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GLUCOSE OVERUTILIZATION IN DIABETES: EVIDENCE FROM STUDIES ON THE CHANGES IN HEXOKINASE, THE PENTOSE PHOSPHATE PATHWAY AND GLUCURONATE-XYLULOSE PATHWAY IN RAT KIDNEY CORTEX IN DIABETES

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SUMMARY: Kidney cortex from alloxan-diabetic rats has an elevated activity of hexokinase, enzymes of the pentose phosphate pathway and of certain enzymes of the glucuronate-xylulose route relative to age-matched control rats. These changes are highly significant when expressed as total kidney units/100 g body weight, a parameter relating biochemical activity to the functional requirement of the whole animal. These changes are discussed in relation to the hypothesis of 'glucose over-utilization' in diabetes in tissues not requiring insulin for glucose uptake [R.G. Spiro (1976) Diabetologia 12, 1-14].

In a recent review Spiro [1] has pointed out that although diabetes has classically been considered a disease of 'glucose underutilization' there are now many indications that in diabetes a shunting of glucose from insulin dependent pathways to those not requiring this hormone may take place. In support of the hypothesis that 'glucose overutilization' might be an important facet of the response of certain tissues, which do not require insulin for glucose uptake, to the high circulating level of glucose in diabetes may be cited: (1) the accumulation of products arising directly from glucose, such as sorbitol and fructose, in lens, nerve and kidney [1-4]; (2) the glycosylation of proteins such as haemoglobin [5] and lens α crystallin [6]; and (3) the raised level of glucose 6-phosphate and derivatives of glucose 6-phosphate such as glycogen and components of basement membranes in tissues such as kidney and white blood cells [1,7,8]. The increased activity of hexokinase in kidney

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cortex [9], intestinal mucosa [10] and lens [11] in diabetes is also consistent with the concept of 'glucose overutilization'.

This hypothesis seemed of particular interest in relation to the kidney growth and to certain of the biochemical changes occurring in kidney cortex in diabetes. In the present study the changes in enzymes involved in the formation and disposal of glucose 6-phosphate among alternative metabolic routes was investigated in kidney cortex, these include the oxidative and non-oxidative routes of pentose phosphate formation (in relation to increased RNA formation) and the glucuronate-xylulose route (in relation to the increased formation of basement membrane and accumulation of glycogen). The profile of enzyme change in diabetes indicated that certain specific biosynthetic routes utilizing glucose are increased in kidney cortex and accords with the postulate of 'glucose over-utilization' proposed by Spiro [1].

METHODS

Adult male albino rats of the Wistar strain were used, the initial body weight was 220-250 g. Diabetes was induced by the subcutaneous injection of alloxan-monohydrate (20 mg/100 g body weight) into rats previously starved for 24h; thereafter insulin was administered (2 units protamine zinc insulin daily for one week) and standard laboratory cube diet and water were allowed *ad lib*. The rats were used four weeks later for measurement of enzyme activities in kidney cortex.

The kidney cortex was removed and 2 g was homogenized in a Potter homogenizer with Teflon plunger in 4 volumes of ice-cold medium containing 0.25M sucrose, 20 mM triethanolamine buffer pH 7.4 and 0.1 mM dithiothreitol. After removal of the nuclei and cell debris by centrifugation at 700 g for 10 min., the mitochondrial fraction was isolated by centrifugation at 12,000 g for 10 min and washed twice with the same buffer. A high speed supernatant fraction and microsomal fraction were obtained by further centrifugation at 105,000 g for 45 min. All fractions were dialysed, with stirring, against the same buffer for 1h at 3°C.

Hexokinase, phosphoglucoseisomerase and enzymes of the pentose phosphate pathway were estimated as previously described [12,13]. The enzymes of the glucuronate-xylulose pathway were determined in essence as described in the literature, the appropriate purified enzymes (Boehringer Corporation Ltd or Sigma Chemical Co) being added to each system to link the reaction to a final step involving a redox change in NAD or NADP. In order these methods were: UDP glucose pyrophosphorylase [14]; UDP glucose dehydrogenase [15], glucuronate reductase [16]; L-xylulose reductase, D-xylulo-reductase [17], Xylulokinase [18]. The Enzyme Commission

Table 1. Blood glucose values, body weight, kidney weight, protein and DNA content for control and alloxan diabetic rats.

	CONTROL	DIABETIC	P
Blood glucose (mM)	5.1 ± 0.4	21.6 ± 1.8	***
Body weight (g)	257 ± 7	188 ± 6	***
Kidney weight	1.90 ± 0.07	2.52 ± 0.08	***
Kidney weight/100 g body weight	0.74 ± 0.02	1.34 ± 0.05	***
Kidney protein content (mg/g wet weight)			
Cytosol	107 ± 2	93 ± 3	*
Mitochondria	40 ± 2	34 ± 2	NS
DNA content (mg/g wet weight)	3.26 ± 0.13	3.02 ± 0.10	NS

Values are given as means ±SEM of not less than six rats. Fisher's P values are shown by asterisks; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N.S. not significantly different.

numbers for these enzymes are given in the legends to Fig. 1 and 2. Where an enzyme was located in two cell fractions, e.g. hexokinase, these have been summated in the presentation of the data in Fig. 1 and 2. A unit of enzyme converts 1 μmole of substrate/min at 25°. Protein was estimated by the method of Lowry et al. [19] and DNA by the diphenylamine reaction as modified by Burton [20].

RESULTS AND DISCUSSION

The kidney hypertrophy occurring in diabetes raises certain problems in the mode of expression of the results. The data in Table 1 summarises the changes in body weight, blood glucose and kidney weight, protein and DNA content of normal and diabetic rats. The basis for comparison used in Fig. 1 and 2 is the total activity in kidney/100 g body weight, a parameter which relates biochemical activity to the functional requirement of the whole animal and which appeared to provide the most significant means of comparison of kidney changes in alloxan-diabetic rats relative to the age-matched controls. The changes in hexokinase and key enzymes of the

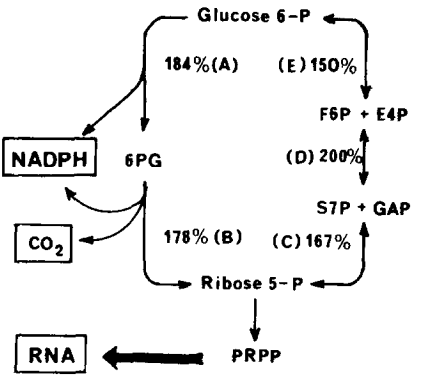

Fig. 1. Changes in enzymes of the pentose phosphate pathway in kidney cortex from alloxan-diabetic rats.

Enzyme activities of kidney cortex from alloxan-diabetic rats are given as a percentage of the age-matched controls, the basis for comparison being total units of activity in the kidney pair/100 g body weight. The values for alloxan-diabetic rats as are shown between appropriate pairs of metabolites. All the values shown for diabetic rats are statistically significantly different from controls with Fisher's P values of < 0.05. The diabetic and control group each contained not less than six values. The enzyme activities of the control group, the Enzyme Commission number, the means ±SEM (units/ kidney pair/100 g body weight) and the code letter in the figure are: (A) glucose 6-phosphate dehydrogenase (EC 1.1.1.49) 1.30±0.11; (B) 6-phosphogluconate dehydrogenase (EC 1.1.1.44) 0.90±0.06; (C) transketolase (EC 2.2.1.1) 0.60±0.05; (D) transaldolase (EC 2.2.1.2) 0.60±0.05; (E) phosphoglucose isomerase (EC 5.3.1.9) 52±3. Abbreviations used: 6PG, 6-phosphogluconate; S7P, sedoheptulose 7-phosphate; GAP, glyceraldehyde 3-phosphate; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; PRPP, phosphoribosyl pyrophosphate.

pentose phosphate pathway and glucuronate-xylulose route are shown in Fig. 1 and 2.

Hexokinase The hexokinase activity of kidney cortex shows a significant increase in diabetes (Fig. 2); this is in accord with the results of Anderson & Stowring [9], who found an increase in the specific activity of this enzyme in kidney cortex from diabetic rats. In the present experiments it was found that the increase was mainly in the Type I isoenzyme of hexokinase in the cytosolic compartment of the cell. The change in hexokinase is consistent with data on the raised glucose 6-phosphate and glycogen content of the kidney in diabetes [7,9].

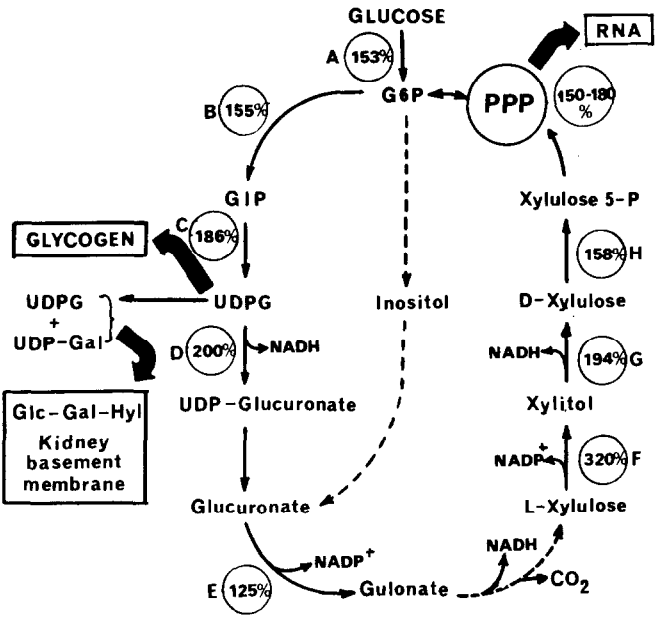

Fig. 2. Changes in enzymes of the glucuronate-xylulose pathway in kidney cortex from alloxan diabetic rats.

Enzyme activities of kidney cortex from alloxan-diabetic rats are given as a percentage of the age-matched controls, the basis for comparison being total units of activity in the paired kidney/100 g body weight. The values for the alloxan diabetic rats are shown in the circles between appropriate pairs of metabolites. All the values shown for diabetic rats are statistically significantly different from controls with Fisher's P values of < 0.05, the exception is reaction (E) glucuronate reductase. The diabetic and control groups each contained not less than six values. The enzyme activities of the control group, the Enzyme Commission number, the means ±SEM (units/kidney pair/100 g body weight) and the code letter in the figure are: (A) hexokinase (EC 2.7.1.1), 1.08±0.06; (B) phosphoglucomutase (EC 2.7.5.1) 5.6±0.20; (C) UDPG pyrophosphorylase (EC 2.7.7.9) 0.75±0.01; (D) UDPG dehydrogenase (EC 1.1.1.22) 0.18±0.02; (E) glucuronate reductase (EC 1.1.1.19) 1.62±0.14; (F) L-xylulose reductase (EC 1.1.1.10) 0.13±0.02; (G) D-xylulose reductase (EC 1.1.1.9) 1.7±0.08; (H) xylulokinase (EC 2.7.1.17) 3.6±0.30; PPP, pentose phosphate pathway, non-oxidative and oxidative reactions leading to pentose phosphate formation, see Fig. 1. Abbreviations used: G6P, glucose-6-phosphate; G1P, glucose 1-phosphate, UDPG, uridine diphosphate glucose; UDP-Gal, uridine diphosphate galactose; Hyl, hyaluronic acid.

Pentose phosphate pathway The enzymes of both the oxidative and non-oxidative routes of pentose phosphate formation are increased in kidney cortex in diabetes (Fig. 1); this is in

line with the increase in RNA synthesis which occurs in diabetic rat kidney [21,22]. The increased flux of glucose into ribose 5-phosphate and RNA would thus be another aspect of glucose overutilization. The second major product of the pentose phosphate pathway, NADPH, may also be important in the biosynthetic mechanisms accompanying kidney hypertrophy in diabetes, in particular with those connected with reductive reactions in lipid synthesis for new membrane formation.

Glucuronate-xylulose route The enzymes converting glucose to UDP-glucose are all increased in diabetic rat kidney as is the UDP-glucose content, which increases from 63±4 nmoles/g in controls to 83±5 nmoles/g in diabetic rat kidney cortex. This observation may be of significance in relation to the raised tissue glycogen, reported to be increased as much as thirty fold [9], and thickened basement membrane. Spiro & Spiro [1,23] have shown an increased glucosyltransferase activity in kidney in diabetes, a change which is reversed by insulin treatment. The observations of Winegrad & Burden [24] that the serum of fasting diabetic patients had an elevated level of L-xylulose, and that this could be decreased by insulin treatment, also suggests that glucose utilization by the glucuronate-xylulose pathway is increased in diabetes.

The increase in enzymes of the glucuronate-xylulose pathway may also be examined from the point of view of the potential transhydrogenase activity of this pathway, resulting from the alternating reactions utilizing NADPH and NAD^+ and producing $NADP^+$ and NADH (see Fig. 2 and ref. [25]). The NADH could, presumably, be used for energy purposes in the cell by the combination with hydrogen shuttle systems and mitochondrial oxidation phosphorylation, thus integrating the pentose phosphate pathway and glucuronate-xylulose pathway with the ATP-generating system of the cell.

Comparison of kidney and liver with respect to 'glucose overutilization' The liver, while not requiring insulin for glucose uptake, does require insulin for maintenance of liver glucokinase and thus for glucose phosphorylation and entry into metabolic pathways. In the absence of insulin glucokinase is decreased [26,27], the glucose 6-phosphate and glycogen content of the tissue fall [28], and there is a decrease in the oxidative and non-oxidative enzymes of the

pentose phosphate pathway [29]. The general pattern is for a depression in these specialized routes of glucose utilization in diabetes in contrast to the profile for kidney. The present results show that, in some tissues which do not require insulin for glucose uptake, changes in enzyme profile occur which would facilitate higher levels of glucose utilization along certain metabolic routes. In this sense they support the hypothesis that, in these tissues, glucose overutilization can occur with overproduction of intermediates and macromolecules arising from glucose 6-phosphate. The increase of hexokinase activity, which parallels the increase already reported for intestinal mucosa [10] and lens [11] could, thus, be an important determinant of the metabolic response to diabetes.

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