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EFFECT OF STREPTOZOTOCIN DIABETES ON SOME UREA CYCLE ENZYMES

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Streptozotocin induced diabetes in rats increased the activities of the three mitochondrial enzymes, carbamylphosphate synthetase, ornithine transcarbamylase and N-acetylglutamate synthetase, but not of the cytosolic N-acetylglutamate deacylase. Levels of both N-acetylglutamate and arginine, which are activators of carbamylphosphate synthetase and N-acetylglutamate synthetase respectively, increased in diabetes. These results serve to explain the increase both of mitochondrial citrulline and urea formation in hepatocytes and the increased urea excretion in diabetes.

The increase in urea excretion and the negative nitrogen balance in diabetes are attributed mainly to increased utilization of amino acids for gluconeogenesis and to decreased protein synthesis (1,2).

As a catabolic cycle, urea synthesis is generally regarded as being regulated only by the availability of substrates (3). Increases in the ammonia load would therefore result in changes in urea synthesis (4). This view fails to explain why N-acetylglutamate (AG), which is an obligatory activator (5) of carbamylphosphate synthetase (CPS), is present in all ureotelic vertebrates, and varies with the metabolic state (6,7,8). The enzyme responsible for AG synthesis is strongly dependent on the concentration of arginine (9).

The picture that emerges from these observations is that CPS is subject to close regulation, although the metabolic advantages

Abbreviations: CPS: carbamylphosphate synthetase, OTC: ornithine transcarbamylase, AG: N-acetylglutamate, AG-synthetase: N-acetylglutamate synthetase, AG-deacylase: N-acetylglutamate deacylase.

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of such fine regulation are not well understood as yet. We present further evidence for the regulation of the synthesis of urea at the level of CPS, by demonstrating increased levels of AG in experimental diabetes.

MATERIALS AND METHODS

Male Wistar rats (200-250 g) were fed "ad libitum" a standard diet containing 18% protein. Diabetes was induced by a single intraperitoneal injection of 65 mg/Kg of Streptozotocin (10). The rats were sacrificed at the same time of day by decapitation.

The tissues were homogenized with an Ultra-turrax under established conditions (11) and diluted appropriately with bi-distilled water. The mitochondria were prepared as described by Hogeboom et al (12), except that mannitol was used instead of sucrose to avoid interference in the determinations of citrulline and urea (13). Hepatocytes were isolated according to Berry and Friend (14).

Mitochondria were incubated as described by McGivan et al (15) in 25 ml closed vials. Hepatocytes were incubated according to Briggs and Freedland (16). Citrulline and urea were assayed according to Hunninghake and Grisolía (17).

CPS and ornithine transcarbamylase (OTC) activities were determined according to Schimke (18) in whole liver homogenate. N-acetylglutamate deacylase (AG-deacylase) was assayed according to Grisolía et al (19). N-acetylglutamate synthetase (AG-synthetase) was determined according to Shigesada and Tatibana (20) in the $(NH_4)_2SO_4$ precipitate of supernatant from sonicated mitochondria which had been centrifuged at 105,000 x g.

AG was determined as described by Shigesada and Tatibana (9), and arginine was determined according to Saheki et al (21). Blood glucose assay was according to Werner et al (22) and protein by a biuret-deoxycholate method (23).

All results have been submitted to statistical analysis and the degree of significance calculated by Student's t test, using an Olivetti-101 programmer.

RESULTS

As anticipated, diabetic rats excreted more urea and had lower liver and body weight (Table I). We expected that the increased urea excretion was the result of increased ammonia availability to the urea cycle enzymes. However, we were surprised to find that hepatocytes isolated from diabetic rats produced urea faster than hepatocytes from control rats when exposed to identical concentrations of ammonia (Table I). This indicated a higher functional capacity of the urea cycle in diabetic rats. Moreover, intact mi-

Table I. Effect of diabetes on blood glucose, body and liver weight, urinary urea excretion, citrulline synthesized in isolated liver mitochondria and urea synthesized in isolated hepatocytes of rat.

	NORMAL	DIABETIC	Student's test
Glucose	157 ± 10	632 ± 33	$p < 0.001$
Body weight	210 ± 20	175 ± 31	$p < 0.001$
Liver weight	7.8 ± 1.2	6.9 ± 1.3	$p < 0.01$
Urinary urea	575 ± 32	927 ± 49	$p < 0.001$
Citrulline synthesized in isolated mitochondria	4.8 ± 0.4	7.7 ± 0.6	$p < 0.001$
Urea synthesized in isolated hepatocytes	3.5 ± 0.7	7.4 ± 0.8	$p < 0.001$

Glucose is expressed in mg/100 ml of blood; body and liver weight in g; urea excretion in mg/24 hours; citrulline in nmol/min/mg protein; urea in µmol/min/g wet weight. Results are expressed as mean ± SD of 8 rats.

tochondria freshly isolated from diabetic rats produced citrulline more rapidly than mitochondria from control rats (Table I), a process which is believed, under most circumstances, to control the rate of operation of the urea cycle. Citrulline synthesis is the result of the sequential action of CPS and OTC, two mitochondrial enzymes of the cycle. Of these two enzymes, the first is much less active, and in addition is only partly operative as a consequence of the non saturating levels of AG "in vivo", which is essential for activity (5). This is illustrated in Table II, which shows that part of the CPS activity is latent in intact mitochondria, and was revealed by disruption of these organelles and assay in the presence of a saturating concentration of AG.

Under the conditions of the assay, the rate of citrulline synthesis by intact mitochondria is determined by the levels of active CPS, which in turn depends on the amounts of enzymatic protein and of AG. As shown in Table II, both CPS and AG were elevated in the

Table II. Effect of diabetes on carbamylphosphate synthetase(CPS), ornithine transcarbamylase(OTC), N-acetylglutamate synthetase(AG-synthetase) and N-acetylglutamate deacylase(AG-deacylase) activities and on the N-acetyl-glutamate(AG) and arginine levels in rat liver.

	NORMAL	DIABETIC	Student's test
C P S	303 ± 77	764 ± 58	$p < 0.001$
O T C	10,550 ± 850	13,100 ± 1100	$p < 0.05$
AG-synthetase	56.7 ± 10.4	90 ± 18.1	$p < 0.001$
AG-deacylase	83.3 ± 8.8	80 ± 7.7	N.S.
A G	29.5 ± 7.3	68.1 ± 12.7	$p < 0.001$
Arginine	69 ± 3.5	306 ± 83	$p < 0.001$

CPS, OTC, and AG-deacylase activities are expressed in µmol/h/g of liver; AG-synthetase in nmol/h/g of liver; AG and arginine in nmol/g of liver. Results are expressed as mean ± SD of 12 rats.

liver of diabetic rats; OTC, the other intramitochondrial urea cycle enzyme, increased slightly (1.2 fold).

AG is synthesized by AG-synthetase (20), an enzyme also located in mitochondria; AG may be split by the very abundant AG-deacylase located in cytosol (19). The level of AG might therefore reflect changes in activity of these two enzymes. When tested, it was found that AG-synthetase activity was increased in diabetic rats while that of AG-deacylase did not change (Table II).

Thus it may be postulated that an increase in the activity of AG-synthetase can account for the increased levels of AG. A second factor may also be invoked for the increased AG levels, although its quantitative importance cannot be evaluated at present, i.e. AG-synthetase is stimulated by arginine, and the arginine levels were found to be higher in the livers of diabetic rats (Table II).

DISCUSSION

The importance of AG in the control of urea cycle activity has been demonstrated (4,8,9,13,20,24). The experiments described in this paper stress this point and show that changes produced by diabetes on the AG concentration, and on AG-synthetase and CPS activities, directly affect the capacity of the mitochondria to synthesize citrulline and the ability of hepatocytes to synthesize urea, explaining the increased excretion of this metabolite.

AG has been measured in total liver homogenate and was found to be increased. The mechanism by which AG is increased by diabetes remains to be elucidated. Some of the possible causes include: 1) a rise in acetyl-CoA, due to activation of lipolysis and subsequent fatty acid oxidation; 2) a rise in arginine, due to stimulated proteolysis or/and inhibition of arginase by accumulating branched-chain amino acids (25,26); and 3) inhibition of mitochondrial AG efflux (8).

Liver acetyl-CoA concentrations increase in diabetes (27), and hepatic arginine also increases, perhaps due to increased proteolysis without increase in arginase activity (28).

Arginine could increase the level of AG by either stimulating AG-synthetase activity (9), or by increasing synthesis and liberation of glucagon (29), since according to Meijer et al (8), glucagon increases mitochondrial AG. The effect of diabetes on the concentration of AG in the liver, may affect the coordination between proteolysis and ureogenesis that appear regulated in part by glucagon (8).

The increased urea excretion can be correlated with the increase in urea synthesis by isolated hepatocytes and also with the increased citrulline synthesis by isolated mitochondria. One of the factors responsible for these changes is the greater CPS activity, which is modulated by changes in the AG levels, and which might be related

to the increases in acetyl-CoA, AG-synthetase and its activator arginine.

All of the changes observed could result from either hypoinsulinemia and/or hyperglucagonemia, absolute or relative (30), which occur in diabetes.

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