Article *NIMHANS Journal*

Ischemia - Induced Changes in the Enzymes of Glutamate Metabolism in Brain

Volume: 08 Issue: 01 January 1990 Page: 57-61

G Y C V S Lakshmi ,

Reprints request

,

- *Department of Neurochemistry, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

V R Sastry Kolluri, - *Department of Neurosurgery, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Abstract

The enzymes of glutamate metabolism in gerbil brain were studied were unilateral cerebral ischemia. Following 10 min of ischemia the activities of aspartate and alanine aminotransferases, glutamate dehydrogenase and glutamine synthetase were inhibited significantly while gulatminase activity was increased. Recirculation for 30 min after 10 min of ischemia enhanced the activities of transaminases but decreased the activities of glutamate dehydrogenase and glutamine synthetase. Glutaminase activity was normalized during recirculation. The results suggest that failure of detoxification mechanisms of glutamate in brain during ischemia might be the cause for glutamate neurotoxicity in ischemic brain damage.

Key words -

Cerebral ischemia,
Enzymes,
Glutamate metabolism

Cerebral ischemia results from a wide variety of cerebrovascular disorders and head trauma. Selective pattern of neurodegeneration in discrete areas of brain following cerebral ischemia was reported [1], [2], [3]. Since the areas of brain that are enriched with glutaminergic inputs were found to be most vulnerable to ischemia [4], [5], [6], and neurodegeneration pattern following ischemia resembles that seen after intraventricular injections of glutamate agonist kianate [7], it was suggested that increased extracellular levels of glutamate may be a causative factor for ischaemic brain damage. Elevated levels of glutamate and aspartate in brain following transient cerebral ischemia were reported [8], [9], [10]. Further, the antagonists of glutamate were found to block ischemia in experimental animals [6], [11], [12]. Elevated levels of glutamate under ischemia could result from altered metabolic pathways either due to increased synthesis and/or decreased detoxification mechanisms. The present study was undertaken to evaluate the relative role of the enzymes of glutamate metabolism in contributing to the metabolic origin of elevated glutamate levels in cerebral ischemia.

Material and Methods

L-aspartate, L-glutamate, L-glutamine, α -ketoglutaric acid, L-alanine, β -NADH, β -NAD^+, malate dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase, ATP were purchased from Sigma Chemical Co., St. Louis. All the other chemicals were obtained locally.

Adult Mangolian gerbils of either sex weighing 70-100 gm on 12 h light/dark cycle were used in the present study. The animals had access to food and water ad libitum.

Unilateral forebrain ischemia was induced in the animals as described elsewhere [10]. Left cerebral hemisphere from same experimental animal served as control. The enzymes, viz., glutamate dehydrogenase [13], aspartate and alanine aminotrasferases [14], glutamine synthetase [15] and glutaminase [16] were assayed in 0.32M sucrose homogenates of brain. Protein was estimated according to the method of Lowry et al [17].

Statistical analysis was carried out using student's 't' test.

Results

Of all the enzymes of glutamate metabolism in brain, the activity of aspartate aminotransferase was found to be very high. Following 10 min of cerebral ischemia the activities of aspartate aminotransferase, glutamate dehydrogenase and glutamine synthetase were found to be inhibited significantly while glutaminase activity was increased. The decrease in alanine aminotransferase activity was not statistically significant (Table I). With recirculation for 30 min after 10 min of cerebral ischemia, the activities of transaminases were increased and glutaminase activity was normalized. However, the activities of glutamine synthetase and glutamate dehyrogenase continued to be inhibited (Table II).

Table I - Changes in the enzymes of glutamate metabolism in gerbil brain following 10 min of cerebral ischemia

Table I - Changes in the enzymes of glutamate metabolism in gerbil brain following 10 min of cerebral ischemia

Values represent mean ± S.D.

Figures in parentheses indicate no. of values obtained from animals.

Activity=μ moles/mg protein/hr.

Table II - Changes in the enzymes of glutamate metabolism in gerbil brain after 10 min of cerebral ischemia followed by 30 min recirculation

Table II - Changes in the enzymes of glutamate metabolism in gerbil brain after 10 min of cerebral ischemia followed by 30 min recirculation

Values represent mean ± S.D.

Figures in parentheses indicate no. of values obtained from animals.

Activity=μ moles/mg protein/hr.

Discussion

The metabolism of glutamine in brain is quite complicated due to the presence of large and small pools localised in neurons and astrocytes [18], [19]. Glutamate in nerve endings is mainly derived from glucose by transamination and reductive amination of α -ketoglutarate by aspartate and alanine aminotransferases and glutamate dehydrogenase respectively. Accordingly, the activities of these enzymes are high in nerve endings compared to neuronal perikarya and astrocytes [20]. The glutamate so formed would be released as neurotransmitter and it is taken up by astroglial cells where it is converted to glutamine by glutaminesynthatase or utilized for metabolic purposes. Glutamate is also formed by the hydrolysis of glutamine by glutaminase which is localized both in astrocytes and in nerve endings [20], [21]

Ischemia for 10 min markedly reduced the activities of transaminases, glutamate dehydrogenase and glutamine synthetase. In the absence of glucose, inhibition of transaminases and dehydrogenase would reduce the utilization of α -ketoglutarate and oxaloacetate and spare them for the operation of citric acid cycle. Inhibition of glutamine synthetase activity might be due to lack of ATP under ischemic condition which would result in the suppression of glutamate detoxification. Stimulation of glutaminase activity is rather difficult to explain as the substrate glutamine would not be available due to inhibition of glutamine synthetase. Suppression of transaminases and dehydrogenase during ischemia suggests that increased glutamate levels during ischemia might be derived from sources other than glucose. Failure of detoxification mechanism due to inhibition of glutamine synthetase and enhanced synthesis due to elevated glutaminase activity might contribute to elevated glutamate levels in brain during ischemia.

Elevation of transminases and continued inhibition of glutamine synthetase during recirculation would result in further enhancement of cerebral glutamate levels. A significant increase in brain glutamate levels with reperfusion following short term ischemia were reported [10]. Inhibition of glutamine synthetase activity during recirculation (though ATP is available) circulation (though ATP is available) suggests the limitation of glutamate availability due to defective reuptake mechanisms in astrocytes and nerve endings. Suppression of glutamate dehydrogenase activity under these conditions might be due to product inhibition as both α -ketoglutarate and glutamate as high concentrations inhibit the enzyme activity [13].

The present observations on the changes in enzymes of glutamate metabolism during ischemia are in agreement with the findings of Drejer et al [22] who reported elevated extracellular concentration of glutamate and aspartate and, inhibition of K^+ stimulation of glutamate uptake during ischemia in cultured brain cells.

In conclusion, the findings of the present study suggest that mainly failure in detoxification mechanisms of glutamate during ischemia and reperfusion contribute to neurotoxic levels of glutamate in brain under these conditions. Further studies on metabolic processes of glutamate at cellular level would help in understanding the glutamate mediated neurotoxicity in ischemic brain damage.

Acknowledgement

This study was supported by NIMHANS Research grants. The authors thank Mr Raghavendran and Mr Ramachandran for their skillful assistance in carrying out the work and Ms Veena for typing the manuscript.

1.Ito U, Spatz M, Walker J T & Klatzo I, Experimental cerebral ischemia in Mangolian gerbils. 1. Light microscopic observations
Acta Neuropathologica (Berlin) Page: 32: 209-223, 1975
2.Kirino T & Sano K, Selective vulnerability in the gerbil hippocampus following tranmsient ischemia
Acta Neuropathologica (Berlin) Page: 62: 201-298, 1984
3.Pulsinelli W A, Brierley J B & Plum F, Temporal profile of neuronal damage in a model of transient forebrain ischemia
Annals of Neurology Page: 11: 491-498, 1982
4.Jorgensen M B & Diemer N H, Selective neuron loss after cerebral ischemia in the rat: Possible role of transmitted glutamate
Acta Neurologica Scandinavica Page: 66: 536-546, 1982
5.Lakshmi G Y C V S, Sastry Kolluri V R, Rao B S S, Das B S & Narayana Reddy G N, The role of glutamate in the pathophysiology of cerebral ischemia
Indian Journal of Clinical Biochemistry Page: 3 (Suppl): 2, 1988
6.Lakshmi G Y C V S, Sastry Kolluri V R, Blockade of NMDA receptors prevents ischemia induced hippocampal neurodegeneration
In: J Kreiglstein (Ed) Pharmacology of Cerebral Ischemia. Suttergart: Wiss Verlag Page: 238-242, 1989
7.Olney J W, Neurotoxicity of excitatory amino acids
In: E G McGear, J W Olney & P L McGear (eds) Kianic Acid as a Tool in Neurobiology New York: Ra Page: 95-121, 1978
8.Benveniste H, Drejer J, Schousboe A & Diemer N H, Elevation of extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis
Journal of Neurochemistry Page: 43: 1369-1374, 1984
9.Hagberg H A, Lehmann M, Sandberg B, Nystrom B, Jacobson I & Hamberger A, Ischemia-induced shift of inhibitory and excitatory amino acids from intra-to extracellular compartments
Journal of Cerebral Blood Flow & Metabolism Page: 5: 413-419, 1985
10.Sastry Kolluri V R & Lakshmi G Y C V S, Changes in regional levels of putative neurotransmitter amino acids in brain unilateral forebrain ischemia
Neurochem Res Page: 14: 621-625, 1989
11.Gill R, Foster A C & Woodroff G N, Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil
Journal of Neurological Sciences Page: 7: 3343-3349, 1986
12.Swan J H, Evan M C & Meldrum B S, Long-term development of selective neuronal loss and the mechanism of protection by 2-amino-7- phosphonoheptanoate in a rat model to incomplete forebrain ischemia
Journal of Cerebral Blood Flow & Metabolism Page: 8: 64-78, 1988
13.Chee P Y, Dahl J L & Fahien L A, The purification and properties of rat brain glutamate dehydrogenase
Journal of Neurochemistry Page: 33: 53-60, 1979
14.Bergmeyer H V & Bernt E, Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase

In: Bergmeyer H V (Ed.) Methods of Enzymatic Analysis. Vol. II New York: Academic Press 1974
15.Rowe W B, Ronzio R A, Wellner V P & Meister A, Glutamine synthetase (Sheep brain)
In H Tabor & C W Tabor (Eds.), Methods in Enzymology, Vol. XVII A, New York: Academic Press Page: 900-910, 1970
16.Nimmo G M & Tipton K F, The soluble and membrane bound forms of glutaminase in pig brain
Journal of Neurochemistry Page: 33: 1083-1094, 1979
17.Lowry O H, Rosenbrough N J, Arr A L & Randall R J, Protein measurement with the folin phenol reagent
Journal of Biological Chemistry Page: 193: 265-275, 1985
18.Weil-Malherbe H, Studies on brain metabolism, I. The metabolism of glutamic acid in brain
Biochemistry Journal Page: 30: 665-676, 1949
19.Benjamin A M & Quastel J H, Fate of L-glutamate in the brain
Journal of Neurochemistry Page: 23: 457-464, 1972
20.Subbalakshmi G Y C V & Murthy Ch R K, Isolation of astrocytes, neurons and synaptosomes of rat brain cortex: Distribution of enzymes of glutamate metabolism
Neurochem Res Page: 10: 239-250, 1985
21.Ward H K, Thanki C M & Bradford H F, Glutamine and glucose as precursors of transmitter amino acids: Ex vivo studies
Journal of Neurochemistry Page: 40: 855-860, 1983
22.Drejer J, Benveniste H, Diemer N H & Schousboe A, Cellular origin of ischemia-induced glutamate release from brain tissue in vivo and in-vivo
Journal of Neurochemistry Page: 145-151, 1985