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Choline Acetyltransferae Activity in the Brain of Alloxan Diabetic Albino Rats: Presence of an Inhibitor of this Enzyme Activity in the Cerebral Extract and Protection by *Cichorium intybus*

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ABSTRACT

Diabetes induced ischaemic manifestations and cerebral disorders have been demonstrated at neurochemical level. However, the pathogenesis still remains to be completely elucidated. The aim of the present study is to demonstrate cerebral manifestations of diabetes as brain is affected by recurrent episodes of hypoglycemia and poor metabolic control during diabetes. Diabetes was induced by alloxan monohydrate (40 mg/Kg i.v.,) in albino rats (300-315 g) and used 48 h after alloxan administration. Activity of Cholin acetyl transferasesterase (ChAT, E C 2.3.1.6) in the cerebrum , cerebellum and medulla oblongata of the brain of rats decreased during diabetes ($P<0.01$, 0.05 and 0.03 respectively). Cerebral extract from alloxan diabetic rats decelerated significantly ($P<0.05$) the activity level of ChAT in discrete regions of the brain of normal animals indicating the presence of an inhibiting factor in the cerebrum of diabetic rats. Diabetic rats when fed with *cichorium intybus* (chicory) for 10 days exhibited significant increase ($P < 0.001$) in ChAT activity., indicating that *Cichorium intybus offered neuro- protection* by virtue of its anti oxidant action.

KEY WORDS: ChAT, brain, alloxan diabetes, inhibitor, cerebral extract, *Cichorium intybus* (Chicory), Biological marker protein

INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with perturbation in glucose metabolism and homeostasis leading to cerebral injury (1,2). During diabetic neuropathy, cerebral uptake of glucose declines significantly (3,4) and the brain suffers from hypoglycaemic episodes(5). Diabetes induced hyperglycaemia augments the extent of neurological disorders due to enzyme protein inactivation (6). Enzymatic activities connected to glucose metabolism (7) and neurotransmission in the CNS of mammals have been studied during diabetes and the changes observed have been attributed to alterations occurring in the levels of RNA and proteins (8). Since diabetes induces significant changes in the levels of aminotransferases and protein levels, it is likely that ChAT activity which is the selective marker of cholinergic system is also affected. Motivated by this, the present study was initiated. The aim of this project is to identify the limiting molecular event whose modification is responsible for specific lesion in the CNS which can be correlated directly to the onset of diabetes in the animal model. Studies have been directed to follow the biochemical lesion and efforts have been made to investigate the possible intervention for arriving at neuroprotection during diabetes. This study is the first to report the presence of a neuroinhibitor in the cerebral extract of diabetic animal model and provide a strategy to revert the inhibitory effect by experimental manipulation utilizing plant extracts. Earlier studies from my laboratory revealed cardiprotective (9) and neuroprotective (10) effects of *Cichorium intybus* (Chicory) leaves extracts during aging and diabetes.We therefore investigated the nueroprotection offered by this plant extract during diabetes keeping inview of the serious biochemical lesions we found in the CNS as manifestations of alloxan induced diabetes during our continuing studies on neurochemical correlates of alloxan diabetes.

MATERIAL AND METHODS

Male albino rats of Wistar strain (280-300 g) were maintained in cages (3 in each cage at 26°C; 12 hr light:dark cycle). The animals were fed on standard pellet diet and water ad libitum. The rats were rendered diabetic with alloxan monohydrate (40 mg/kg; i.v.).

The study protocol was approved by the Institutional Animal Ethics committee.

The rats were divided into the following groups:

Gr I : Controls

Gr II : Diabetic

Gr III: Diabetic fed on dried leaf powder of *Cichorium intybus* (500 mg/kg) for 10 daysas reported earlier(9).

The animals were killed by decapitation and the brains were quickly removed and washed in ice-cold saline. Different regions of the brain were separated with sterilized fine bent forceps and scalpel, weighed in an electric balance in mammalian Ringer and immediately used for the determination of cholinacetyl transferase (ChAT) activity by the method of Morris(11). A final volume of 1ml incubation medium consisted of 12 mM choline chloride; 300mM NaCL, 0.26 mM eserine sulphate, 40 mM phosphate citrate buffer (pH 7.5), 0.35 mM acetyl CoA and the homogenate. The incubation medium was adjusted to pH 7.5 with 0.3 N NaOH at 37 °C for 2 min before addition of CoA. After the period of second incubation for 15 min at 37 °C the ACh content was estimated by the colorimetric method of Hestrin (12)..Homogenate (10% w/v) of different regions of the brain was prepared in ice-cold 0.02 M phosphate buffer (pH 7) centrifuged for 20 min at 6000 rpm and the supernatant was used for the assay.

Effects of *in vitro* administration of the aqueous cerebral extract

(0.5ml) from alloxan diabetic rats on the activity level of brain ChAT of normal animal were studied. It was noted from preliminary experiments that only the cerebral extract from the diabetic animal was capable of causing significant decrease in enzyme activity of brain in normal animals and therefore the effects of *in vitro* administration of the extracts from cerebellum, medulla and whole brain of diabetic animals were not studied.

The assay systems on addition of aqueous extract of the brain from normal animals constituted controls. Preliminary experiments indicated negligible difference between the activity levels of controls prepared on addition of cerebral extract from diabetic animals to the assay system after terminating the enzyme reaction and those receiving the extract of the brain from normal animals. Hence the latter were used as the experimental controls. The assay systems with the aqueous cerebral extract from the diabetic rat were the experimental samples.

Studies on neuroprotection by intervention with *Cichorium intybus*

Diabetic rats after administration of ***Cichorium intybus*** were used to demonstrate the neuroprotection by investigating ChAT activity in the brain. For this, ChAT isozymic spectrum was resolved on polyacrylamide gel electrophoresis and compared with that of control and diabetic pattern in the cerebral cortex.

50% (w/v) homogenates of the cerebral cortex were prepared in deionized distilled water and centrifuged at 7000 rpm for 1 hr at 4° C. Ten µl of the supernatant was used for the electrophoretic run. The gels were run at 6 m Amps/tube (D.C. power supply) for 2 hr using Tris-Boric acid-EDTA buffer at pH 8.5.

After the run, the gels were cut into 2.5 mm bits and each bit was homogenized in phosphate buffer, centrifuged and the supernatant was used for determination of ChAT activity colorimetrically.

RESULTS AND DISCUSSION

The weight of the animal and brain exhibited insignificant decrease as a function of the disease (Table 1). The blood sugar level demonstrated 200.5% elevation as a function of the disease (Table 1)

From the values given in Table 2, it is clear that the activity level of ChAT decreased in the cerebrum, cerebellum and medulla oblongata from diabetic rats. It is also seen (Table 2) that the decrease in the level of ChAT activity showed variation in relation to the region of the brain. The activity level of ChAT was the highest in the cerebrum of both normal and alloxan diabetic rats compared to the other regions of the brain. Further, the cerebrum showed marked response for the change in the activity level of ChAT and the brain stem the least (Table 2).

The decrease in the activity level of ChAT during diabetes may be a reflection of the remarkable inhibition of cholinergic system as ChAT is responsible for the synthesis of the neurotransmitter, **ACh** and thus acts as a bio-marker of cholinergic neurons. The decrease in the activity levels of ChAT in different regions of the brain of alloxan diabetic rats may be either due to the inhibition of the enzyme synthesis by the altered cellular environment prevailing in the brain, or due to a decrease in the rate of enzyme synthesis. Earlier studies also indicated an inhibition of ChAT activity leading to a decline in the synthesis of ACh in the brain of rats with alloxan diabetes. It was suggested that the cause of inhibition of ACh synthesis is inadequate permeation of glucose across the cell membrane.

Activity levels of ChAT in the cerebrum, cerebellum and medulla oblongata of normal animals determined

Table 1 Changes in glucose levels in blood of albino rats as a function of alloxan diabetes

Body wt. (g)	Brain wt. (g)	Blood Glucose (mg/100ml)
	Control	
80±2.3	1.7±0.08	102±3.2
	Diabetic	
75±1.6	1.5±0.6	326±7.5
@ -6.2 NS	@ -4.9 NS	@ +200***
@ NS not significant		*** P < 0.001

Values are mean ± SD of 26 obervations

Table 2 Activity levels of chat in different regions of the brain of control and diabetic albino rats

Cerebrum	Cerebellum	Medulla Oblongata
	Control	
3.4±0.9	3.8±0.02	1.5±0.01
	Diabetic	
0.96±0.01	1.2±0.025	0.63±0.01
@ 73.32***	@ -32.2*	@ -57.4**
***P<0.001	*P<0.05	** P < 0.01

@ Percentage change ; [ChAT activity expressed as milli moles ACh synthesized/min/mg protein. Values are Mean ± SD of 9 observations.

Table 3 : Effect of cerebral extract of diabetic rat on the chat in different regions of the control and diabetic albino rats

Nature of the extract used	Cerebrum	Cerebellum	Medulla Oblongata
Normal cerebral extract			
Treated tissue (**Controls**)	3.30 ± 0.01	1.38± 0.02	1.50± 0.012
Diabetic cerebral extract			
Treated tissue (**Exptls**)	0.69 ± 0.01	0.09 ± 0.01	0.71±0.025
Percentage Change	*** -79.1	*** -93.47	** -46.00
	*** P > 0.001		** P<0.01

Activity expressed as milli moles ACh synthesized/min/mg protein. Values are Mean ± SD of 11 observations

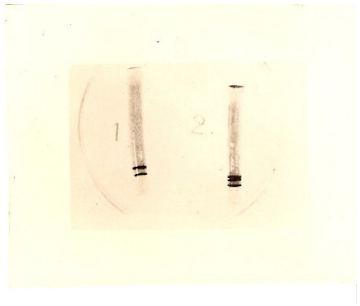

***Fig** 1. Photograph of hydrosoluble proteins in the cerebra cortex of brain resolved on Polyacrylamide gel electrophoresis*

A- Control
B- Diabetic
C- Diabetic fed on Chichorium intybus (see the abolition of the fastest moving protein band)

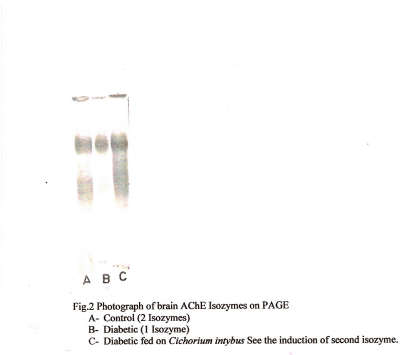

Fig 2.

in the presence of the cerebral extract from diabetic animals were significantly low (Table 3). The changes produced by the nervous extract of diabetic rats indicated the **presence of an inhibiting substance/s in the cerebral extract of alloxan diabetic rats** capable of depressing ChAT activity of normal animal on ***in vitro*** **administration**.

In order to characterize the inhibitory factor present in the cerebral extract of the diabetic rat, polyacrylamide gel electrophoresis of the hydro-soluble proteins was done and protein bands were stained with Amido Black (Fig.1). It is evident from Fig.1 that there is a fast moving protein band which is induced during diabetes and hence is absent in the control cerebral extract. This fast moving hydro-soluble protein is inhibitory in nature and is capable of inhibiting the activity of ChAT when added to the incubation medium thereby decreasing the level of ACh. This band is designated as **biological marker protein of diabetes (BMP-D).** This protein is capable of perturbing the Cholinergic synaptic dynamics by disturbing calcium homeostasis and inducing neuronal injury by creating neurological blocks/biochemical lesions due to accumulation of glutamate (220%) and calcium overload (unpublished observations; form the subject of next paper in this series).

In the CNS, changes in calcium fluxes across synaptic membrane are known to mediate important events underlying synaptic transmission (13). Cholinergic synaptic dynamics involves ACh synthesis, its storage and release. Biosynthesis of ACh occurs from acetate and choline by the mediatin of ChAT. In cholinergic synaptic vescicles, calmodulin (CaM) is required for stimulation of calcium transport and protein phosphorylation for the synaptic dynamics(14). Calcium over load in the neurons is damaging thus culminating in neuronal injury and dysfunction during diabetes. *Cichorium intybus* appears to have provided neuro-protection by virtue of its anti-oxidant effects as indicated in our earlier studies (9,10). Induction of an isozyme of ChAT of diabetic rats on feeding with chicory for 10 days stands testimony to the neuroprotection offered by Cichorium intybus (Fig. 2 and Table 3).

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