Neurochemical correlates of alloxan diabetes: Gamma amino butyric acid of amphibian brain

Nayeemunnisa and J. S. Nagaraj[1]

Department of Zoology, Bangalore University, Bangalore 560001 (India), 26 April 1977

Summary. The levels of gamma aminobutyric acid decreased, while glutamic acid and aspartic acid levels increased in the forebrain, and decreased in the mid and hind brain regions of frog, Rana cyanophlictis during alloxan diabetes. Since glutamic acid and GABA are intimately involved in the central nervous system (CNS) functions, the alterations occurring in their levels during alloxan diabetes may be significant in bringing about a correlation between the diabetic state and the altered functional dynamics of the CNS.

Gamma aminobutyric acid (GABA) is known to act as an inhibitory transmitter in the brain of vertebrates[2]. It has been demonstrated that GABA imitates qualitatively the action of the cortical inhibitory transmitter and that there is an active uptake mechanism by which it is removed from extracellular spaces and accumulated in mammalian cells[3,4]. Egaña[5] reported that GABA is specific substrate of the vertebrate central nervous system (CNS) and that it supports oxidative phosphorylation. A possible role of GABA in the homeostatic control of brain metabolism under hypoxic conditions has been explained[6].

However, information is lacking on the changes occurring in the level of GABA in the broad compartments of the amphibian brain during diabetes. The presence of GABA exclusively in the neural tissues and the preponderance of glutamic acid, GABA and aspartate (which form more than $^2/_3$ of alpha amino nitrogen of the cerebral pool) have induced the present study. The present investigation is an attempt to evaluate the possible effects of alloxan diabetes on the regional GABA levels to understand its role in the functional dynamics of the amphibian CNS.

Frogs, Rana cyanophlictis, of medium size (21–26 g) were purchased and maintained in the laboratory in glass aquaria at 24 ± 2 °C. These animals were force-fed once in 3 days on the leg muscle of frog.

Diabetes was induced by i.m. injections of freshly prepared aqueous solution of alloxan monohydrate (40 mg/kg b.wt)[7]. Animals were analyzed 96 h after alloxanization. They were killed by decapitation and the brains were quickly removed and washed in ice-cold saline (to remove the adhering blood). The fore, mid and hind brain regions were separated with sterilized fine bent forceps and scalpel at 0 °C, weighed in an electric balance in amphibian Ringer[8] at 0 °C and immediately used for analyses.

Table 1. Blood glucose level and body and brain weight of control and alloxan diabetic frogs, R. cyanophlictis

	Blood glucose (mg/100 ml)	b. wt (g)	Brain wt (mg)
Controls (Normal frogs)	33.8 ± 3.3	26.0 ± 3.2	77.2 ± 8.4
Diabetics (96 h)	61.7 ± 4.0	22.9 ± 2.1	70.0 ± 6.5

Each value represents mean ± SD of 8 observations.

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Table 2. Changes in GABA, glutamic acid and aspartic acid levels in the fore, mid and hind brain regions of controls and alloxan diabetic frogs

Amino acid determined	Forebrain Control	Forebrain Diabetic	Midbrain Control	Midbrain Diabetic	Hindbrain Control	Hindbrain Diabetic
GABA (μmoles/g wet wt)	6.1 ± 0.4 (12)	2.9 ± 0.08 (12)	4.0 ± 0.8 (12)	2.3 ± 0.2 (12)	7.2 ± 0.6 (12)	2 ± 0.6 (12)
	−52.5* $p < 0.01$		−42.5* $p < 0.01$		−72.3* $p < 0.01$	
Glutamic acid (μmoles/g wet wt)	39.9 ± 4.2	59.0 ± 7.0	51.3 ± 6.1	36.1 ± 5	34.2 ± 4.8	26.6 ± 3.3
	+47.6* $p < 0.01$		−29.6* $p < 0.05$		−22.2* NS	
Aspartic acid (μmoles/g wet wt)	11.2 ± 2.4	17.5 ± 3	18.2 ± 2.5	16.0 ± 4	22.7 ± 5.6	21.6 ± 4.6
	+56.3* $p < 0.05$		−12.1* NS		−4.8* NS	

Each value represents mean ± SD of 8 observations, for glutamic and aspartic acids. * Percentage change, +indicating an increase in the level and, −indicating a decrease in the level with respect to controls. NS, not significant. Alloxan diabetic frogs: Animals exhibiting 80–85% elevation in the blood sugar levels 96 h after alloxanization. Numbers in parentheses indicate number of observations.

GABA was determined by the paper chromatographic method of Robert and Frankel as given by Colowick and Kaplan[9]. Descending chromatograms were run on whatman No. 1 paper utilizing water saturated phenol as solvent for 24 h. The dried sheets were sprayed on both sides with a 0.1% solution of ninhydrin in butanol and the colour development allowed to take place for 30 min at 90 °C. The developed spots were cut out in standards and experimental samples. Suitably chosen paper blanks were always included. The pieces of paper containing the spots were cut into small strips and eluted with 5 ml of glass distilled water. The OD of the eluate was measured in a DU2 Beckman spectrophotometer at 570 nm. Changes in glutamic and aspartic acid levels were followed by circular chromatography (on whatman No. 1 circles of 32 cm diameter), as described by Giri and Rao[10]. For quantitative estimation, the amino acid band was cut and eluted with 75% ethanol and 0.1% copper sulphate (5:1) solution. The OD of the eluate was measured in a spectrophotometer at 510 nm. The concentrations of amino acids were evaluated from standard graphs, prepared using standard glutamic and aspartic acids (BDH England). The levels of glucose in the blood (collected by cardiac puncture) were determined colorimetrically[11].

Results and discussion. It is evident from the data given in table 1 that the weight of the animal and brain exhibited considerable decrease as a function of alloxan diabetes. The blood sugar levels demonstrated 80–85% elevation as a function of disease (table 1). The level of GABA in general showed considerable decrease in the fore, mid and hind brain regions on alloxanization (table 2). It is also clear that the level of GABA exhibited regional specificity. It was more in the brain stem region and less in the mid-brain of control frogs (table 2). On inducing diabetes, the level of GABA decreased remarkably (− 72.3%) in the hindbrain, hence, this region showed the highest response during the diabetic state (table 2). It is therefore obvious that the brain stem is the region which is highly susceptible to the effects of alloxan diabetes, thus safegarding the fore- and mid-brain regions with their highly significant functional assignments.

The level of glutamic acid increased in the forebrain and decreased in the mid- and hind-brain regions on alloxanization (table 2). Following the same trend, the level of aspartic acid also exhibited an increase in the forebrain and a decrease in the mid- and hind-brain regions (table 2). The decrease in the level of GABA and a corresponding decrease in glutamic and aspartic acid levels in the mid- and hind-brain regions as a function of alloxan diabetes indicate a decrease in the production of the inhibitory transmitter in these regions. In support of this, considerable decrease in the total free amino pool of mid- and hind-brain regions was observed on inducing alloxan diabetes in frogs (unpublished observations of Nayeemunnisa, 1976). It is therefore likely that the glutamic acid conversion to GABA was inhibited by the decrease in the precursor substrate. This in turn is related to the higher respiratory rate of acute diabetic state[12] and would allow certain synaptic pathways to be facilitated and synaptic ratio to be altered so that through conduction pathways would be established[13].

The substantial decrease in the level of GABA and corresponding increase in glutamic and aspartic acid levels in the fore-brain during diabetes (table 2) indicate that the production of the inhibitory transmitter is decreased in this region probably due to substrate inhibition as the total free amino acid pool increases in the fore-brain during diabetes (unpublished observations of Nayeemunnisa, 1976). This may perhaps be related to the enhanced protein catabolism in this region during diabetes. Earlier reports of Nayeemunnisa[7] indicated that the protein metabolism is affected in the brain of frog during diabetes. It is therefore possible that an increase in protein catabolism may result as a consequence of diabetic state, which is well indicated by the increase of free amino acid levels of the fore-brain (table 2 and unpublished observations of Nayeemunnisa). Since glutamic acid and GABA are intimately involved in the CNS function, the alterations brought about in their levels by the diabetic state emphasize a correlation between the diabetic state and the altered functional dynamics of the central nervous system.

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Electrical uncoupling by Sr action potentials in cardiac muscle[1]

R. Ochi

Department of Physiology, Jichi Medical School, Minamikawachi-machi, Tochigi-ken (Japan 329-04), 11 March 1977

Summary. Sr action potentials elicited in guinea-pig papillary muscle under Na-free conditions decreased the space constant and increased the input resistance. It suggests that Sr current induced intercellular uncoupling by increasing $[Sr]_i$.

The propagation of cardiac action potential requires electrical coupling between cells. The coupling is interrupted by Ca, Sr[2] and Na[3] ions electrophoretically injected into the myoplasm of Purkinje fibres as in epithelial cells[4] and by ouabain treatment in ventricular muscle[5]. I report here that similar electrical uncoupling was produced by Sr action potentials[6] which could increase $[Sr]_i$ by Sr current through the general membrane.

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