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EFFECT OF EXPERIMENTAL DIABETES ON THE ACTIVITY OF HEXOKINASE IN RAT LENS: AN EXAMPLE OF GLUCOSE OVERUTILIZATION IN DIABETES

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SUMMARY: Hexokinase activity of lens increases two-fold in alloxan-diabetic rats four weeks after induction of diabetes; both type I and type II isoenzymes of hexokinase are increased. This increase in lens hexokinase is considered in relation to alternative routes of glucose metabolism.

Changes in the content, subcellular distribution and isoenzymic forms of hexokinase (ATP : D-glucose 6-phosphotransferase; EC 2.7.1.2) in experimental diabetes have been reported for a number of tissues, and an association between insulin sensitivity and a high proportion of hexokinase type II has been proposed [1-3]. A decreased hexokinase activity, both bound and free forms, and a fall in the type II isoenzyme has been demonstrated for some insulin-sensitive tissues including mammary gland and adipose tissue [1-6]. An apparent glucose overutilization and glycosylation of proteins by certain tissues not requiring insulin for glucose uptake has been reported for a number of tissues including kidney and red blood cells [7,8].

The present study of the effect of diabetes on the activity distribution and molecular species of hexokinase in lens was prompted by a number of observations: (1) that lens contains 70% of hexokinase in the type II form [9]; (2) that a part of lens hexokinase is in the bound or latent form [10,11]; (3) that the ratio soluble : bound form can be modified by alterations in glucose availability, as in hypoglycaemia [11]; and (4) that glucose overutilization may occur in lens and vitreous humour as

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shown by the presence of glycosylated proteins e.g. glycosylated α crystallin [8].

The present finding that hexokinase is significantly increased in rat lens a few weeks after induction of diabetes focusses attention on the potential increased flux of glucose through the multiple pathways arising from glucose 6-phosphate.

METHODS

Adult male albino rats of the Wistar strain were used, the initial body weight was 220-250g. Diabetes was induced by the subcutaneous injection of alloxan-monohydrate (20mg/100g body weight) into rats previously starved for 24 hr; 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 hexokinase activity of lens.

Rat lens tissue pooled from 3 rats, was homogenised in a Potter homogeniser with Teflon plunger in 20 volumes of ice-cold medium containing 0.25M sucrose, 20mM triethanolamine buffer pH 7.4, 0.1mM dithiothreitol and defatted bovine serum albumin 0.5% final concentration. The resulting homogenate was centrifuged at 105,000g for 45 min. Supernatant and pellet fractions were obtained and the pellet was resuspended in the same homogenising medium in an amount equivalent to the initial volume used. These fractions were dialysed, with stirring, for 2 hr at 4°C. Hexokinase activity was estimated as previously described [4] using the radioactive procedure, based on the release of $^{14}CO_2$ from [1-^{14}C]glucose, for measurement of enzyme activity of pellet and soluble fractions; the spectrophotometric procedure was used for the evaluation of the isoenzymic forms of hexokinase in the soluble fraction of lens homogenates. The heating procedure (45°C for 1 hr) in the absence of glucose was used to differentiate between type I and type II hexokinase [4, 9, 12]. Values are given as means ±SEM.

RESULTS AND DISCUSSION

Hexokinase activity and distribution. Table 1 presents data on the activity of lens hexokinase from control and alloxan-diabetic rats. There is a significant rise, of approximately two-fold, in the total hexokinase of the lens from alloxan-diabetic rats at a relatively early stage of diabetes, four weeks after cessation of insulin treatment, before cataracts develop. The rise is seen in both soluble hexokinase and in that part of hexokinase associated with the pellet fraction, moreover it is not restricted to a particular isoenzymic form, both heat stable (type I) and heat labile (type II) forms are increased in the lens in diabetes.

The present values for hexokinase activity of lens and the high proportion of the heat-labile type II isoenzyme in this

Table 1. Activity of hexokinase in lens from normal and alloxan-diabetic rats

	Control	Alloxan-diabetic	Diabetic as % of control	P
Blood glucose (mg %)	92±10 (9)	565±18 (11)	614	***
Body weight (g)	389± 9 (9)	249±11 (11)	65	***
Lens weight (mg)	93± 3 (9)	82± 5 (11)	88	N.S
Hexokinase activity (Units/g)				
Soluble fraction				
Total	0.151±0.007 (9)	0.222±0.021 (11)	147	**
Type I	0.053±0.004 (9)	0.088±0.009 (11)	166	**
Type II	0.098±0.008 (9)	0.134±0.018 (11)	137	N.S
Pellet fraction				
Total	0.070±0.010 (7)	0.219±0.013 (5)	313	***
Type I	0.048±0.011 (7)	0.123±0.019 (5)	256	**
Type II	0.022±0.012 (7)	0.095±0.019 (5)	432	**
Whole lens activity	0.221	0.441	200	

Values are given as means ±SEM. Fisher's P values are shown by asterisks; *P 0.05; **P 0.01; ***P 0.001; N.S not significantly different. Number of separate observations are given in parentheses, each observation is the mean of lens tissue pooled from 3 rat.

organ are in accord with data of van Heyningen [13] and Chylack [9]. The distribution of hexokinase between the two major fractions obtained from whole lens homogenate would seem to be dependent upon the species, the homogenising technique and the medium used [13-15]. The effect of ionic environment on the binding of hexokinase to subcellular fractions is strikingly illustrated by the experiments of Hernandez and Crane [16] and Vallejo et al. [17] using heart and brain.

In the present experiments approximately 30% of lens hexokinase of normal rats was associated with the pellet fraction; this increased to 50% in the alloxan-diabetic group. The nature and significance of the binding of hexokinase in rat lens is not entirely clear from the present experiments. Hexokinase has been found in lens epithelium in soluble, particulate and latent forms [18] and redistribution of the hexokinase occurs in epithelial cells exposed to varying glucose concentrations [11]. In the present experiments the high proportion of hexokinase associated with the pellet fraction may represent non-specific binding of hexokinase to insoluble lens proteins or to cold precipitable protein fractions of the lens [19, 20]. It may be noted that an increase in the ratio of insoluble : soluble protein generally occurs in mammalian lens during cataract formation [19-21].

The present results showing an increase in hexokinase in lens in diabetes are in sharp contrast to the many examples of a decrease in hexokinase which occurs in tissues such as liver, adipose tissue, mammary gland and muscle [1-6] in this condition. However, there is some evidence for an increase in the activity of hexokinase and of a change of iso-enzymic profile in certain tissues not requiring insulin for glucose uptake. Tyrell and Anderson [22] found a substantial rise in hexokinase in intestinal mucosa of diabetic rats fed ad lib; reduction of food intake to that of the control largely abolished this effect. Sochor et al. [23] have found that hexokinase activity of kidney cortex is significantly increased in alloxan-diabetic rats when the values are calculated on the basis of the whole organ.

Hexokinase and the regulation of glycolysis. Many authors have drawn attention to the relatively low activity of hexokinase in lens and have suggested a 'pacemaker' role for this enzyme in the regulation of glycolysis [9, 13-15]. The present finding that

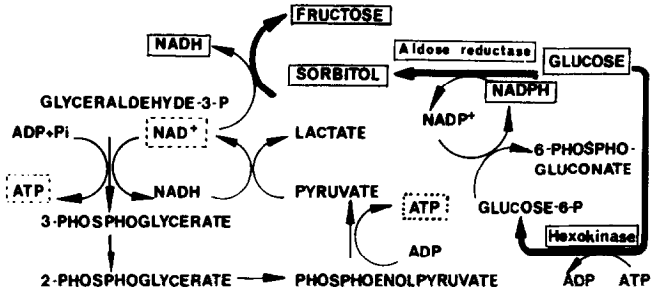

Fig. 1. Interrelationships among pathways of glucose metabolism in lens. The effect of diabetes on interlocking metabolic pathways is shown by heavy arrows, which indicate enzyme reactions that are increased in lens in diabetes. Enzymes, metabolites and co-enzymes enclosed in solid lines are those known to increase in diabetes, those enclosed in broken lines decrease in diabetes.

hexokinase is increased in lens in diabetes would have the corollary that there would be a potential increase in flux of glucose through pathways originating at glucose 6-phosphate. In the lens in diabetes there would thus appear to be a paradox of an increase in a rate limiting enzyme of the glycolytic pathway associated with a decreased flux through that pathway, as shown by the fall in lens ATP in diabetes [24], the glycolytic route being the major source of ATP in this tissue [see 25].

Among the explanations that could be advanced to account for these findings is a decline in the activity of other key enzymes of the glycolytic pathway in diabetes, in particular of phosphofructokinase. In the present experiments no changes were observed in the activity of phosphofructokinase, measured under optimal conditions, the activities in control and diabetic rat lens being 0.99±0.06 and 1.02±0.06 units/g respectively (mean ±SEM of 6 values). This result does not preclude regulation of phosphofructokinase by metabolites or by covalent modification. It is noteworthy that aldolase was decreased to 62% of the control activity in diabetic rat lens in the present experiments, a result in accord with data of Sippel [26].

The glyceraldehyde 3-phosphate dehydrogenase reaction is another possible site for restriction of the activity of the glycolytic pathway in lens in diabetes [27]. This reaction, the first ATP-generating step in the glycolytic pathway, is regulated, in part, by the redox state of NAD [28]. Varma and

Kinoshita [29] have observed a decrease in NAD^+ and an increase in both NADH and NADPH in lens in diabetes.

The combined effect of increased hexokinase activity, the increased aldose reductase activity [29] and the massive reserve potential of the oxidative enzymes of the pentose phosphate pathway for generation of NADPH [30], would be such as to increase the overall transhydrogenase activity of lens and would be consistent with the observed shifts in redox state of the lens in diabetes and fall in ATP content [24, 29]. These inter-relationships are illustrated in Fig. 1, which is an extension of schemes proposed by van Heyningen [31], Kuck [27] and Kinoshita et al. [30].

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