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REGULATION OF GLUCOSE METABOLISM IN RAT ADRENAL GLAND IN ALLOXAN-DIABETES: THE POSSIBLE ROLE OF FRUCTOSE 2,6-BISPHOSPHATE

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SUMMARY: The level of fructose 2,6-bisphosphate is markedly decreased in the rat adrenal gland in diabetes, falling to 23 % of the control value. There is parallel decrease in the flux of 14C-labelled glucose through the glycolytic route and tricarboxylic acid cycle. Only minimal changes in hexokinase (EC 2.7.1.1.), a 22 % decrease in Type I hexokinase of the soluble fraction, were observed, highlighting the probable significant involvement of fructose 2,6-bisphosphate in the regulation of glycolysis in the adrenal. In contrast, there was evidence for a marked rise in the flux of glucose through the pentose phosphate pathway, which may be linked to enhanced corticoid synthesis in the diabetic state.

In addition to the many reports of disturbances of carbohydrate and lipid metabolism in a variety of tissues in diabetes [see 1-3], some evidence appears to suggest that there are also effects on adrenal function, such as adrenal hypertrophy and increased corticoid biosynthesis [4-6]. The adrenal hyperfunction in early stages seems to be of a mixed (gluco- and mineralcorticoid) type while, in later stages, it may be a response to the chronic stress resulting from the metabolic consequences of the diabetic state [4].

Recently, a novel metabolite, fructose 2,6-bisphosphate, has been reported to be a potent stimulator of phosphofructokinase and the regulation of its concentration by hormones an important factor in the control of glycolysis and gluconeogenesis [7-9]. In the present study we have examined the effect of diabetes on the different pathways of glucose metabolism in the adrenal gland by the use of differentially labelled ^{14}C-glucose, the activity and distribution of the isoenzymic forms of hexokinase and the tissue level of fructose 2,6-bisphosphate, and discuss the possible regulatory role of the metabolite in the carbohydrate metabolism of the adrenal gland.

Abbreviations: PPP, pentose phosphate pathway; PMS, phenazine methosulphate.

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METHODS

Adult male albino rats of the Wistar strain were used, the initial body weight of which was 220-250g. 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 six weeks later.

The conversion of ^{14}C-labelled glucose to $^{14}CO_2$ by adrenal glands was estimated as described previously [10,11]. Paired adrenals were cut in half and incubated for 1h in 2.5 ml Krebs-Ringer bicarbonate medium containing 20 mM glucose and 0.5μCi of ^{14}C-glucose. The gas phase was O_2/CO_2:95/5.

For enzyme estimation adrenal glands from 4 rats were pooled and homogenised in 20 vol of 0.25 M sucrose, 20 mM triethanolamine buffer, pH 7.4, containing 0.1 mM dithiothreitol. Hexokinase activity was estimated in the high-speed supernatant fraction (105,000 x g for 45 min) and high-speed pellet fraction which was suspended in the same buffer to the original volume. Both fractions were dialysed for 1 h against the same buffer before use. Hexokinase (EC 2.7.1.1) was estimated spectrophotometrically as described previously [12]. A unit of enzyme converts 1 μmole of substrate/min at 25°C.

The level of fructose 2,6-bisphosphate was determined by the method of Van Shaftingen et al [13]. The rats were anaesthetized (60 mg/kg of Na pentobarbital i.p.) and adrenals quickly removed and frozen in liquid N_2. Fructose 2,6-bisphosphate was assayed by its stimulatory action of potato tuber PPi: fructose 6-phosphate 1-phosphotransferase prepared by the method of Van Shaftingen et al [13].

RESULTS AND DISCUSSION

As shown in Table 1, there is marked decrease in the rate of $^{14}CO_2$ production from [U-^{14}C]glucose by adrenal glands from alloxan-diabetic rats. The specific decline in the $^{14}CO_2$ yields from [3,4-^{14}C]glucose and from [6-^{14}C] glucose may be interpreted as indicating a fall in the flux of glucose through the glycolytic route plus pyruvate dehydrogenase reaction and the tricarboxylic acid cycle respectively. Each of these parameters falls by approximately 50% in the alloxan-diabetic rat adrenal gland.

In marked contrast, there is evidence for an increase in the functional activity of the pentose phosphate pathway (PPP) in the adrenal gland of diabetic rats as shown by the increase in the C_1/C_6 quotient and by the difference in the yields of $^{14}CO_2$ from [1-^{14}C] glucose and [6-^{14}C] glucose. It may be noted that the fully stimulated PPP, measured in the presence of the artificial electron acceptor phenazine methosulphate, is some 20-fold higher than the basal activity and remains unchanged in diabetes. This differentiation

Table 1 Conversion of ^{14}C-labelled glucose to $^{14}CO_2$ by adrenals from control and alloxan-diabetic rats

Labelled substrate	$^{14}CO_2$ production		Diabetic as % of control
	CONTROL	DIABETIC	
	(μmol/g/h)		
[1-^{14}C] glucose	2.03 ± 0.26	1.42 ± 0.12	70 *
[1-^{14}C] glucose + PMS	10.2 ± 0.8	9.7 ± 0.9	95
[1-^{14}C] glucose + insulin	2.05 ± 0.19	1.40 ± 0.13	68 **
[2-^{14}C] glucose	1.69 ± 0.19	1.46 ± 0.12	86
[3,4-^{14}C] glucose	4.66 ± 0.60	2.36 ± 0.40	51 **
[6-^{14}C] glucose	1.62 ± 0.19	0.75 ± 0.10	46 ***
[U-^{14}C] glucose	2.48 ± 0.24	1.09 ± 0.06	44 ***
C_1/C_6	1.25 ± 0.10	1.89 ± 0.14	151 **
Approximate PPP (C_1-C_6)	0.41 ± 0.04	0.67 ± 0.07	163 **
PMS stimulation	504%	683%	

The results shown are given as means ± SEM of 6 observations. The glucose concentration was 20 mM. Phenazine methosulphate (PMS) was present in a final concentration of 0.1 mM. When insulin was added it was a final concentration of 0.4 units/ml medium. Fisher's P values are shown by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

between the functional activity and the total capacity for NADPH generation via the PPP may be related to the requirement for NADPH in the reductive stages of steroid synthesis and is in line with reports of increased corticoid biosynthesis and an increment in the serum corticosterone levels of diabetic animals [4,6,14,15]. In addition to its function in reductive synthetic reactions, the PPP also plays a role in cell metabolism in providing ribose 5-phosphate for nucleotide and nucleic acid synthesis and is, thus, involved in cellular growth processes. In the present experiments we have observed only a modest increase in adrenal weight at this stage of diabetes(Table 2) but the increased activity of the PPP would be consistent with the reported adrenal hypertrophy in this condtion [4,5].

In seeking an explanation for the depression in the rate of glucose utilization via the glycolytic route and tricarboxylic acid cycle, measurements were made of the hexokinase activity, isoenzyme form and distribution, in the adrenal glands of normal and diabetic rats since this is one site involved in 'glucose underutilization' in diabetes in liver, adipose tissue and mammary gland [16-18] and of 'glucose overutilization' in lens and kidney [19,20]. As

Table 2 Activity of hexokinase in adrenals of control and alloxan-diabetic rats

	CONTROL	DIABETIC	Diabetic as % of control
Hexokinase (EC 2.7.1.1)	(units/g)		%
Soluble fraction			
Type I	0.537 ± 0.035	0.410 ± 0.063	76 *
Type II	0.740 ± 0.080	0.744 ± 0.080	94
Total	1.18 ± 0.12	1.07 ± 0.11	91
Pellet fraction			
Type I	0.481 ± 0.041	0.470 ± 0.051	98
Type II	0.557 ± 0.048	0.460 ± 0.051	83
Total	0.872 ± 0.081	0.759 ± 0.077	87
Blood glucose (mM)	6.4 ± 0.4	25 ± 2	390 ***
Body weight (g)	344 ± 11	242 ± 10	70 ***
Adrenal weight (mg)	60 ± 3	70 ± 4	116
Adrenal weight (mg) / 100g body weight	17.5 ± 1.0	29.0 ± 2.0	166 ***

Values are given as means ± SEM of 6 observations.
Fisher's P values are shown by asterisks: * $P < 0.05$; *** $P < 0.001$

shown in Table 2, the only significant change was a 24% decrease in the hexokinase Type I isoenzyme located in the soluble fraction of the adrenal homogenates, itself a small component of the total adrenal hexokinase activity. While this change may be important in a particular cell type or region of the adrenal gland, it did not appear to offer an explanation for the 50% decline in glycolytic flux nor for the increased activity of the PPP.

The potent effect of fructose 2,6-bisphosphate as an allosteric modifier of phosphofrutokinase [7-9] and the recent reports of a decreasee in hepatic fructose 2,6 bisphosphate content in diabetes [9,21], prompted the present investigation of changes in the level of this compound in the adrenal glands from normal and diabetic rats. The results of these experiments are shown in Table 3 from which it may be seen that the fructose 2,6-bisphosphate content of the adrenals from alloxan-diabetic rats falls to only 23% of that found in the glands of control animals. This striking change offers an attractive hypothesis to account for the differential effects of diabetes on the alternative routes of glucose metabolism in the adrenal gland. The decline of the flux through

Table 3 Effect of alloxan-diabetes on the level of fructose 2,6-bisphosphate in rat adrenal gland

	CONTROL	DIABETIC	Diabetic as % of control
	(nmol/g of tissue)		
Fructose-2,6-bisphosphate:	2.12 ± 0.32 (6)	0.48 ± 0.08 (6)	23 ***

The values represent the means ± SEM for the number of animals in parentheses. Fisher's P values are shown by asterisks: *** $P < 0.001$

the glycolytic route would be entirely consistent with the dramatic fall in fructose 2,6-bisphosphate content while the glucose 6-phosphate, generated at the hexokinase reaction, would be available for utilization via the PPP. Whether the apparent increase in the PPP (Table 1) is solely linked to the increased corticoid synthesis, or whether there is another dimension, in the involvement of the transhydrogenase reactions of the sorbitol route [22, 23], remains to be determined as does the more detailed study of the possible role of the cortical and medullary regions and of factors, e.g. cAMP [8], involved in the regulation of the tissue fructose 2,6-bisphosphate level.

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