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**BRAIN GLUTAMATE DEHYDROGENASE CHANGES IN STREPTOZOTOCIN
DIABETIC RATS AS A FUNCTION OF AGE**

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SUMMARY

Kinetic parameters of brain glutamate dehydrogenase (GDH) were compared in the brain stem, cerebellum and cerebral cortex of three weeks and one year old streptozotocin (STZ) induced four day diabetic rats with respective controls. A single intrafemoral dose of STZ (60mg/Kg body weight) was administered to induce diabetes in both age groups. After four days the blood glucose levels showed a significant increase in the diabetic animals of both age groups compared with the respective controls. The increase in blood glucose was significant in one year old compared to the three weeks old diabetic rats. The V_{max} of the enzyme was decreased in all the brain regions studied, of the three weeks old diabetic rats without any significant change in the K_m . In the adult the V_{max} of GDH was increased in cerebellum and brain stem but was unchanged in the cerebral cortex. The K_m was unchanged in cerebellum and cerebral cortex but was increased in the brain stem. These results suggest there may be an important regulatory role of the glutamate pathway in brain neural network disturbances and neuronal degeneration in diabetes as a function of age.

Key words: Glutamate dehydrogenase, Neuronal disturbance, Diabetes, Streptozotocin, Cerebellum, Brain stem, Cerebral cortex, Ageing.

INTRODUCTION

Glutamate dehydrogenase (GDH, E C 1.4.1.2-4) catalyses the reductive inter-conversion of α -ketoglutarate and L-glutamic acid. Transamination between this α -amino acid and α -keto acids determines the amount of this amino acid in the brain (1). Glutamate is a putative neurotransmitter and a precursor of the inhibitory neurotransmitter γ -aminobutyric acid (GABA). Diabetes mellitus is associated with peripheral as well as central nervous system neuropathy (2,3).

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An increased glutamate content is reported to cause neuronal degeneration (4-6). Glutamate, which causes excitotoxic neuronal damage, increases calcium influx through N-methyl-D-aspartate receptors in postsynaptic neurones, leading to phospholipase A₂ mediated arachidonic acid release (7). The increase in arachidonic acid in brain may mediate neuronal injury by inhibiting the sodium ion channels (8). Earlier work showed that GDH activity in the adult rat brain was increased during diabetes (9). The return of glutamate levels to normal reduces the diabetic complications in the brain. In this study 3 weeks and one year old streptozotocin induced diabetic rats were used to investigate the glutamate functional pathway alterations and their significance in diabetes as a function of the age of the animals.

MATERIALS AND METHODS

Animals

Three weeks old male Wistar rats, weighing ~50 grams and one year old animals weighing ~300 grams were used for the experiments. They were fed lab chow and water *ad libitum* and maintained under a 12 hr light and 12 hr dark cycle. Each age group was divided randomly into two sets.

Materials

Streptozotocin was purchased from Sigma Chemical Co. USA. and all other biochemicals used were of analytical grade. Glucose kit (GOD-POD) was purchased from Glaxo India Ltd.

Induction of diabetes, Surgical & Analytical methods

One set in each of the age groups received a single intrafemoral vein injection of streptozotocin (60 mg/Kg of body weight) dissolved in citrate buffer (pH 4.5) under ether anaesthesia. The second set of the group were injected with buffer only and served as the control. The blood glucose level was estimated with GOD-POD method using the Glucose kit. Protein concentration was estimated (10) using BSA as the standard. After four days the rats were sacrificed by decapitation, brain tissues were dissected (11) and the tissues were stored immediately at -70°C until used.

Enzyme Assay

Glutamate dehydrogenase activity was measured in the crude extract of the brain regions (12). Sample extracts were prepared by making 5% homogenate of the tissue in cold distilled water and the supernatant fluid was collected after centrifugation at 10,000 xg for 20 minutes. The enzyme activity was measured in supernatant fluid as follows: The reaction mixture in the experimental and reference cuvettes contained 0.04M triethanolamine buffer of pH 8.0, 2.6mM EDTA, 105mM Ammonium acetate and 100 µl of the enzyme sample extract of appropriate concentration. The reaction mixture of 1ml volume was assayed at 366 nm using a Milton Roy Genesis spectrophotometer by adding saturating concentrations of α-ketoglutarate and 10mM NADH. Decrease in optical density (O.D.) due to the oxidation of NADH was measured at 15 second intervals for two minutes at room temperature. The decrease in absorbance was linear during the course of all assays. One unit of enzyme activity is equal to the change in O.D. of 0.1 in 100 seconds at 366 nm. Activity of enzyme was expressed as specific activity represented by units/mg protein. Kinetic parameters, V_{max} and K_m, were calculated from the data of GDH activity measured at substrate concentrations of 0.5mM, 1mM, 2mM and 4mM of α-ketoglutarate. The data were analysed using students *t*-test (13).

RESULTS

The blood glucose levels were significantly increased in diabetic rats of both age groups compared to their respective controls (Table-1). The extent of hyperglycaemia was seen to be higher in one year old rats compared with that of the 3 weeks old rats. The V_{max} of GDH in the cerebellum was decreased in young and increased in adult animals without any change in the K_m value compared with the values for the respective control rats (Table-2). The brain stem samples of both groups showed similar trend in GDH activity, the V_{max} of the enzyme was decreased very significantly ($P < 0.01$) in young and both the K_m and V_{max} were increased significantly in adult

TABLE-1
Blood glucose level of Streptozotocin treated rats (mg/dl).

Young rats (3 Week old)		
	Initial	Final
Control	59.73 ± 2.78	71.88 ± 2.35
Diabetic	77.26 ± 2.89	152.03 ± 22.90*
One year old rats		
Control	86.77 ± 3.27	85.34 ± 5.94
Diabetic	87.3 ± 1.42	361.32 ± 17.37*†

* $p < 0.01$ when compared to controls.

† $p < 0.05$ when compared to young diabetic rats.

Values are mean ± S.E.M. of 4-6 separate determinations.

TABLE-2

Kinetic parameters of glutamate dehydrogenase in the cerebellum of 3 week and one year old diabetic rats. Diabetes was induced by streptozotocin and was of four day duration

	V_{max} (Units/mg protein)	K_m (mM)
Control (3 week)	42.84 ± 1.68	0.34 ± 0.02
Diabetic (3 week)	$34.01 \pm 1.69^*$	0.35 ± 0.03
Control (1 year)	36.89 ± 1.79	0.26 ± 0.02
Diabetic (1 year)	$49.24 \pm 4.47^*$	0.26 ± 0.02

* $p < 0.05$ compared to the respective control

Values are mean \pm S.E.M. of 4-6 separate determinations.

animals ($P < 0.05$, Table-3). In the cerebral cortex, the V_{max} of the enzyme was decreased in young rats without any change in the K_m and in adult the kinetic parameters were unchanged (Table-4).

DISCUSSION

The glucose content was more significantly increased in the one year old diabetic rats than that of the young diabetic rats. This suggests that young rats may have more resistance to streptozotocin induction of diabetes than one year old rats. The differential glucose content observed may be due to the regenerative capacity of the pancreatic β -cell of the young compared to the adult (14). The decreased V_{max} of GDH in the cerebellum of young rat, without any change in K_m , compared to the control, suggests a decreased number of enzyme molecules and the increased V_{max} without change in K_m in the adult indicates an increase in the number of enzyme molecules. The results for the brain stem also exhibited the same trend to both age groups but the decrease in V_{max} in young was the more significant. The increased V_{max} and K_m of the enzyme in

TABLE-3

Kinetic parameters of glutamate dehydrogenase in the brain stem of 3 week and one year old (four) day diabetic rats

	V_{\max} (Units/mg protein)	K_m (mM)
Control (3 week)	9.97 ± 0.21	0.55 ± 0.06
Diabetic (3 week)	$4.64 \pm 0.25^{**}$	0.75 ± 0.05
Control (1 year)	4.88 ± 0.34	0.56 ± 0.04
Diabetic (1 year)	$7.99 \pm 0.88^*$	$0.80 \pm 0.03^*$

* $p < 0.05$ compared to the respective control

** $p < 0.01$ compared to the respective control

Values are mean \pm S.E.M. of 4-6 separate determinations.

this region of the adult rats are consistent with the induction of GDH during severe diabetes. In the cerebral cortex the decrease in the V_{\max} of GDH was significant while in the adult there was a slight but insignificant increase in V_{\max} . The results suggest that in young rats the compensatory regulatory pathway of GDH is functioning to decrease the synthesis of glutamate whereas this is not seen in the adult rats. It is of note that the extracellular glutamate concentration should be low in order to avoid diabetes associated brain changes; since excessive glutamate receptor activation can lead to neuronal damage (15). Since the immature brain is less vulnerable to glutamate induced neurotoxicity than the mature brain (16), the increased GDH activity observed in the adult rats may be one of the reasons for adult diabetic neuropathy. In diabetes the GABA content decreases in the brain regions of adult rats (17). In the brains of alloxan diabetic amphibians the content of GABA is reported to decrease and glutamate to increase. This may be due to an inhibition of glutamate decarboxylase, which catalyses the conversion of glutamate to GABA, by α -ketoglutarate. GABA is involved in the release of various neurotransmitters through GABA_B receptors (18). The change in neurotransmitter content during severe diabetes

TABLE-4

Kinetic parameters of glutamate dehydrogenase in the cerebral cortex of 3 week and one year old (four) day diabetic rats

	V_{max} (Units/mg protein)	K_m (mM)
Control (3 week)	3.26 ± 0.04	0.55 ± 0.05
Diabetic (3 week)	$2.67 \pm 0.09^*$	0.64 ± 0.05
Control (1 year)	2.67 ± 0.12	0.52 ± 0.03
Diabetic (1 year)	2.09 ± 0.06	0.54 ± 0.09

* $p < 0.05$ compared to the respective control

Values are mean \pm S.E.M. of 4-6 separate determinations.

may be due to the imbalance between the two major CNS neurotransmitters- glutamate and GABA (19). The results of this study suggest that the regulatory mechanism functioning in young diabetic rats avoids glutamate toxicity in the brain whereas in the adult the toxicity is uncontrolled. This has clinical implications in the mechanism of maturity onset diabetes.

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