-maintained diabetic) nmol · 10 min^{-1} · mg^{-1} protein (means ± SE). Both the diabetic and insulin-maintained diabetic groups were significantly different from the normal group (P < 0.05). The diabetic and insulin-maintained groups were not significantly different from each other when examined at each phosphate concentration (possibly due to the small number of observations), but when these data were examined at all phosphate concentrations by analysis of variance followed by Student-Newman-Keuls test, they were found to be different at the 5% level.

DISCUSSION

Diabetes is characterized by a decrease in, or total lack of, insulin action, and a relative increase in counterregulatory hormones such as glucagon. Previous studies using STZ-induced diabetic rats have found significant increases in

TABLE 2
Flux through glutaminase in isolated hepatocytes from control, insulin-maintained diabetic, and diabetic rats

	Control	Insulin-maintained diabetic	Diabetic
No addition	5.55 ± 0.64	3.56 ± 0.34	7.10 ± 0.65‡
+glucagon	5.62 ± 0.64	5.19 ± 0.33	8.03 ± 0.72†‡
+epinephrine	8.10 ± 0.69	6.57 ± 0.94	11.58 ± 1.64‡
0.5 mmol/l NH_4Cl	6.98 ± 1.28	5.43 ± 1.58	13.74 ± 2.09†‡
+glucagon	9.58 ± 0.60*	12.63 ± 0.60*†	19.85 ± 3.41*†
+epinephrine	13.73 ± 0.95*	19.02 ± 1.10*†	30.67 ± 4.70*†

Data are means ± SE. Glutaminase flux is expressed as nmol CO_2 produced · mg dry wt $cells^{-1}$ · 30 min^{-1}; n = 4 for control and diabetic; n = 3 for insulin-maintained diabetic. Final concentrations: glucagon 10^{-7} mol/l, epinephrine 10^{-6} mol/l, [1-^{14}C]glutamine 1 mmol/l. *P < 0.05 versus no added hormone by paired t test; †P < 0.05 vs. control (Tukey's multiple comparison test); ‡P < 0.05 versus insulin-maintained (Tukey's multiple comparison test).

TABLE 3
Glutaminase flux in isolated mitochondria

	2.5 mmol/l Pi	10 mmol/l Pi	20 mmol/l Pi	RCR
Control, nondiabetic	2.15 ± 0.82	10.65 ± 1.79	18.88 ± 2.99	4.2 ± 0.3
Insulin-maintained diabetic	11.78 ± 4.92*	27.88 ± 5.59*	39.63 ± 5.92*	4.6 ± 0.3
Diabetic, off insulin 6 days	25.73 ± 6.17*	44.85 ± 7.66*	55.05 ± 6.41*	4.2 ± 0.2
Ratio of flux at 20 mmol/l Pi/flux at 2.5 mmol/l Pi				
Control	9.88 ± 2.26	—	—	—
Insulin-maintained diabetic	—	5.25 ± 1.34	—	—
Diabetic	—	—	2.90 ± 0.87*	—

Data are means ± SE and are nmol glutamate produced · mg^{-1} mitochondrial protein · min^{-1}. RCR, respiratory control ratio of intact mitochondria using α-ketoglutamate as substrate. Glutaminase flux was determined using 20 mmol/l glutamine and the indicated phosphate concentration. *$P < 0.05$ versus control.

plasma glucagon levels in untreated diabetic rats (15,31). A major problem associated with diabetes is continued production of glucose by gluconeogenesis, even in the presence of hyperglycemia. Glutamine, being a glucogenic amino acid, can be converted to glucose in the liver. The first step in this conversion involves the formation of glutamate from glutamine, a conversion catalyzed by the mitochondrial enzyme glutaminase.

Diabetes results in an increase in glutaminase activity due to more glutaminase protein (32,33). Previously, however, little attention has been given to the involvement of hormonal changes in the regulation of hepatic glutaminase in diabetes, even though this enzyme is known to be stimulated by gluconeogenic hormones (8,12). Our studies indicated that glutaminase flux is increased in hepatocytes isolated from the diabetic compared with the nondiabetic control group. Maintaining the diabetic rats on daily insulin injections prevented the increase in glutaminase flux in isolated hepatocytes. Similar results were found in mitochondria from diabetic rats, in that glutaminase flux was also higher than in mitochondria from control rats, with insulin-maintained diabetic rats having intermediate rates. There is also an increased sensitivity of glutaminase toward phosphate in intact mitochondria (Table 3). This is in agreement with the previous results obtained for both glucagon-injected rats (13) and high-protein–fed rats (30). This provides evidence for the hormonal activation of hepatic glutaminase in diabetes.

Increased metabolic disposal of amino acids is a well-known phenomenon in diabetes. The old description "melting the flesh into urine" is not an inaccurate description of the loss of the body protein and increased urinary nitrogen excretion in uncontrolled diabetes. The protein anabolic actions of insulin are well established, involving both increased protein synthesis and decreased proteolysis in liver and muscle (34). However, changes in protein synthesis and degradation, in themselves, do not result in increased urinary nitrogen loss unless the amino acids not incorporated into protein are catabolized. The increased amino acid oxidation in diabetes has been examined by Lariviere et al. (35), who found that IDDM patients intensively treated with insulin exhibited significantly elevated obligatory nitrogen losses. In fact, they reported that "even mildly elevated average blood values, well within the guidelines for intensive therapy, were strongly correlated with high rates of urinary N excretion." Based on these results, Hoffer (36) has argued for a reassessment of protein requirements in diabetes, being concerned that, with increased rates of amino acid catabolism occurring even in well-controlled diabetes, diabetic individuals may be at risk for protein malnutrition should protein intakes be further reduced, as has been suggested to retard the progression of renal disease. Thus the study of amino acid catabolism in diabetes is of considerable importance. In this regard, it must be emphasized that glutamine plays a major role in the nitrogen economy of mammals. It is, quantitatively, one of the principal amino acids released from muscle in catabolic states (37). Nissim et al. (38), using ^{15}N-labeled amino acids, showed that hepatocytes use glutamine as the dominant source of nitrogen for urea synthesis, even when supplied by all 20 amino acids at their physiological concentrations.

The involvement of hyperglucagonemia in increased amino acid oxidation and urea production has recently been demonstrated (39). In that study, one group of STZ-induced diabetic rats was injected with a specific antibody against pancreatic glucagon, whereas a second group received an injection of nonimmune rabbit serum. Urea production increased 2.5-fold in the serum-treated diabetic rats, whereas the production in the glucagon antibody-treated diabetic rats was identical to the nondiabetic controls (39). Such a study clearly demonstrates the importance of glucagon in increasing hepatic amino acid catabolism in diabetes.

The increase in glutaminase flux is not entirely unexpected, since the glutaminase activity, measured in broken mitochondria, is also increased with diabetes (32), as we have shown above. Smith and Watford (33) also reported an approximate fourfold increase in relative abundance of hepatic glutaminase mRNA in 6-day STZ-induced diabetic rats. However, at a near-physiological phosphate concentration of 2.0–2.5 mmol/l, there was a 12-fold increase in glutaminase flux in intact mitochondria (Table 3). Such an increase can be only partially accounted for by the threefold increase in glutaminase activity in mitochondria. In fact, the total glutaminase activity was elevated in the insulin-treated diabetic rats, yet the glutaminase flux in hepatocytes was not increased. In mitochondria from insulin-treated diabetic rats, flux through glutaminase is only about 40% of that seen in mitochondria from diabetic rats at a physiological phosphate concentration. Thus the activation of glutaminase is at least as important as the increase in glutaminase protein in contributing to the increased catabolism of this amino acid in diabetes.

Although the focus of this study was the regulation of glutaminase, it is of interest to consider the ultimate fate of the glutamine carbon. Essentially, it could be directly oxidized or converted to glucose. It is well established that glucagon stimulates gluconeogenesis from glutamine in rat hepatocytes (40) and in isolated perfused rat liver (41). Glutamine is a major gluconeogenic amino acid in humans (42). Consoli et al. (43) examined the contribution of glutamine to glucose production in fasting NIDDM patients, since it is known that increased gluconeogenesis is the predominant mechanism by which blood glucose is elevated in such patients. Experiments with labeled glutamine clearly showed a doubling in the rate of conversion of glutamine to glucose, even though its turnover was unchanged (44). Thus it appears that decreased glutamine oxidation in certain tissues must also occur to permit increased gluconeogenesis from glutamine in liver and kidney. We are unaware of any comparable experiments in IDDM patients.

ACKNOWLEDGMENTS

This study was supported by a grant from the Canadian Diabetes Association and a grant from the Medical Research Council of Canada (J.T.B.). S.A.S., H.S.E., and C.M. were supported by graduate fellowships from the School of Graduate Studies, Memorial University.

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