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Localization of glucose-6-phosphatase and fructose-1-6-diphosphaiase in subcellular fractions from different regions of the rat brain

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Abstract. Two key enzymes of gluconeogenesis, glucose-6-phosphatase and fructosp-1-6-diphosphatase, were present in the cerebral hemispheres, the cerebellum and the brain stem of the rat brain. Significant activities of these-enzymes were associated with the particulate fraction.

Keywords. Glucose-6-phosphatase; fructose-1-6-diphosphatase; brain; subcellular localization.

Introduction

Gluconeogenesis comprises the synthesis of glucose from non-carbohydrate sources like lactate, pyruvate, glycerol and amino acids. The activity, regulation and general properties of gluconeogenic enzymes have been well studied in the liver and the kidney (Exton, 1972). However, the existence and activity of gluconeogenic enzymes has not been adequately studied in the brain. The presence of these enzymes in the brain has attracted the attention of investigators only recently (Cheng and Cheng, 1972; Patel, 1974). In the present study, an attempt has been made to localize two gluconeogenic enzymes, glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, E.C. 3.1.3.7) and fructose-l-6-diphosphatase (fructose-l-6-diphosphate I phosphohydrolase, E.C. 3.1.3.11) in three regions of the rat brain, namely, cerebral hemispheres, the cerebellum and the brain stem.

Glucose-6-phosphatase occupies an unique metabolic position, catalyzing the terminal reaction not only in gluconeogenesis but also in glycogenolysis (Ashmore and Weber, 1959). Its presence in chicken brain has recently been reported by Richardson *et al.* (1979). Fructose diphosphatase catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of this process (Tejwani *et al.,* 1976).

Materials and methods

Adult rats of Holtzman strain weighing between 180-220 g were used. The animals were killed by cervical dislocation and the brains dissected out immediately. Each

brain was divided into three regions, cerebral hemisphere, cerebellum and brain stem. Tissues were homogenized (1:10) using a Potter Elvehjem-type homogenizer fitted with a teflon plunger in 0.25 M sucrose-buffered with 20 mM triethanolamine (p H 7.4) containing 0.1 mM dithiothreitol. Supernatant and crude mitochondrial fractions were prepared by differential centrifugation according to the method of Whittaker (1959). The mitochondrial fraction was washed once with the homogenizing medium before assaying for enzyme activity.

Glucose-6-phosphatase was estimated according to the method of Baginski *et al.* (1974) with some modifications. The reaction mixture contained the following components in a final volume of 0.4 ml; Tris-HCl bufer, 135 mM (pH 6.5); EDTA, 5 mM and glucose-6-phosphatase, 20 mM. The reaction was initiated by the addition of enzyme extract (100 µl in the case of brain region and 50 µl in the case of liver and kidney were used per assay) and incubated for 15 min at 30°C. The reaction was stopped by adding 1 ml of 10% ice-cold trichloroacetic acid. The precipitate was centrifuged (800 *g*) and 0.3 ml of the clear supernatant was used for the estimation of inorganic phosphate (P_i) by the method of Chen *et al.* (1956). Absorbance was recorded at 820 nm in a Carl Zeiss Spectrophotometer.

Fructose diphosphatase activity was determined in the different regions of the brain according to the method of Toshima and Yoshimura (1975) with some modification. The reaction mixture contained the following components in a final volume of 0.4 ml: Tris-HCl buffer, 135 mM (pH 7.5); $MgCl_2$, 14 mM and fructose diphosphate, 5 mM. The reaction mixtures were assayed at 30°C for 15 min and P_i liberated was estimated as described for the assay of glucose-6-phosphatase.

Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. All chemicals were of Analar grade. Substrates were obtained from Sigma Chemical Co., St. Louis, Missouri, USA.

Results and discussion

The activities of glucose-6-phosphatase and fructose diphosphatase (mitochondrial and soluble) in different regions of the rat brain are presented in table 1, along with protein values. The activities of the two enzymes were also assayed in liver and kidney.

The results obtained in the present study showed that high percentages of these two gluconeogenic enzymes were present in the mitochondrial fraction (glucose-6-phosphatase was 52.4%, 47.6% and 48.5% and fructose diphosphatase 47.7%, 31% and 30.2% in the cerebral hemisphere, the cerebellum and the brain stem respectively, normalizing the activity in the supernatant+mitochondrial fraction to 100%). Recently it was shown that glucose-6-phosphatase is present in hepatic mitochondrial preparations of some vertebrates (Vorhaben and Campbell, 1979). It is well known that the amount of gluconeogenesis in the adult brain is very low (Balazs, 1970) as compared to liver and kidney. The other two gluconeogenic enzymes, pyruvate carboxylase (EC 6.4.1.1) and phosphenol pyruvate carboxykinase (EC 4.1.1.32) have already been shown to be present in brain (Cheng and Cheng, 1972; and Patel, 1974) and both these enzymes in brain have been shown to be associated with the mitochondrial fraction. The present data also shows

Table 1. Glucose-6-phosphatase and fructose diphosphatase activites in different regions of rat brain.

	Glucose-6-phosphatase nmol/g tissue/min		Fructose diphosphatase nmol/g tissue/min		Protein mg/g tissue	
	A	B	A	B	A	B
Cerebral hemispheres	186±20	203± 7	350±40	320±80	56± 5.1	84.3±23
Cerebellum	220±72	200± 9	400±60	180±50	48± 7.1	63.8±9.9
Brain stem	220±60	190±70	370±85	160±70	56.3±13.8	76.8±7.2

A = Supernatant fractions; B = Mitochondrial fraction.

Each value is a mean ± SEM of more than four values. The activity of glucose-6-phosphatase in liver and kidney is 6.04 ±1.24 and 7.1 ±2.02 and fructose diphosphatase, 2.11 ±0.47 and 5.0 ±0.87 μmol/g/min respectively.

that glucose-6-phosphatase and fructose diphosphatase which control the rate of gluconeogenesis are present in significant amounts in the mitochondria. Also, some key glycolytic enzymes like hexokinase, pyruvate kinase and aldolase are localized in the particulate fraction of the brain (MacDonnell and Greengard, 1974).

The presence of all the gluconeogenic enzymes in the brain indicates that the complete pathway is operative and may be of significance under conditions where enough intracellular glucose is not available for utilization, although the circulating blood glucose levels may be high. One such hormonal condition is diabetes where the absence of insulin adversely influences the normal transport of glucose into the cells. The availability of these enzymes may also be beneficial to the brain under conditions like hypoglycemia that may be caused by drugs.

Preliminary data from our laboratory (to be published elsewhere) show a differential effect of alloxan diabetes on the activities of the two enzymes in the three regions of the rat brain at different time intervals after the onset of diabetes. Administration of insulin to diabetic animals showed a differential effect in all the three regions. Based on these observations it can be postulated that glucose-6-phosphatase and fructose diphosphatase may have a functional role in carbohydrate metabolism in the brain.

References

Ashmore, J. and Weber, G. (1959) *Vitam. Horm.*, **17,** 91.

Baginski, E. S., Piero, P. F. and Bennie Zak (1974) *Methods of enzymatic analysis,* ed. H. U. Bergmeyer (New York: Academic Press) **2,** 876.

Balazs, (1970) *Handbook of neurochemistry*, ed. A. Lajtha (New York: Plenum Press) **3,** 1.

Chen, P. S. and Toribara, T. Y. and Warner, H. (1956) *Anal. Chem.,* **28,** 1756.

Cheng, S. C. and Cheng, R. H. C. (1972) *Arch. Biochem. Biophys.*, **151,** 501.
Exton, J, H. (1972) *Metabolism*, **21**, 945.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265.
MacDonnel, P. C. and Greengard, O, (1974) *Arch. Biochem. Biophys.,* **163,** 644.
Patel, M. S. (1974) *J. Neurochem.*, **22,** 717.
Richardson, R. J., Davis, C. S. and Johnson, M. K. (1979) *J. Neurochem*., **32,** 607.
Tejwani, G. A., Pedrosa, F. O., Pontremoli. S. and Horecker, B. L. (1976) *Arch. Biochem. Biophys.*, **177,** 253.
Toshima, Y. and Yoshimura, N. (1975) *J. Biol. Chem.*, **78,**1181.
Vorhaben, J. E. and Campbell, J. W. (1979) *Comp. Biochem. Physiol*., **B62,** 85.
Whittaker, V. P. (1959) *Biochem. J.*, **72,** 694.