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Glutamate dehydrogenase induction in the brain of streptozotocin diabetic rats

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Brain glutamate dehydrogenase (GDH) activity was compared between streptozotocin induced diabetic rats and insulin treated diabetic rats using crude and purified preparations from rat brain. V_{max} of GDH increased significantly ($p<0.05$) while K_m remained same in diabetic state when compared to control. Insulin treatment restored V_{max} to normal values. The observed changes in the present study indicate disturbance in the metabolic pathway of glutamate which may bear importance in view of the physiological and cognitive disorders associated with diabetes.

In diabetes glucose metabolism is increased in brain and increase in circulating ketone bodies supplement the increased fuel requirement to a certain extent[1,2]. Moreover the free accessibility of brain cells to glucose makes it different from other cells[3]. When ketone bodies are available in the circulating blood, it is used in proportion to their plasma concentration[2]. Mans *et al.*[1], while studying whether glucose utilization is decreased as a compensatory effect to ketone body utilization, checked glutamate and glutamine level during first week and after four weeks. They found that glutamate level remained the same and glutamine increased significantly, while all other intermediary metabolites remained unaltered. Glutamate is a putative neurotransmitter and a precursor of GABA. Transamination between these alpha-amino acid and alpha ketoacid in the brain decides the amount of this amino acid in the brain[6]. In this paper we report glutamate dehydrogenase (E.C. 1.4.1.2-4) activity in diabetic and insulin treated diabetic rats.

Sprague-Dawley rats were purchased and acclimatised in the laboratory for two weeks before using them for experiments. Streptozotocin (STZ) was purchased from Sigma (St. Louis) and DEAE cellulose from Pharmacia. All other chemicals used were of AR grade.

Adult male rats of same age and weight (~200g) were used for all experiments. The rats were divided randomly into two groups. One group received single intrafemoral vein injection of streptozotocin (45 mg/kg of body wt) dissolved in a citrate buffered vehicle (*p*H 4.5) . A second group, injected with vehicle alone served as control. The first group was again divided into two and one group was given insulin injection daily according to a reported protocol[7]. The other group remained without any treatment. The blood glucose was estimated using glucose oxidase enzyme kit (Merck). The animals were given food and water *ad libitum*. After two weeks rats were sacrificed by decapitation, brain tissue were collected and stored immediately at –70°C till used.

The activity of glutamate dehydrogenase was determined by a continuous assay[8]. The incubation mixture contained alpha-ketoglutarate (saturating concentrations), NADH (1.5×10^{-4} *M*) triethanolamine (0.04*M*), EDTA (0.0026*M*), ammonium acetate (0.105*M*), and an appropriate concentration of enzyme solution of 0.2ml to start reaction. The final volume of the mixture was 2 ml. The decrease in optical density was measured at 366 nm.

GDH was purified from the three experimental groups (diabetic, insulin treated diabetic and control) by the method of Corman *et al.*[9]. The original procedure which designed to isolate GDH from liver tissue, was adopted with slight modifications. The purified enzyme was run on a non-dissociating-discontinuous disc gel (7.5%) and visualised using enzyme specific stain[10]. Separate gels were run for Coomassie staining for each step.

Protein was estimated by the method of Lowry *et al.*[11] with bovine serum albumin (BSA) as stan-

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dard. The data were statistically analysed using one way analysis of variance (ANOVA) and least significant difference (1sd)[12].

Blood glucose of diabetic rats were significantly elevated when compared to control values ($p<0.05$) on the third day of injection. The same was reversed to normal by insulin treatment (Table 1). The body weight of diabetic group reduced significantly and was reversed with insulin treatment .

Glutamate dehydrogenase was purified from the brains of control, diabetic and insulin treated diabetic rats (Table 2). The recovery of activity varied between 26 to 34%. The final preparation from DEAE column was pooled and used for kinetic studies.

As shown in Table 3, in diabetic rats the K_m remained unaltered while V_{max} showed a significant increase (~54%) ($p<0.05$) when compared to control values. Insulin treatment, although did not completely reverse the V_{max} a reversal to control value was observed. The K_m showed a significant decrease ($p<0.05$) in insulin treated group.

The results obtained with crude extract (Table 4) indicated a similar trend of increase in V_{max} in diabetic state as was observed with pure preparation, but there was no reversal with insulin treatment.

Increase in the V_{max} of GDH observed during conversion of α-ketoglutarate to glutamate, indicates an increase in glutamate content in brain. In similar studies reported earlier[1,2,13], in Long Evans rats, the level of glutamate remained the same for 4 weeks but the glutamine level increased after one week and later got stabilised. Glutamate which formed as a result of increased conversion from alpha-ketoglutarate may be channelised to its subsequent pathways (i) to glutamine and (ii) gammaaminobutyrate (GABA). In the latter case GABA may be recycled through succinic semialdehyde to succinate.

In diabetic rats without insulin treatment, the increase in V_{max} without any change in K_m indicate the possibility of increased number of enzyme molecules rather than an alteration in the enzyme itself. Earlier workers did not report change in glucose transport mechanism in STZ diabetic state[14]. When Sprague-Dawley rats were compared to Long-Evans, the former was found to develop severe ketosis than the later[1]. Same authors observed increased utilisation of glucose (when ketosis occur) for energy by brain in diabetic rats.

Table 1—Blood glucose levels of diabetic and insulin treated diabetic rats

[Data are mean±S E M of 6-7 separate estimations]

Experimental group	Body weight (Grams)	Blood glucose (mg/dl)
Control	183±3	111.7±7.1
Diabetic	172±2*	532.6±13.9*
Insulin treated diabetic	190±4	151.8±17.5

*$p<0.05$ with respect to control

Table 2—Purification of glutamate dehydrogenase from brain of diabetic and insulin treated diabetic rats

Steps	Specific activity (μ moles×10^3/min/mg protein) Control	Diabetic	Insulin treated diabetic
Acetone powder extract	0.45	0.72	0.47
Sodium sulphate precipitate (17-22%)	1.55	3.26	2.48
Ammonium sulphate precipitate (25-33%) +Dialysis	40.88	72.01	21.91
DEAE cellulose column chromatography pooled fraction	236.88	549.29	341.01

Table 3—V_{max} and K_m of purified GDH

[Data are mean±SEM determined from 4-6 separate estimations]

Experimental group	V_{max} (Units/mg of protein×10^3)	K_m (m*M*)
Control	236.68±16.50	18.75±2.1
Diabetic	549.20±35.30*	20.00±0.9
Insulin treated diabetic	341.01±21.51†	13.75±4.6*

*$p<0.05$ with respect to control
†$p<0.05$ with respect to diabetic group

Table 4—V_{max} and K_m of glutamate dehydrogenase in crude preparations from rat brain

[Data are mean±SEM of 6-7 separate estimations]

Experimental group	V_{max} (Units/mg of protein)	K_m (m*M*)
Control	505.10±107.5	16.5±3.1
Diabetic	778,.69±46.8*	19.8±4.3
Insulin treated diabetic	739.58±20.2*	13.8±0.8

*$p<0.05$ with respect to control

Nucleotide triphosphates act as inhibitors of GDH in the presence of NADH while nucleotide diphosphates act as activators in the presence of reduced coenzyme[15-17]. This is possible by a shift between the oligomeric form with little GDH activity and a polymeric form with high activity. A balance between the tri and dinucleotide in the brain and allosteric effect of coenzyme play an important role in the activation of GDH. So in diabetic state a shift in the dynamic equilibrium from triphosphates to diphosphate resulting from increased energy consumption may partially contribute to the increase in enzyme activity[1,2].

In crude preparation, insulin treatment did not reverse the increased V_{max} although such reversal is evident in pure enzyme preparation, when compared to diabetic group (Table 3). So the present observation shows that change in the enzyme function is partially reversible by insulin treatment. The K_m value in insulin treated diabetic rats showed a significant decrease when compared to control and diabetic group. This shows that insulin while being a good hypoglycemic agent, its effects on reversing metabolic derangement is not complete. With crude enzyme preparation from insulin treated diabetic rats, V_{max} showed no reversal as seen in pure preparations. Under untreated conditions changes in the glutamate metabolism can lead to deleterious effects resulting from glutamate toxicity[18,19].

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