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Hexokinase isoenzymes in diabetes

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Abstract. Hexokinase is present in the tissues in four isoenzymic forms. Cerebral tissue contains predominantly Type I hexokinase which is believed to be insulin-insensitive. In cerebral tissue about 60 to 70% of the hexokinase is bound to the particulate fraction. The changes in the distribution of hexokinase Type I and Type II together with the bound and free hexokinase have been studied in control, diabetic and diabetic animals treated with insulin. The results indicate that the presence of insulin is essential for the normal binding of the hexokinase to the particulate fraction. In heart tissue, Type II hexokinase bound to the pellet shows a significant decrease in diabetes, which is reversed on insulin administration.

Keywords. Hexokinase isoenzymes; alloxan diabetes; insulin; cytosol; total particulate fraction.

Introduction

Hexokinase (EC 2.7.1.1) exists in tissues in four isoenzymic forms (Grossbard and Schimke, 1960; Katzen and Schimke, 1965; Katzen *et al*., 1975). Type II hexokinase which is known to be insulin-sensitive is found predominantly in adipose tissue and mammary gland (McLean *et al*., 1967; Katzen, 1967; Walters and McLean, 1968). Cerebral tissues contain predominantly Type I hexokinase (Wilson, 1968, 1972). Recently, we have reported the distribution of hexokinase in brain and demonstrated the presence of about 45% Type II isoenzyme in brain (Ali and Baquer, 1980). A substantial proportion of cerebral hexokinase is bound to sub-cellular particles (Wilson, 1968; Thompson and Bachelard, 1977; Ali and Baquer, 1980).

In the present work an attempt has been made to study the distribution of hexokinase isoenzymes in the brain and heart of control and diabetic rats. Administration of insulin seems to reverse the diabetic effects indicating that the presence of insulin is essential for the stability and binding of hexokinase.

Materials and methods

Animals

Adult female rats of Holtzman strain weighing between 175-200 g maintained on a standard pelleted diet in our Department were used for the experiments.

Alloxan treatment

A group of rats was starved for 48 h and each rat received a single subcutaneous injection of alloxan monohydrate prepared freshly in acetate buffer, pH 4.5

20 mg/100 g body wt). Protamine zinc insulin injections (1 unit/rat) were given intraperitoneally for five days after the alloxan injection. This treatment prevents the high mortality rate of the animals caused by alloxan toxicity. The animals were used three weeks after insulin withdrawal. Rats were provided food and water *ad libitum*.

Most of the chemicals and purified enzymes were from Sigma Chemical Company, St. Louis, Missouri, USA, unless otherwise mentioned.

Tissue extracts

Rats were sacrificed by cervical dislocation and tissues were excised immediately, weighed and homogenized as described earlier (Baquer *et al*., 1976). The homogenates were dialyzed for 1 h in the cold against the homogenizing buffer (sucrose 0.25M, triethanolamine, 20 mM; dithiothreitol, 0.1 mM, pH 7.4) to remove low molecular weight compounds like glucose. The extracts were centrifuged at 15,000 g for 40 min, and pellets were suspended in the homogenizing buffer and were referred to as the total particulate fraction in the text. To liberate the latent and bound enzymes, the total particulate fraction was treated in the cold for 30-60 min with Triton X-100 (final concentration 0.5%). Protein was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin as the standard.

Determination of blood sugar

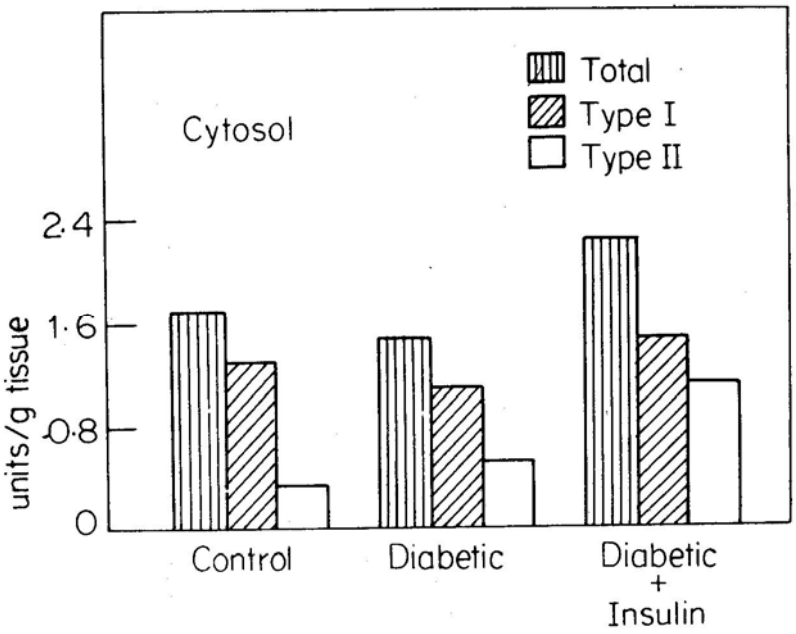
Blood (50 μl) was drawn by heart puncture into 0.2 ml of distilled water. The samples were frozen overnight and thawed the next day. The samples were centrifuged and the supernatant used for the determination of blood glucose by the hexokinase reaction (Bergmeyer *et al*., 1974).

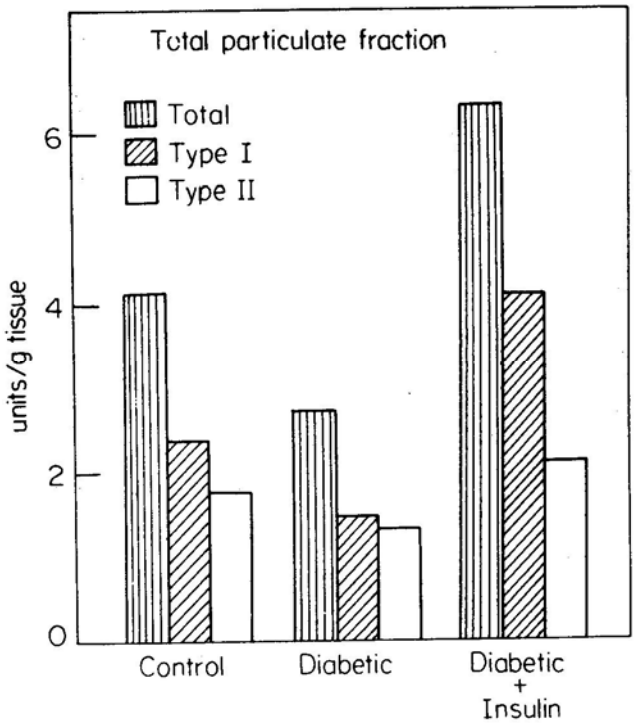
Enzyme assay

Hexokinase was estimated essentially according to the method of Sharma *et al*. (1963), as modified by Gumaa and McLean (1972). For the estimation of Type II activity, a portion of each fraction was heated at 45°C for 1 h (Katzen and Schimke, 1965). The reaction mixture contained the following components: Tris-HCl buffer, 20mM; (pH 7.4); $MgCl_2$,8 mM (pH 7.0); NADP, 0.4 mM, ATP/Mg^{2+} , 8 mM/2 mM (pH 7.2); glucose, 5 mM and one unit of purified glucose-6-phosphate dehydrogenase. One unit of activity of hexokinase was defined as the amount required to form one μ mol of NADPH per min at 25°C.

Results

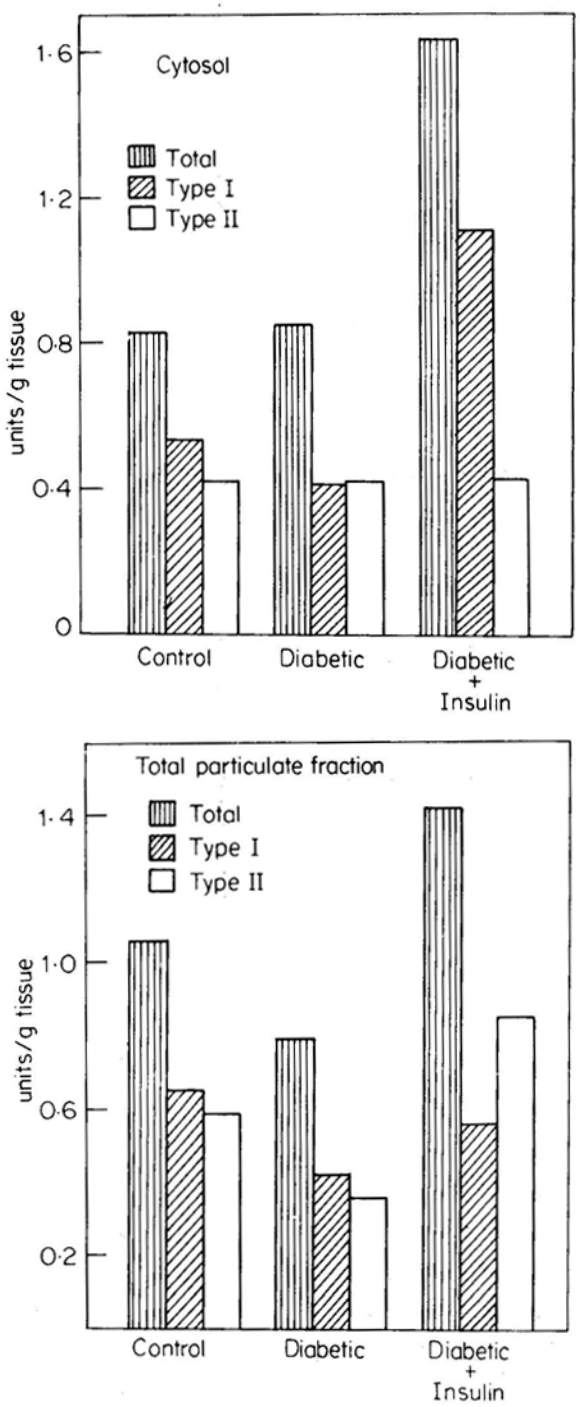
The results of the present experiment on the distribution of the isoenzymes of hexokinase are presented in figures 1 to 4. Figures 1 and 2 show the pattern in cerebral hemispheres. As can be seen, there was a significant decrease in the amount of Type I bound to the pellet which was increased to values larger than of the controls on the administration of insulin. There was also a marginal (20%) change in the Type II bound to the total particulate fraction, which again increases to more than the control value on insulin administration similar to the pattern observed with Type I. The soluble hexokinase, however, was not altered markedly, only the administration of insulin to diabetic animals activated the enzymes to some extent.





Figures 1 and 2. Distribution of hexokinase isoenzymes in cerebral hemisphere.

The distribution pattern of heart isoenzymes is shown in Figures 3 and 4. Unlike the hexokinase from cerebral tissue, the total particulate fraction Type I did not



Figures 3 and 4. Distribution of hexokinase isoenzymes in heart.

alter significantly, but the Type II bound to the total particulate fraction was decreased in diabetes and the effect was reversed by the administration of insulin. The soluble, total and Type I also increased in activity, similar to the changes in the brain.

The distribution pattern of the hexokinase between the soluble and the total particulate fraction in brain is shown in table 1. A significant decrease in the

Table 1. Distribution of hexokinase in the soluble and particulate fractions of brain tissues of the rat

Condition	Total hexokinase	Soluble	Total particulate fraction
		(units/g tissue)	
Control	5.8±0.94	1.69±0.26	4.16+0.71
Diabetic	4.59±0.44 (NS)	1.46+0.13 (NS)	2.73±0.29 (NS)
Diabetic+ insulin	8.59±0.70[a]	2.25±0.39 (NS)	6.34±0.33[a]

Each value is a mean ± SEM of at least four determinations.
[a] : $p < 0.05$
NS – not significant

enzymes bound to the total particulate fraction occurred with alloxan diabetes. Administration of insulin increased the bound hexokinase accompanied by the activation of the soluble hexokinase. Similar results were found in heart hexokinase as shown in table 2, except that the inactivation of bound hexokinase in diabetes was not as significant as that found in the case of brain.

Table 2. Distribution of heart hexokinase in the soluble and particulate fractions

Condition	Total hexokinase	Soluble fraction	Total particulate fraction
		(units/g tissue)	
Control	1.89±0.10	0.83±0.18	1.06±0.14
Diabetic	1.64±0.17 (NS)	0.85±0.10 (NS)	0.79±0.13 (NS)
Diabetic+ insulin	2.89±0.45 (NS)	1.63±0.09[a]	1.42±0.23 (NS)

Each value is a mean ± SEB of atleast four determinations.
[a] $p = 0.02$.

The percentage of hexokinase bound to the total particulate fraction and the changes with diabetic conditions and insulin administration are shown in figure 5.

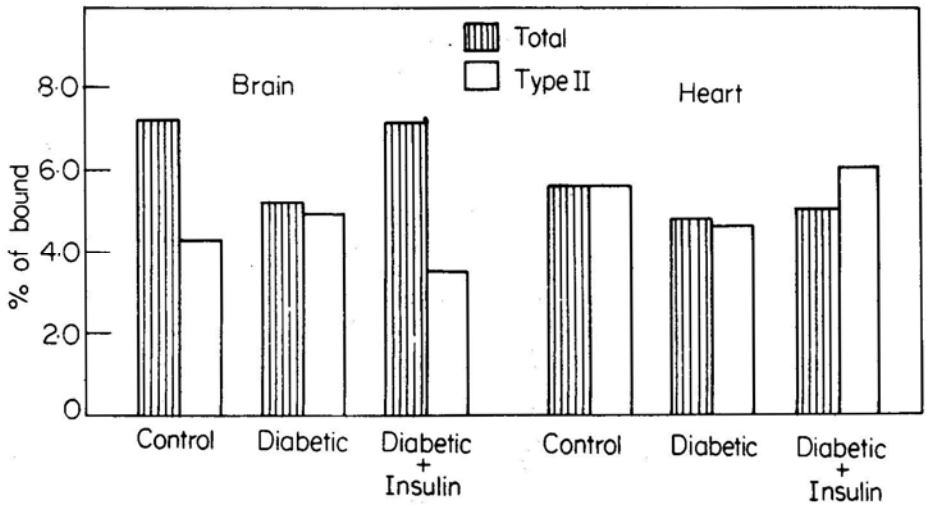


Figure 5. Percentage of hexokinase bound to total particulate fraction.

The total amount of hexokinase bound to the total particulate fraction in control brain and heart tissues of control animals were 72% and 56% respectively. Induction of diabetes reduced the amount bound to 52% and 48% in the brain and heart. The administration of insulin to diabetic animals restored the reduced levels of brain hexokinase to control values. The percentage of Type I and Type II bound are also shown in figure 5. The weight of the rats, protein values together with blood glucose values are presented in table 3.

Table 3. Weight of rats, protein and glucose content of blood, brain and heart tissues of control, diabetic and insulin-treated rats

Treatment	Weight	Blood glucose (mg)	Protein content (mg/g)			
			Brain		Heart	
			Soluble	Pellet	Soluble	Pellet
Control	201±17	77.4±9.9	58±5.0	65.2±5.4	72.0±8.6	57.9±4.8
Diabetic	164±9	270±35	64.0±3.5	66.9±3.1	70.9±3.7	62.3±3.9
Diabetic+ insulin	125±11	168±11.5	42.4±4.1	55.0±5.3	51.4±5.6	61.2±7.9

Each value is a mean ± SEM of at least four determinations.

Discussion

Katzen and Schimke (1965) and Katzen (1966) from a survey of the multiple forms of hexokinase in different normal tissues and from changes found in starved or alloxan diabetic rats for the first time suggested that there might be a correlation between hexokinase Type II and the action of insulin. Several workers (Moore *et al*., 1964; Katzen, 1966; McLean *et al*., 1966) had previously demonstrated that the activity of Type II enzyme in adipose tissue decreased in starvation and diabetes, conditions characterized by low blood insulin levels. Katzen (1967) later proposed that the amount of hexokinase Type I relative to that of Type II might be an important factor in determining insulin sensitivity. A relative deficiency in Type I appeared to be correlated with insulin requirement.

Diabetes induced by alloxan is known to cause changes in cerebral metabolites (Thurston *et al*., 1975). The most significant changes were the levels of glucose-6-phosphate and phosphocreatine. Wilson (1968) had earlier demonstrated the solubilization of brain hexokinase by glucose-6-phosphate. Taking into consideration the above two observations, it was demonstrated that induction of diabetes reduced the amount of hexokinase bound to the total particulate fraction, in both brain and heart (tables 1 and 2). There was, however, no concomitant increase in the soluble enzyme in both the tissues. The increased content of glucose-6-phosphate in cerebral tissues of diabetic animals might be one of the factors responsible for releasing the enzyme from the particulate fraction. It is also probable that the lack of insulin (due to the diabetic state) might cause the inactivation of the hexokinase in the soluble fraction. On insulin administration, the bound enzyme increased in both heart and brain and an increase in activity of the soluble enzyme was also observed. The relative percentage of Type I and Type II bound to the total particulate fraction in brain and heart can be seen clearly in figure 5. Type I isoenzyme was also modified in the diabetic state in both the tissues, the changes in Type II isoenzyme of insulin-sensitive tissues, in a diabetic state is already known and was discussed earlier (Moore *et al*., 1964; Katzen, 1966; McLean *et al*., 1966).

Insulin may be of importance both in the binding of hexokinase to cell membranes, in particular to mitochondria and in the stability of hexokinase Type II (Waiters and McLean, 1968). The observation reported here substantiates the above view that insulin is an important factor for the normal physiological functioning of the tissues, like the brain and heart which are responsive to the *in vitro* addition of the hormone.

The ability of insulin to increase the *in vitro* content of hexokinase in epididymal adipose tissue has been demonstrated by Hansen *et al*. (1970). Insulin by itself was found to be ineffective but in the presence of an energy source like glucose, it caused a significant stimulation. The increased activity of hexokinase in the diabetic state with insulin administration, as shown in the present results (table 1) may be due to the presence of insulin in a condition with high circulating glucose levels.

Further work is in progress on the activity of other enzymes and metabolites controlling the process of brain glycolysis in diabetes.

Acknowledgements

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