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Lactate dehydrogenase and glutamate dehydrogenase activities in the circumventricular organs of rat brain following neonatal monosodium glutamate

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Abstract. Glutamate (glu) an excitatory neurotransmitter amino acid, is present in high concentrations in the mammalian central nervous system and is the most abundant amino acid in our daily diet. In the present study the activities of lactate dehydrogenase (LDH) and glutamate dehydrogenase (GDH) were evaluated in the circumventricular organs (CVO) of the brain in 25-day-old rats following MSG administration at a dose of 4 mg/g b.wt during the first ten days of life. The results show the LDH activity increased to 265% of that in the control ($p < 0.001$), whereas GDH activity was significantly decreased ($p < 0.05$), The great elevation in LDH, a cytoplasmic marker enzyme, is apparently due to cytoskeletal changes brought about as a consequence of glu toxicity, whereas lowered GDH activity indicates altered glu homostasis in the blood-brain-barrier deficient areas following neonatal exposure to glu.
Key words. Monosodium glutamate; LDH; GDH; excitotoxin; blood-brain barrier.

Evidence now exists for the neurotoxic actions of glutamate (glu), an excitatory transmitter and the most abundant amino acid in the mammalian brain[1,2,3] as well as in most dietary proteins[4]. It has also been shown that several structural analogs of glu possess both neuroexcitatory and neurotoxic properties, and these have therefore been called excitotoxins[5,6,7]. Moreover, recent reports including our own indicate a generalized defect in glu metabolism in patients with motor neuron diseases[8,9]. Monosodium glutamate (MSG), the sodium salt of glu, has been shown to cause lesions in the developing brain of several animal species following its oral or systemic application in excessive amounts during the neonatal period[6,7]. The brain damage resulting from peripheral glu is selective for certain brain regions which are collectively known as circumventricular organs (CVO). The blood-brain barrier (BBB) is deficient in these areas, owing to anatomical peculiarities[10].

Most of the earlier studies focused on ultrastructural or neuroendocrine changes caused by systemic glu administration[7]. The present study was designed to evaluate some biochemical mechanisms of glu toxicity by measuring the activities of lactate dehydrogenase (LDH), a cytoplasmic enzyme marker, and glutamate dehydrogenase (GDH), a key enzyme of glu metabolism, in organs of the CVO, including the arcuate hypothalamus-median eminence (AH-ME), following neonatal exposure of rats of MSG.

Materials and methods

Animals and housing. Adult Wistar rats weighing 200–250 g were housed under controlled conditions of light (12 h light:12 h dark) and temperature 22 ± 2 °C. They were fed with rat pellets (Lipton, India) and tap water ad libitum. Males of proven fertility and sexually mature females were allowed to cohabit. Presence of sperm in the vaginal smear was taken as day 1 of pregnancy. Mated rats were left in individual cages until term. The litter size was adjusted to 8 uniformly by redistribution among mothers which had delivered within a span of 2 days. Litters from each rat were divided into two groups, control and experimental. MSG was obtained from Sigma Chemical Co., USA, and administered to the pups by subcutaneous injection at a dose of 4 mg/g b.wt during the first ten days of the postnatal period. This dose had earlier been shown to produce neuroendocrine ablations in rats[7]. It was 100 to 1000 times higher than the daily per capita intake of MSG, world wide[4]. An equal volume of physiological saline was given to control animals.

Pups were sacrificed on day 25 by cervical dislocation and the brains rapidly dissected out on a cold tray. The midbrain region, which included the circumventricular organs: the AH-ME, the organum vasculosum of the lamina terminalis, the subfornical organ and the subcommisural organ, were dissected out. A piece of frontal cortex was used as tissue control. Tissues were homogenized in 10% w/v 0.32 M sucrose and centrifuged as 12000 RPM for 20 min, and the supernatant was used as enzyme source. LDH was assayed[11] by determining the rate of oxidation of NADH in the presence of buffered substrate, i.e. pyruvate, which is converted to lactate. The 3 ml reaction medium contained 0.1 ml supernatant, 2.9 ml 0.01 M sodium pyruvate in 0.1 M phosphate buffer and 0.1 ml 3.5 μmoles NADH. The reaction was initiated by rapidly adding

pyruvate and transferring the contents into a cuvette. The reduced coenzyme (NADH) absorbs strongly at 340 nm while the oxidized form (NAD) does not, so the progress of the reaction could be monitored by measuring the decrease in extinction at 340 nm for 2 min at 25 °C.

GDH was assayed[12] using the same principle. The 3 ml reaction medium contained 0.1 ml enzyme extract pretreated with Triton X-100, 1 ml 100 µmoles Tris containing 30 µmoles EDTA, 1 ml 300 µmoles $(NH_4)_2SO_4$, 0.4 ml 0.1 µmoles NADH and 0.5 ml 20 µmoles 2-oxoglutarate. The reaction was initiated by the addition of 2-oxoglutarate while the blank contained no 2-oxoglutarate. GDH catalyses the reversible oxidative deamination of glutamate to 2-oxoglutarate. The enzyme activities were expressed as n-moles NADH oxidized/min/mg protein. Protein was estimated by the method of Lowry et al.[13].

Results

The table indicates the levels of LDH and GDH in the CVO of 25-day-old rats following MSG treatment. It can be seen that the activity of LDH significantly increased ($+265\%$, $p < 0.001$) in the MSG-treated group with respect to the saline-treated controls, whereas the activity of GDH was significantly decreased (-19%, $p < 0.05$) in the BBB-deficient areas of MSG-treated animals as compared to the control group. No significant alterations in the above parameters in MSG treated rats in comparison to saline-injected animals were observed in the frontal cortex (data not shown).

Discussion

The present study indicates that neonatal MSG exposure in rats significantly increased the activity of LDH, whereas GDH activity was decreased in the CVO. LDH is an important enzyme regulating energy metabolism in the cell, and is also known to be a cytoplasmic enzyme marker. The large increase in this enzyme activity implies that MSH causes altered plasma membrane permeability, and consequent cellular damage. An earlier report[14] showed that LDH leaks from the slice into the medium when sagittal slices of brain are incubated with various glutamate agonists, indicating that these excitotoxins bring about rupture of neuronal plasma membranes. Our in vivo finding corroborates this in vitro report. At low pH caused by lactic acidosis there is denaturation of structural and integral proteins, which might be responsible for disruption of neuronal plasma membranes[15].

The activity of GDH, a key enzyme of glu metabolism, is decreased. This suggests that neonatal MSG treatment caused alterations in glu homeostasis in these brain areas. Depending upon several factors, GDH can catalyze glutamate breakdown or formation[16]. Disturbances in brain glu metabolism were reported earlier during excessive neuronal activity, e.g. convulsions[17], and also under conditions of ischemia/hypoxia[18]. Common to both is a decline in the energy level of the brain and a reduction in transmembrane ion gradients, which may result ultimately in the reductive amination of 2-oxo glutarate and the formation of glu. The decreased activity of GDH in the present study indicates that oxidative deamination is reduced. This finding is in agreement with an earlier report[19] showing increased tissue levels of glu in AH-ME following neonatal MSG treatment. It was also shown[20,21] that in patients with recessive, adult-onset olivo-ponto-cerebral degeneration associated with a partial GDH deficiency, plasma glutamate rises. Furthermore, in this disease state, decreased catabolism of glutamate at the nerve terminals results in an increased retention in the synaptic cleft of the amino acid released during neuronal activity. This, in turn, could cause direct damage to post-synaptic neurons. These data also strongly support the view that in vivo GDH is intimately involved in glutamate disposal.

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Table. LDH and GDH activities in the CVO of day 25 rats following neonatal MSG application. All values represent mean ± SEM. Student's 't' test was used to evaluate statistical significance. A 'p' value less than 0.05 was considered significant.

Enzyme	Units	Control	Treated	% increased (+) or % decreased (−) vs control	p value vs control
LDH	n moles NADH oxidized/min/mg protein	350 ± 40 (6)	930 ± 50 (6)	+265%	<0.001
GDH	n moles NADH oxidized/min/mg protein	2090 ± 190 (6)	1700 ± 90 (6)	−19%	<0.05

Numbers in parentheses indicate the number of animals in each group.

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