J. Biosci., Vol. 9, Numbers 1 & 2, September 1985, pp. 35–40. © Printed in India.

Changes in the subcellular distribution of brain and heart hexokinase isoenzymes during alloxan diabetes

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MS received 7 August 1984; revised 11 February 1985

Abstract. Changes in the subcellular distribution of hexokinase activity from three brain regions and heart were studied during alloxan induced diabetes. There was an overall decrease in the particulate hexokinase with an increase in the soluble form, after different time intervals of the onset of diabetes. Administration of insulin to the diabetic rats showed a partial counteraction of the enzyme changes. A possible regulation of brain hexokinase by metabolite changes is proposed.

Keywords. Hexokinase; isoenzymes; brain; insulin; diabetes.

Introduction

Adult brain is dependent on glucose as the major substrate for energy metabolism (Mcllwain and Bachelard, 1971). The key position of hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) in the cellular metabolism of glucose makes the study of this enzyme of great interest. Hexokinase in the brain is predominantly localized in the particulate fraction (Crane and Sols, 1953; Wilson, 1968) and, *in vivo* particulate and soluble forms of the enzyme remain in a state of dynamic equilibrium, contributing to the regulation of glycolysis in the central nervous system (Knull *et al.,* 1973).

The present results demonstrate the changes in the amount and subcellular distribution of the two isoenzymic forms of hexokinase from different regions of the rat brain under conditions of short and long term diabetes. Since effects of diabetes on heart tissue are well documented, heart tissue was also included in the study for comparison.

Materials and methods

Animals

Albino rats of Wistar strain, weighing between 200 and 300 gm were used for all the experiments. The rats were made diabetic with alloxan and were divided into two

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Abbreviation used: TPF, Total particulate function.

groups (8–12 rats were used in each group for the different time intervals), as described earlier (Murthy and Baquer, 1981). The first group of rats formed the diabetic group. To the second group, protamine zinc insulin was injected each day (2 units/day) until the day of sacrifice and these were designated as diabetic rats treated with insulin. Both groups were given food and water *ad libitum.*

Preparation of homogenates

Rats were killed by cervical dislocation and cerebral hemispheres, cerebellum, brain stem and heart were excised immediately and weighed. Tissue homogenates (1:10) were prepared as described earlier (Murthy and Baquer, 1981). The extracts were centrifuged at 12,000 *g* for 40 min. The 12,000 *g* supernatant was used as the soluble fraction. The pellet was washed once and then resuspended in the homogenizing medium, and formed the total particulate fraction (TPF).

Enzyme assay

Hexokinase was estimated essentially according to the method of Sharma *et al.* (1963) as modified by Gumaa and McLean (1972). The TPF was treated with Triton-X-100 (final concentration 0·5 %) in the cold for 30–60 min to liberate the latent and bound enzymes. For the estimation of Type II hexokinase activity, aliquots of each fraction were heated at 45°C for 1 h (Grossbard and Schimke, 1966). This process destroyed hexokinase Type II completely. A unit of hexokinase activity was defined as the amount required to form 1 μmol of NADPH per min at 25°C.

Blood glucose was estimated according to the method of Bergmeyer *et al.* (1974).

ATP, glucose-6-phosphate dehydrogenase, NADP and Tris were obtained from Sigma Chemical Co., St Louis, Mossouri, USA. All the other chemicals were from British Drug House, UK and were of analar grade.

Results

The severity of diabetes was established by the determination of blood glucose levels, which increased significantly from control values of 5·5 ± 0·3 mM to 26·5 ± 2·3, 28·8 ± 0·9 and 31·6 ± 1·2 mM at 3, 8 and 15 days, respectively after induction of diabetes. Administration of insulin to diabetic rats (D + I group) decreased the blood glucose level to 15·4 ±0·8, 14·6 ± 1·3 and 12·9 +0·9 mM on 3, 8 and 15 days, respectively.

Changes in hexokinase activity in brain regions

The total hexokinase from TPF in cerebral hemispheres showed a significant decrease in activity at 3, 8 and 15 days of alloxan treatment, whereas, the total enzyme activity from the soluble fraction increased at all the three time intervals (tables 1 and 2). Hexokinase Type I and II isoenzymes from both the fractions followed the same pattern of changes as that of the total enzyme activity. A significant decrease in TPF hexokinase Type II isoenzyme activity (40–60 %) was observed at all the three time intervals after diabetes ($P < 0{\cdot}001$). The soluble Type II isoenzyme, however, did not show any significant change. Results are shown in tables 1 and 2, and figure 1A.

Table 1. Effect of alloxan diabetes and insulin administration on total particulate hexokinase from brain and heart.

	Cerebral hemispheres		Cerebellum		Brain stem		Heart	
Time (days)	D	D+I	D	D+I (units/gm)	D	D+I	D	D+I
3	$7{\cdot}4^{c} \pm 0{\cdot}4$	$8{\cdot}6 \pm 0{\cdot}4$	$5{\cdot}6 \pm 0{\cdot}3$	$6{\cdot}1 \pm 0{\cdot}3$	$3{\cdot}1 \pm 0{\cdot}2$	$2{\cdot}7 \pm 0{\cdot}2$	$0{\cdot}78^{c} \pm 0{\cdot}07$	$1{\cdot}2^{b} \pm 0{\cdot}1$
8	$7{\cdot}3^{c} \pm 0{\cdot}3$	$9{\cdot}7 \pm 0{\cdot}6$	$5{\cdot}3 \pm 0{\cdot}2$	$5{\cdot}3 \pm 0.02$	$2{\cdot}8 \pm 0{\cdot}2$	$3{\cdot}2 \pm 0{\cdot}2$	$1{\cdot}3^{a} \pm 0{\cdot}08$	$1{\cdot}8 \pm 0{\cdot}03$
15	$7{\cdot}5^{c} \pm 0{\cdot}1$	$9{\cdot}3 \pm 0{\cdot}4$	$5{\cdot}8 \pm 0{\cdot}2$	$5{\cdot}9 \pm 0{\cdot}4$	$3{\cdot}1 \pm 0{\cdot}2$	$3{\cdot}4 \pm 0{\cdot}04$	$1{\cdot}3 \pm 0{\cdot}1$	$1{\cdot}6 \pm 0{\cdot}04$
Control	$9{\cdot}1 \pm 0{\cdot}2$		$5{\cdot}9 \pm 0{\cdot}2$		$3{\cdot}1 \pm 0{\cdot}2$		$1{\cdot}6 \pm 0{\cdot}1$	

Each value is a mean ± S.E.M. of four or more experiments done in triplicates.
D = Diabetes and D + I = diabetes + insulin.
[a] $P < 0{\cdot}05$; [b] $P < 0{\cdot}02$; [c] $P < 0{\cdot}001$.

Table 2. Effect of alloxan diabetes and insulin administration on total soluble hexokinase from brain and heart.

	Cerebral hemispheres		Cerebellum		Brain stem		Heart	
Time (days)	D	D+I	D	D+1 (units/g)	D	D+I	D	D+I
3	$3{\cdot}6^{e} \pm 0{\cdot}3$	$3{\cdot}3 \pm 0{\cdot}8$	$3{\cdot}9^{e} \pm 0{\cdot}2$	$3{\cdot}1 \pm 0{\cdot}1$	$3{\cdot}3^{e} \pm 0{\cdot}05$	$2{\cdot}8 \pm 0{\cdot}12$	$2{\cdot}8^{b} \pm 0{\cdot}1$	$3{\cdot}2 \pm 0{\cdot}2$
8	$3{\cdot}6^{a} \pm 0{\cdot}2$	$3{\cdot}2 \pm 0{\cdot}1$	$3{\cdot}2^{b} \pm 0{\cdot}1$	$2{\cdot}8 \pm 0{\cdot}1$	$3{\cdot}0 \pm 0{\cdot}2$	$2{\cdot}8 \pm 0{\cdot}1$	$2{\cdot}7^{d} \pm 0{\cdot}1$	$3{\cdot}6 \pm 0{\cdot}2$
15	$4{\cdot}1^{e} \pm 0{\cdot}1$	$3{\cdot}6 \pm 0{\cdot}3$	$3{\cdot}5^{c} \pm 0{\cdot}2$	$3{\cdot}4^{c} \pm 0{\cdot}3$	$3{\cdot}0^{e} \pm 0{\cdot}1$	$3{\cdot}1^{a} \pm 0{\cdot}2$	$3{\cdot}1 \pm 0{\cdot}2$	$3{\cdot}5 \pm 0{\cdot}2$
Control	$3{\cdot}1 \pm 0{\cdot}1$		$2{\cdot}8 \pm 0{\cdot}1$		$2{\cdot}6 \pm 0{\cdot}1$		$3{\cdot}6 \pm 0{\cdot}2$	

Each value is a mean ± S.E.M. of four or more experiments done in triplicates.
D = Diabetes and D +I = diabetes + insulin.
[a] $P < 0{\cdot}05$; [b] $P < 0{\cdot}02$; [c] $P < 0{\cdot}01$; [d] $P < 0{\cdot}002$; [e] $P < 0001$.

In the cerebellum, total and Type I hexokinase activity from TPF did not change. In the soluble fraction, however, total as well as Type I isoenzyme activity increased significantly after 3,8 and 15 days (tables 1 and 2). A significant decrease in hexokinase Type II isoenzyme activity was observed at 8 days ($P < 0{\cdot}05$), with a subsequent increase in the soluble form at 8 and 15 days ($P< 0{\cdot}05$ and $P < 0{\cdot}01$, respectively). Results are given in tables 1 and 2 and figure 1B.

The total and Type I hexokinase activity from particulate fraction in the brain stem did not show any change with diabetes, but the enzyme activity in the soluble fraction increased significantly at 3 and 15 days after alloxan treatment. Hexokinase Type II activity in TPF showed a significant decrease at 3 days ($P < 0{\cdot}05$) with no change in the soluble form. The results are presented in tables 1 and 2 and figure 1C.

Administration of insulin to the diabetic rats restored the enzyme activity to near control levels in all the brain regions.

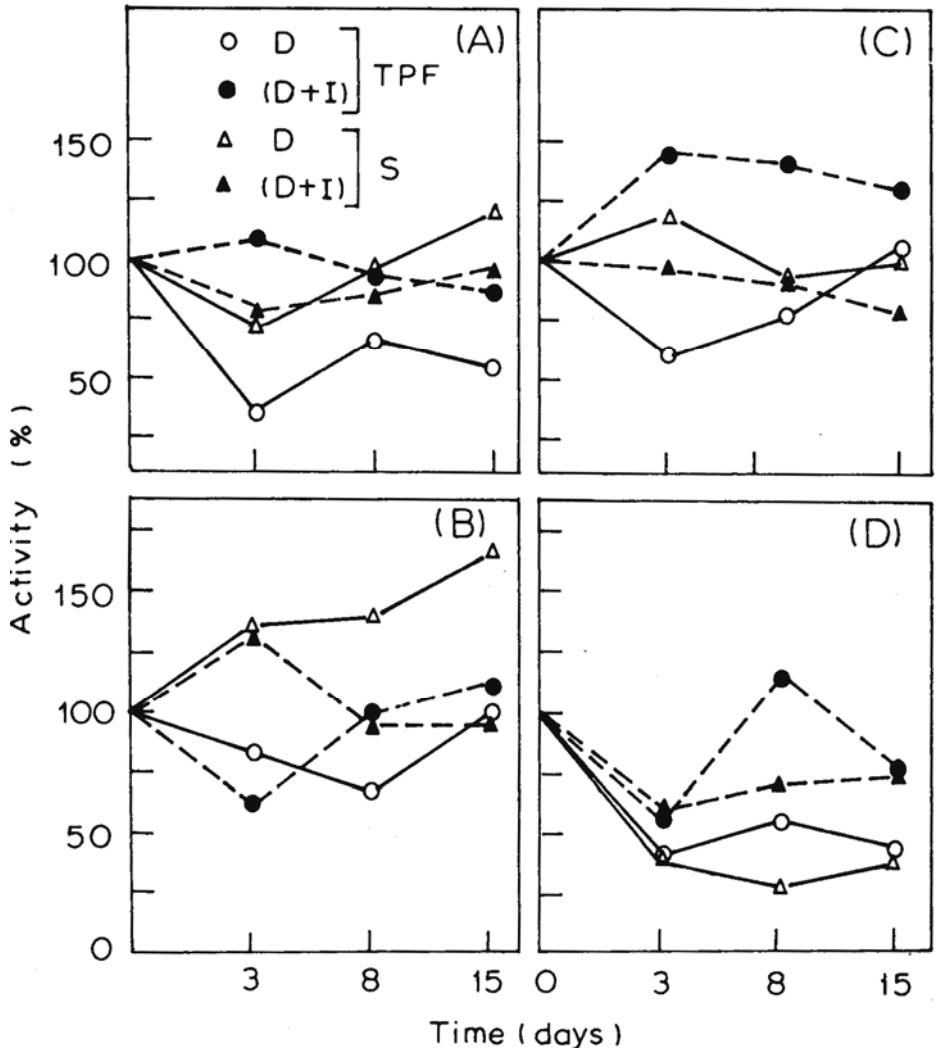

Figure 1. Effect of alloxan diabetes and insulin administration on hexokinase Type II isoenzyme in (A) cerebral hemispheres (CH); (B) cerebellum (CB); (C) brain stem (BS) and (D) heart (H). Absolute activities of hexokinase Type II in control rats from TPF are 1·35 ±0·08; 0·6 ±0·06; 0·4 ±0·03 and 0·41 ±0·07 in CH, CB, BS and H respectively and from soluble fraction are 0·8 ± 0·04; 0·5 ± 0·06; 0·45 ± 0·05 and 1·3 ± 0·1 in CH, CB, BS and H, respectively. (S, Soluble; D, Diabetes, D + I, diabetes + insulin).

Changes in hexokinase activity in heart tissue

A significant decrease in the total hexokinase activity from the TPF and the soluble fraction in heart tissue was observed after 3, 8 and 15 days of alloxan treatment. Hexokinase Type I isoenzyme activity from TPF showed a significant decrease at 3 and 15 days of diabetes, with no change in the soluble form. Significant changes were, however, found in hexokinase Type II, with a significant decrease in the activity from

TPF and soluble fraction at all the three time intervals ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively). Administration of insulin to diabetic animals restored the activity of total and Type I hexokinase in heart tissue to near control levels. Results are shown in tables 1 and 2 and figure 1D.

As hexokinase Type I followed the same pattern of changes as total enzyme activity, results of total and Type II isoenzyme are presented in tables and figure, respectively.

Discussion

Significant changes in the intracellular distribution of brain hexokinase with alloxan diabetes were observed during the present investigation. The increase in the activity of the soluble hexokinase found in our experiments may be due to the partial solubilization of the particulate hexokinase, occurring as a consequence of the increased levels of glucose-6-phosphate in the rat brain (Blackshear and Alberti, 1974; Thurston *et al*.,1975). Wilson (1968) has reported that *in vivo,* the soluble:mitochondrial hexokinase equilibrium is very much sensitive to metabolite control, in particular to glucose-6-phosphate level.

Previous kinetic studies from bovine brain hexokinase have shown that mitochondria-bound hexokinase has a higher K_i for glucose-6-phosphate and a lower K_m for ATP as compared to the soluble form, suggesting that the bound form of the hexokinase in brain is the more active form (Copley and Fromm, 1967). The results obtained in the present experiments, therefore, suggest that the more active form (particulate) of hexokinase in the rat brain, may be converted to a less active (soluble) form under conditions of reduced cerebral metabolism.

The increased levels of free fatty acids in the brain during hypoxia cause solubilization of the brain hexokinase *in vivo* (Domanska-Janik *et al.,* 1978). Diabetes leads to the increased levels of free fatty acids in the blood (Schonfeld and Kipnis, 1968; Hall *et al.,* 1976), which are permeable through the blood brain barrier (Galli *et al*., 1971). The decreased activity of hexokinase in the particulate fraction of brain with diabetes may partly be a consequence of the solubilizing action of free fatty acids on particulate bound hexokinase with an increase in the soluble enzyme activity.

Katzen (1967) has reported that Type II isoenzyme is the adaptive form of hexokinase and is affected more in diabetes, as compared to Type I, in insulin sensitive tissues such as heart and adipose tissue. During the present experiments, hexokinase Type II, although present in very low amounts (15–20%) in brain, showed more significant changes than Type I and followed similar pattern of changes as that in the heart tissue.

Insulin administration reverses the effect of alloxan diabetes on hexokinase activity. The recovery in the enzyme activity may be due to the decrease in the free fatty acids level after insulin administration (Hall *et al.,* 1976), and correction in most of the metabolite changes seen in the brain of alloxan diabetic rats, especially glucose-6-phosphate (Thurston *et al.,* 1975).

Acknowledgements

One of the authors (G. K.) thanks the University for financial support in the form of a Fellowship. N.Z.B. was a Homi Bhabha fellow during the course of this work.

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