The regulation of phosphate-activated glutaminase activity and glutamine metabolism in the streptozotocin-diabetic rat

Malcolm WATFORD, Eleanor M. SMITH and Emily J. ERBELDING

Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology of the Division of Biological Sciences, Savage Hall, Cornell University, Ithaca, NY 14853, U.S.A.

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The activity of phosphate-activated glutaminase was increased in the kidney, liver and small intestine of rats made diabetic for 6 days with injection of streptozotocin (75 mg/kg body wt.). Insulin prevented this increase in all three tissues. Treatment with NaHCO_3 , to correct the acidosis that accompanies diabetes, prevented the increase in renal glutaminase activity, but not that in liver or small intestine. Chemically induced acidosis (NH_4Cl solution as drinking water) or alkalosis (NaHCO_3 solution as drinking water) increased and decreased, respectively, glutaminase activity in the kidney, but were without significant effect on the activity in liver and small intestine. The increase in glutaminase activity in the small intestine during diabetes was due to an overall increase in the size of this organ, and was only detectable when activity was expressed in terms of whole organ, not mucosal scrapings or isolated enterocytes. Prolonged diabetes (40 days) resulted in an even greater increase in the size and glutaminase activity of the small intestine. Despite this marked increase in capacity for glutamine catabolism, arteriovenous-difference measurements showed a complete suppression of plasma glutamine utilization by the small intestine during diabetes, confirming the report by Brosnan, Man, Hall, Colbourne & Brosnan [(1983) *Am. J. Physiol.* **235**, E261–E265].

Glutamine functions as a major carrier of both nitrogen and carbon between tissues in the mammalian body. In the rat it is well established that the major site of glutamine synthesis is skeletal muscle (Ruderman & Berger, 1974; Schrock & Goldstein, 1981). Under normal physiological conditions the small intestine is probably the most important site of glutamine utilization; the kidney does not appear to produce or use glutamine; the role of the liver is uncertain. Glutamine utilization by other tissues is not of quantitative importance (see Lund, 1980, for review). Evidence from arteriovenous-difference measurements points to the liver as a site of glutamine production under physiological conditions (Aikawa *et al.*, 1973; P. Lund & L. G. Welt, unpublished work reported in Lund & Watford, 1976; Rémésy *et al.*, 1978), but a major site of glutamine utilization in diabetes (Schrock & Goldstein, 1981; Brosnan *et al.*, 1983) or after 'high-protein' feeding (Rémésy *et al.*, 1978; Yamamoto *et al.*, 1974).

In metabolic acidosis the kidney becomes a major site of glutamine utilization. The nitrogenous end product, ammonia, is used to titrate

urinary acids as ammonium salts, thereby conserving other cations. This increased utilization of glutamine by the kidney would rapidly deplete the plasma glutamine pool if its metabolism elsewhere remained unchanged. Since plasma glutamine concentrations are maintained, there must be increased production and/or decreased utilization of glutamine by extrarenal tissues.

Models of chronic metabolic acidosis have usually involved exogenous administration of NH_4Cl or HCl . An endogenously generated acidosis is that accompanying uncontrolled diabetes mellitus. From arteriovenous-difference measurements, Schrock & Goldstein (1981) report increased glutamine uptake by the kidney and increased release by skeletal muscle in both chemically induced acidosis and diabetic ketoacidosis. They also report that the liver, normally a site of glutamine utilization, changes to glutamine production in chemical acidosis, but increases glutamine removal in diabetes. More recently, Brosnan *et al.* (1983) have confirmed increased hepatic and renal glutamine utilization in diabetic rats. They also report a complete cessation

of glutamine utilization by the small intestine, whereas Schrock & Goldstein (1981) find no change in this tissue.

The regulation of glutamine metabolism could involve adaptations in the activity of key enzymes in the pathway of glutamine catabolism. There are a number of enzymes capable of using glutamine as a substrate. Of these, phosphate-activated glutaminase (L-glutamine aminohydrolase, EC 3.5.1.2), sometimes known as glutaminase I or phosphate-dependent glutaminase, is the only true glutaminase (Meister, 1972), in that it catalyses the stoichiometric formation of ammonia and glutamate from the hydrolysis of glutamine. It is now accepted as the major enzyme of glutamine breakdown in most mammalian tissues, with the possible exception of lactating mammary gland [for reviews, see Lund (1980) and Kovacevic & McGivan (1983)]. There are two distinct forms of phosphate-activated glutaminase (hereafter referred to as glutaminase), as first noted by Krebs (1935). The enzyme in adult liver is unique, and all other glutaminases are of the 'kidney' type. The two forms are characterized by different immunochemical and kinetic properties (see Kovacevic & McGivan, 1983; Snodgrass & Lund, 1984; Patel & McGivan, 1984).

The work described in the present paper was designed to determine if adaptive changes in glutaminase activity occur in response to diabetes and, if so, whether they are due to the associated acidosis or to some other aspect of the diabetic state. The results show distinct differences in the response of the enzyme in different tissues. The relevance of these changes to the regulation of glutamine metabolism is discussed. Some of these results have been presented in preliminary form (Watford & Smith, 1983).

Materials and methods

Dithiothreitol was from Calbiochem-Behring (LaJolla, CA, U.S.A.). Streptozotocin, glutamine, glutaminase (grade V) and bovine serum albumin (fraction V) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other enzymes and coenzymes were products of Boehringer Corp. (Indianapolis, IN, U.S.A.). Insulin was a mixture of bovine and pig zinc-insulin (Lente, Iletin; Eli Lilly and Co., Indianapolis, IN, U.S.A.).

Rats

Male Sprague-Dawley rats (150–250 g), obtained from Blue-Spruce (Altamont, NY, U.S.A.), were maintained on a standard diet (Purina) and water *ad libitum*. Chronic metabolic acidosis and alkalosis were induced by replacement of drinking water with 1.5% (w/v) solutions of NH_4Cl and

NaHCO_3 . Rats were made diabetic by injection of streptozotocin (75 mg/kg body wt., in 8.1% $\text{NaCl}/50\text{ mM}$ -sodium citrate, pH 4.5) directly into a tail vein. For some experiments insulin was administered (subcutaneously) twice daily at doses which maintained blood glucose concentrations and rates of body-weight gain comparable with those in control animals. Rats receiving insulin also received 5% (w/v) glucose solution as drinking water. Some of the streptozotocin-treated rats also received NaHCO_3 solution as drinking water to correct the acidosis associated with diabetes (Kamm *et al.*, 1974).

Preparation of tissue homogenates

Rats were killed by a blow on the head, immediately followed by decapitation and exsanguination. The liver and kidneys were quickly removed, freed of connecting tissue, rinsed in ice-cold saline (0.9% NaCl), blotted dry, and weighed. The small intestine, beginning at the junction with the duodenum and ending at the caecum, was removed, trimmed of connecting tissue, and the lumen contents were flushed out with 40 ml of ice-cold saline. Residual saline in the lumen was removed by squeezing the intestine gently along its length. Approx. 1 g of liver and one whole kidney were homogenized in 10 vol. of ice-cold 0.3M-sucrose/1 mM-EDTA/5 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.4, with an Ultra-Turrax Tissue-miser (Tekmar, Cincinnati, OH, U.S.A.). The whole small intestine, mucosal scrapings (Warburg, 1924) and isolated enterocytes (Watford *et al.*, 1979) were homogenized in 5 vol. of the medium, with the addition of 2 mM-dithiothreitol to disperse mucus. Homogenates were filtered through cheesecloth and frozen and thawed five times to disrupt mitochondria.

Determination of glutaminase activity

Glutaminase activity in kidney and intestine homogenates was determined by the method of Curthoys & Lowry (1973). This method involves the use of 20 mM-glutamine as substrate. The hepatic enzyme has a relatively high K_m for glutamine (6–40 mM; Huang & Knox, 1976; Horowitz & Knox, 1968; McGivan *et al.*, 1980; Patel & McGivan, 1984), so that the glutamine concentration in the original assay was limiting when used for liver. In preliminary experiments we found that >100 mM-glutamine was needed to saturate the assay system for hepatic glutaminase activity. Final conditions for the assay were 132 mM-glutamine, 125 mM-potassium phosphate, 0.16 mM-EDTA, 16.7 mM-Tris, pH 8.6. Under these conditions the addition of known activators (ATP, NH_4Cl , HCO_3^-) of hepatic glutaminase was with-

out effect. Snodgrass & Lund (1984) have reported that the spectrophotometric assay of glutamate in the presence of high glutamine concentrations is subject to drift, owing to contamination of commercial glutamate dehydrogenase preparations with glutaminase activity. This was also a problem in the present studies when glutaminase was assayed by the enzymic determination of glutamate production; however, we were able to correct for drift by the use of glutamate standards (see Snodgrass & Lund, 1984). In addition, we have compared some of our results obtained by this assay with the glutaminase assay of Curthoys & Shapiro (1982), in which [$U\text{-}^{14}\text{C}$]glutamine is hydrolysed and the glutamate produced is detected by scintillation counting. There were no apparent differences in the activities measured by either method. Results reported in the present paper were determined by the method of Curthoys & Lowry (1973). All assays were carried out at 37°C with at least two different concentrations of enzyme, and activities were linear with time.

Isolated enterocytes

Enterocytes were isolated and incubated as previously described (Watford *et al.*, 1979). For the determination of glutaminase activity, approx. 1 g of enterocytes was prepared by centrifugation of the enterocyte suspension and homogenized as described above.

Arteriovenous-difference measurements

Rats were anaesthetized with diethyl ether, the abdominal cavity was opened and blood was drawn into heparinized syringes fitted with 26-gauge needles. Blood was drawn simultaneously from the aorta and the portal vein. Samples (0.5–1.0 ml) were quickly added to 1.0 ml of ice-cold HClO_4 (10% v/v). Assays were performed on the neutralized deproteinized supernatants.

Determination of metabolites

Glutamine was determined as glutamate after hydrolysis with glutaminase (Lund, 1974). Glut-

amate was measured by the method of Bernt & Bergmeyer (1974), ammonia by that of Kun & Kearney (1974) and alanine by that of Williamson (1974). Glucose was measured by the glucose oxidase-peroxidase method (Sigma kit no. 510). Protein was determined by the method of Lowry *et al.* (1951).

Results

Rats made diabetic with streptozotocin, including those also receiving NaHCO_3 , had blood glucose concentration in excess of 20 mM. All other rats, including those treated with streptozotocin plus insulin, had blood glucose concentrations of less than 5 mM. The diabetic and diabetic/ NaHCO_3 -treated rats lost body weight during the course of the experiments (results not shown). Acidotic rats showed no change in body weight, and all other groups gained body weight.

Glutaminase activity in the kidney

Renal glutaminase activity was markedly increased by acidosis and slightly decreased by alkalosis (Table 1). Diabetes caused a slight increase in activity, which was prevented by treatment with either insulin or NaHCO_3 . The weight of the kidneys, when expressed as a percentage of body weight, was also increased by diabetes and diabetes plus NaHCO_3 .

Glutaminase activity in the liver

The weight of the liver, expressed as a percentage of body weight, was unaffected by any of the treatments (Table 2). The activity of hepatic glutaminase was not changed by acidosis or alkalosis (Table 2). Diabetes resulted in a 4-fold increase in hepatic glutaminase activity, which was prevented by treatment with insulin, but not with NaHCO_3 .

Glutaminase activity in the small intestine

The activity of glutaminase in rat small intestine has been reported to decrease slightly in starvation,

Table 1. *Glutaminase activity in rat kidney*

Rats were treated for 6 days. Activities were determined as described in the Materials and methods section and are expressed as μmol of glutamate formed/min at 37°C. Results are means $\pm$ S.E.M., where n is the number of experiments, or are the means of two experiments.

Treatment group	n	Kidney weight (% of body wt.)	Glutaminase activity		
			(units/g of tissue)	(units/100 g body wt.)	(munits/mg of protein)
Control	7	0.76 ± 0.02	19.81 ± 1.85	15.05 ± 1.54	181 ± 18
Acidotic	4	0.90 ± 0.03	71.78 ± 7.60	64.16 ± 6.50	533 ± 34
Alkalotic	5	0.76 ± 0.02	14.57 ± 2.04	11.36 ± 1.90	118 ± 11
Diabetic	4	1.28 ± 0.07	27.77 ± 1.04	32.87 ± 1.46	229 ± 24
Diabetic + NaHCO_3	5	1.20 ± 0.03	20.66 ± 0.75	26.14 ± 1.09	150 ± 16
Diabetic + insulin	2	0.78	22.22	17.45	205

Table 2. *Glutaminase activity in rat liver*

Rats were treated for 6 days. Activities were determined as described in the Materials and methods section and are expressed as μmol of glutamate formed/min at 37°C . Results are means $\pm$ S.E.M., where n is the number of experiments, or are the means of two experiments.

Treatment group	n	Liver weight (% of body wt.)	Glutaminase activity		
			(units/g of tissue)	(units/100g body wt.)	(munits/mg of protein)
Control	7	3.96 ± 0.08	4.12 ± 0.40	16.36 ± 1.65	24 ± 2.6
Acidotic	4	3.48 ± 0.10	4.02 ± 0.55	13.98 ± 1.87	23 ± 3.0
Alkalotic	5	4.00 ± 0.15	3.51 ± 0.43	14.06 ± 1.79	22 ± 2.2
Diabetic	4	3.95 ± 0.08	16.52 ± 0.52	67.97 ± 3.36	86 ± 8.7
Diabetic + NaHCO_3	5	4.10 ± 0.12	16.31 ± 0.55	63.60 ± 1.76	78 ± 5.5
Diabetic + insulin	2	3.80	5.86	21.81	32

Table 3. *Glutaminase activity in rat small intestine*

Rats were treated as described in the Materials and methods section for the numbers of days indicated in parentheses. Activities were determined as described in the Materials and methods section and are expressed as μmol of glutamate formed/min at 37°C . Results are means $\pm$ S.E.M., where n is the number of experiments, or are the means of two experiments.

Treatment group	n	Small-intestine weight (% of body wt.)	Glutaminase activity		
			(units/g of tissue)	(units/100g body wt.)	(munits/mg of protein)
Control	27	3.30 ± 0.14	1.05 ± 0.16	3.31 ± 0.46	11.41 ± 1.77
Acidotic (6 day)	9	3.30 ± 0.11	0.44 ± 0.13	1.30 ± 0.33	6.45 ± 2.41
Alkalotic (6 day)	10	3.5 ± 0.11	1.34 ± 0.38	3.68 ± 1.16	9.03 ± 2.53
Diabetic (6 day)	23	4.48 ± 0.22	2.03 ± 0.35	8.38 ± 1.33	20.39 ± 3.42
Diabetic + NaHCO_3 (6 day)	9	4.63 ± 0.25	2.83 ± 0.47	13.28 ± 2.59	29.13 ± 5.13
Diabetic + insulin (6 day)	2	4.10	0.12	0.47	1.95
Diabetic (40 day)	4	6.77 ± 0.56	4.82 ± 0.25	33.16 ± 1.35	41.78 ± 2.61

but generally not to adapt to other conditions (McFarlane-Anderson *et al.*, 1976; Pinkus & Windmueller, 1977; Alleyne *et al.*, 1982; Budohoski *et al.*, 1982). Such results were obtained by using separate components of the small intestine, enterocytes or mucosal scrapings, and may not reflect changes owing to adaptations in the amount of mucosa or number of enterocytes. The results presented in Table 3 were obtained with whole small intestine (mucosal plus muscle layers). Acidosis and alkalosis respectively resulted in very slight decreases and increases in glutaminase activity. The results obtained with rats made diabetic for 6 days showed great variation. Diabetes (6 days) resulted in an increase in the size of the small intestine and an increase in glutaminase activity. Furthermore, the increase in glutaminase activity was prevented by insulin treatment, but not by NaHCO_3 . When diabetes was prolonged for periods of 40 days, the small

intestine was visibly enlarged and markedly increased in size when expressed as a percentage of body weight (Table 3). In these long-term diabetic rats the glutaminase activity of the small intestine was further increased (Table 3).

By using preparations *in vitro* (cortex slices, isolated tubules, perfused organ) of kidney from chronically acidotic rats, it is possible to demonstrate increased rates of glutamine utilization and of ammonia and glucose production (see Alleyne *et al.*, 1982). Similar increases in glutamine utilization and urea synthesis can be observed in hepatocytes isolated from diabetic rats (M. Watford & E. J. Erbeling, unpublished work). Although such results cannot be taken to imply a regulatory role for glutaminase adaptation, they do at least indicate that changes in glutaminase activity are reflected in the intact cell. The increase in glutaminase activity in the small intestine of diabetic animals (Table 3) could be due to an

increase in activity per enterocyte. If this were so, then enterocytes isolated from such rats might metabolize glutamine at faster rates than those from control rats. The results presented in Table 4 show no apparent differences in the rates of glutamine utilization or formation of the major nitrogenous products (glutamate, alanine, ammonia) between enterocytes isolated from control or diabetic (6 days) rats.

The results presented in Tables 3 and 4 suggests that the increase in glutaminase activity observed in the small intestine of diabetic rats was due to an increased number of enterocytes rather than to increased glutaminase activity per enterocyte. The results in Table 5 compare the specific activity of glutaminase in homogenates of whole small intestine,

mucosal scrapings and isolated enterocytes (all from the same animal) for control and 6-day-diabetic rats. The diabetic rats all exhibited increased glutaminase activity (> 9 munits/mg of protein) when the whole organ was assayed. This increase in activity was not observed when mucosal scrapings or isolated enterocytes were used.

Glutamine metabolism by the small intestine

Various reports suggest that, in the streptozotocin-diabetic rat, net glutamine utilization by the small intestine either ceases (Brosnan *et al.*, 1983) or is unchanged (Schrock & Goldstein, 1981). The findings reported in the present paper appear to contradict these reports, because the

Table 4. *Effect of diabetes on glutamine metabolism in isolated enterocytes*

Enterocytes were isolated and incubated as described by Watford *et al.* (1979). The results are expressed as μmol of metabolite removed (–) or produced (+) and are the means $\pm$ S.E.M. for four separate experiments for control and diabetic (6 days) rats.

		Metabolite removal (–) or production (+) ($\mu\text{mol}/\text{min}$ per g dry wt.)			
Substrate added		Glutamine	Glutamate	Alanine	NH_4^+
Glutamine (5mM)	Control	-11.77 ± 0.11	$+3.78 \pm 0.25$	$+2.35 \pm 0.30$	$+9.02 \pm 0.48$
	Diabetic	-11.35 ± 0.25	$+3.63 \pm 0.60$	$+2.70 \pm 0.29$	$+9.82 \pm 0.43$
Glutamine (5mM) + glucose (10mM)	Control	-12.00 ± 0.03	$+2.63 \pm 0.05$	$+3.50 \pm 0.93$	$+6.87 \pm 0.61$
	Diabetic	-11.71 ± 0.02	$+3.16 \pm 0.52$	$+4.44 \pm 0.61$	$+7.96 \pm 0.23$

Table 5. *Specific activity of glutaminase in various preparations of rat small intestine*

Tissues were prepared and homogenized as described in the Materials and methods section. Specific activities are expressed as nmol of glutamate formed/min per mg of protein and are the means $\pm$ S.E.M. of four separate experiments.

	Glutaminase activity (munits/mg of protein)		
	Whole small intestine	Mucosal scrapings	Isolated enterocytes
Control	6.91 ± 0.9	63.8 ± 21.4	107.6 ± 23.5
Diabetic (6 days)	27.2 ± 7.9	66.0 ± 8.0	117.1 ± 15.7

Table 6. *Arteriovenous-difference measurements for glutamine, glutamate, alanine and ammonia across the portal-drained viscera in the rat*

Measurements were made as described in the Materials and methods section and are expressed as μmol of metabolite/ml of whole blood. Results are means $\pm$ S.E.M., where n is the number of experiments.

	n	Arterial glutamine concn.	Arteriovenous difference			
			Glutamine	Glutamate	Alanine	NH_4^+
Control	10	0.595	+0.090	–0.043	–0.141	–0.329
		± 0.033	± 0.035	± 0.028	± 0.028	± 0.058
Diabetes (40 days)	3	0.293	–0.013	–0.018	–0.056	–0.119
		± 0.051	± 0.034	± 0.001	± 0.065	± 0.010

large increase in glutaminase activity observed in the small intestine of diabetic rats suggests increased capacity for glutamine utilization by this organ. Arteriovenous-difference measurements across the portal-drained viscera of control and long-term (40 days) diabetic rats are presented in Table 6. In control rats there was a net removal of glutamine from the circulation, with a production of alanine and ammonia and a slight production of glutamate. In the diabetic animals arterial glutamine concentration was markedly depressed and there was no net difference for glutamine across the portal-drained viscera. Production by the small intestine of glutamate, alanine and ammonia was also decreased in the diabetic animals. It is worth noting that long-term (40 days) diabetic rats were severely emaciated and markedly ketotic (total blood ketone concentration $> 6 \text{ mM}$).

Discussion

Kidney and liver

In the rat, chronic metabolic acidosis is accompanied by increased renal uptake of plasma glutamine for the production of urinary ammonia (see Tannen, 1978, for review). This is accomplished, in part, by adaptive increases in the activities of certain enzymes involved in renal glutamine catabolism. The results presented in this paper confirm that acidosis increases renal glutaminase activity (Davies & Yudkin, 1952; Rector *et al.*, 1955). This is due to increased amounts of glutaminase protein (Curthoys *et al.*, 1976), and the 4-fold increase in activity observed in the whole kidney reflects a greater than 20-fold increase in the proximal-convoluted-tubule cells (Curthoys & Lowry, 1973). The increase in renal glutaminase activity (expressed as units/100g body wt.) observed during diabetes was due, in part, to a general increase in the relative size of the kidney, as indicated by the results in Table 1. But increased kidney size alone was not sufficient to increase glutaminase activity, as treatment with NaHCO_3 suppressed the induction by diabetes of glutaminase activity, but had no effect on kidney hypertrophy. This finding indicates that long-term regulation of this enzyme is related to changes in acid-base status. Renal glutaminase activity has also been reported to increase after consumption of a protein-rich diet (Katunuma *et al.*, 1970; Brosnan *et al.*, 1978; Watford *et al.*, 1984). Again, correction of the associated acidosis prevented the increase in enzyme activity (Brosnan *et al.*, 1978).

The activity of hepatic glutaminase was not affected by long-term changes in acid-base status, but was increased by diabetes. This increase was prevented by treatment with insulin but not NaHCO_3 . This indicates that the response in liver

was due not to the acidosis of diabetes, but to some other aspect of the diabetic state. There are few reports of long-term changes in hepatic glutaminase activity in the rat. Consumption of a protein-rich diet (Freedland & Taylor, 1964; Katunuma *et al.*, 1970; Watford *et al.*, 1984), treatment with thyroxine (Freedland & Taylor, 1964) and starvation (Watford *et al.*, 1984) have been reported to increase hepatic glutaminase activity. Consumption of a protein-deficient diet results in decreased hepatic glutaminase activity (Simell, 1974). These responses of glutaminase are similar to those of other adaptive enzymes involved in hepatic glutamine catabolism: the activities of the five urea-cycle enzymes and the gluconeogenic enzyme phosphoenolpyruvate carboxykinase all increase in response to a high-protein diet, starvation and diabetes.

Small intestine

The role of glutamine metabolism in the small intestine is as a respiratory fuel for enterocytes (Windmueller & Spaeth, 1974; Watford *et al.*, 1979). From various studies it appears that the rate of glutamine utilization by the small intestine and the glutaminase activity of this organ do not change in response to acidosis (Pinkus & Windmueller, 1977), starvation (Pinkus & Windmueller, 1977), consuming carbohydrate- or protein-rich diets or supplementation of drinking water with glutamine for 7 days (Pinkus & Windmueller, 1977). Most of these findings were obtained from studies using intestinal segments, mucosal scrapings or isolated enterocytes, and may not reflect changes in glutamine metabolism of the whole organ. Results in the present paper show a marked increase in the glutaminase activity of the small intestine in response to diabetes. This increase was not seen when activity was measured in mucosal scrapings or isolated enterocytes, and the increase in activity was most pronounced when expressed in terms of body weight (see Table 3). On the basis of these findings, a different form of regulation in this organ must be considered. The total number of enterocytes can be changed rapidly (see Klein & McKenzie, 1984), but potential glutamine utilization (glutaminase activity) per cell may not alter. Increased intestinal size is characteristic of prolonged diabetes in the rat (Jervis & Levin, 1966; Schedl & Wilson, 1971); this was noted in the present study as an increase (36%) in the relative weight of the small intestine after 6 days of diabetes. Furthermore, prolonged diabetes (40 days) resulted in even greater increases in small-intestine size (105%) and glutaminase activity. These results are inconsistent with reports of either little change in (Schrock & Goldstein, 1981) or complete cessation of (Brosnan *et al.*, 1983)

glutamine utilization by the small intestine *in vivo* during diabetes. The present results, however, may not be directly comparable with previous reports. There is evidence to suggest that an early response to streptozotocin injection is a decrease in food intake and intestinal size (Schedl & Wilson, 1971). Later adaptation involves a large increase in food intake and intestinal size (Schedl & Wilson, 1971; Lal & Schedl, 1974; Hanson & Parsons, 1978). The reports of glutamine utilization cited above were obtained with streptozotocin-diabetic rats, which had been maintained on insulin for some days and then withdrawn from insulin for 48–90 h to induce diabetes. Such rats would have low initial glutaminase activity in the small intestine (see results for insulin-treated rats in Table 3), and after only 48–90 h of diabetes the activity may have been decreased further, owing to lowered food intake. In the present studies with long-term diabetes, in contrast, the small intestine was enlarged and total glutaminase activity markedly increased. The results illustrate that it is important to take into account the stage of diabetes and also to measure activity of an enzyme in the whole small intestine when comparisons are made. Measurements in isolated mucosa or enterocytes may not represent overall changes.

Glutamine metabolism in vivo

The changes in glutaminase activity in kidney and liver presented here are consistent with reported changes in glutamine catabolism in these organs during diabetes, acidosis or alkalosis. During diabetes there is increased utilization of glutamine by both kidney and liver (Brosnan *et al.*, 1983). During chemically induced acidosis the kidney removes glutamine from the circulation, and the liver becomes a site of glutamine production (Schrock & Goldstein, 1981). To date, there are no reports of significant changes in glutamine metabolism by the small intestine in response to chemically induced acidosis (Hanson & Parsons, 1977; Pinkus & Windmueller, 1977; McFarlane-Anderson *et al.*, 1976). Enterocytes isolated from acidotic rats do not metabolize glutamine at rates different from those obtained from control rats (Watford, 1977; Alleyne *et al.*, 1982).

The finding by Brosnan *et al.* (1983) that glutamine utilization by the small intestine is suppressed in the diabetic rat has been confirmed in the present study (Table 6) in long-term diabetes. This effect is physiologically consistent with a sparing of glutamine for use by other tissues, such as kidney and liver. In the present work the decreased removal of glutamine by the small intestine could have been due to two factors: first, low arterial concentration of glutamine, resulting in substrate unavailability; second, the sparing of

glutamine by an alternative respiratory fuel. Possible alternative fuels could be circulating ketones (Windmueller & Spaeth, 1974, 1978; Hanson & Parsons, 1978; Brosnan *et al.*, 1983) or amino acids absorbed from the lumen (Windmueller & Spaeth, 1980; Hanson & Parsons, 1977). If, in fact, there is little utilization of glutamine by enterocytes during diabetes, then this is a significant finding. Most rapidly dividing mammalian cells have been thought to have an absolute requirement for high rates of glutamine catabolism (see Zielke *et al.*, 1984; Krebs, 1980; McKeehan, 1982).

Conclusions

The results of this and other studies indicate that long-term adaptations in glutaminase activity occur in several tissues in the rat. Phosphate-activated glutaminase in kidney appears to respond only to changes in acid-base status. Such changes, however, are without direct effect on the long-term regulation of glutaminase activity in liver (Table 2; Ardawi & Newsholme, 1984), heart and lymphoid tissues (Ardawi & Newsholme, 1984) and small intestine (Table 3; McFarlane-Anderson *et al.*, 1976; Pinkus & Windmueller, 1977). Glutaminase activity of some tissues also adapts in response to hormonal signals. Diabetes results in increased activity in both liver and small intestine. Increases in glutaminase activity are consistent with known patterns of glutamine catabolism for both liver and kidney. The response observed in the small intestine, however, illustrates that a marked increase in enzyme activity does not necessarily result in increased flux through a pathway: the capacity of this organ to catabolize glutamine is increased 10-fold in long-term diabetes, yet glutamine removal from the circulation could not be detected.

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