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Aspartate aminotransferase and alanine aminotransferase activities of rat brain during crush syndrome

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ABSTRACT

Crush syndrome develops due to muscle crush injury often found in patients extricated from prolonged compression after disasters. It leads to rhabdomyolysis, kidney failure and hypovolemic shock, followed by decreased blood supply, to tissue under compression and general body parts including brain. In the present study, experimental model of crush syndrome in albino rats was induced by, 2 h of compression followed by 48 h of decompression, of femoral muscle tissue. Aspartate and alanine aminotransferase activities of rat brain regions during crush syndrome were investigated. After exposure to 2 h compression in comparison to normal/control levels, both cytosolic AST and ALT activities reduced. Cytosolic AST activity reduced by 31.2%, 26.1% and 19.4% in olfactory lobes, cerebral cortex and cerebellum, respectively, whereas cytosolic ALT activity decreased by 51.1%, 52.4%, 47.4% and 36.9% in olfactory lobes, cerebral cortex, cerebellum and medulla oblongata, respectively.

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Crush syndrome (CS), a muscle crush injury, is common to the limbs and is found in patients extricated from prolonged compression after disasters [18]. Pathogenesis is characterized by acute hemodynamic shock, ischemia, myoglobinuria, renal failure and also diabetes [7,8,24]. Muscle crush stimulus opens nonselective, stretch-activated channels of the sarcolemma. This leads to influx of Na^+ and Ca^{2+} in cytosol of the myocyte followed by water influx [5], which leads to cell swelling and autolysis. Initial attempts to restore cell volume through the Na^+/K^+-pumps deplete the cellular ATP. Cell lysate products and matrix thus formed finds its way in circulation [25]. Pathogenesis also involves generation of iNOS and augmentation of eNOS, which leads to NO-dependent vasodilation in muscle injury region and also hypovolemic shock [27]. This is followed by decreased blood supply to compressed tissue and general body parts including brain resulting in altered consciousness and sometimes coma. Toxic metabolites of protenious nature accumulate in the brain, after muscle crush involving sciatic nerve, with elevation of enzyme activity such as adenosine deaminase in cerebrum, cerebellum and hypothalamus of rat [11]. We have reported changes in Na^+, K^+-ATPase activity of brain regions of rat subjected to crush syndrome [9]. The reduction of blood supply to brain during crush syndrome could lead to altered metabolism of brain and thus energy crisis. Therefore, under such condition other pathways of metabolism could be opened. One of the pathways is protein metabolism involving aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2). AST transfers an amino group from aspartate whereas ALT transfers an amino group from the alanine, to alpha-ketoglutaric acid to produce glutamate and pyruvate or the reverse reaction for which pyridoxal 5-phosphate (PLP) is required as a coenzyme [3,15]. The pyruvate thus generated could be useful for energy production either by its conversion into lactic acid and then to glucose or could be oxidatively decarboxylated to enter into Kreb's cycle. Neuronal AST and ALT are active brain enzymes that are involved in glutamate metabolism. AST activity mediates connection between amino acids and saccharide metabolism, also participates in the malate–aspartate shuttle [15]. AST and ALT exists in cytosolic and mitochondrial forms [3,10] and ALT is also involved in synthesis of glutamate neurotransmitter in glutamatergic neurons [22].

Changes in alanine–glutamate ratio during and after asphyxia [12] are also reported. Besides, decrease in glutamate uptake in brain was found to be associated with crush syndrome [14]. Since olfactory lobes, cerebral cortex, cerebellum and medulla oblongata constitute functionally important regions of CNS performing different functions, it was felt necessary to investigate cytosolic and mitochondrial AST and ALT activities involved in glutamate metabolism of these regions. Hence, glutamate levels of these brain regions as well as of CSF were also studied.

Experiments were carried out on Swiss albino rats (100–150 g) and were performed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA) of India and were approved by the animal Ethics

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Committee of Goa University, India. Crush syndrome was induced by unilateral compression of femoral tissues of anesthetized rats on a special press exerting a force of 100 kg per kg of animal weight, for a period of 2 h as described by Knaryan et al. [14]. The animals were divided into three groups. Each group consisted of five animals, Group I: control group, without exposure to compression; Group II: exposed to 2 h of compression; Group III: exposed to 2 h of compression, followed by decompression for 48 h.

Following decapitation, the whole brain was rapidly removed and placed in ice-cold 0.32 M sucrose, blotted dry. The olfactory lobes, cerebral cortex, cerebellum and medulla oblongata were dissected and transferred to tubes for storage, at −20 °C, until the preparation of homogenate. Frozen tissue was homogenized with a Teflon-glass homogenizer. For estimation of glutamate, 10% (w/v) homogenate, of each of the brain regions, was prepared in 0.25 M cold sucrose. CSF was collected using routine techniques. Briefly, 100 μl of CSF was aspirated from sub-arachnoid space of cisternae magna through the atlanto-occipital membrane [23]. Glutamate was measured after developing the flurophores by ninhydrin with the protein free filtrate [6].

For estimation of cytosolic enzymes, homogenate was prepared in 0.32 M sucrose containing 5 mM Hepes buffer (pH 7.4) and centrifuged at 4 °C for 20 min at 12,000 × *g*. Supernatants were used for enzyme assay. Whereas for mitochondrial enzymes, each brain region homogenate prepared in nine volumes of 0.32 M sucrose was centrifuged at 1000 × *g* for 10 min to yield a pellet which was washed and applied on a discontinuous sucrose gradient consisting of 0.8, 1.0, 1.2 and 1.4 M sucrose. After centrifugation at 75,000 × *g* for 2 h, the mitochondrial pellet obtained at the bottom of the tube was used as source for estimation of mitochondrial enzymes. AST and ALT kits were obtained from Span diagnostics, India (AST, Code No. 25913; ALT, 25912). All other chemicals were of analytical grade and were purchased from standard commercial suppliers. AST and ALT were assayed according to the method described by Reitman and Frankel [26]. Briefly, 1 ml of the substrate (2 mM α-ketoglutarate, 0.2 M D,L-aspartate for ALT and 2 mM α-ketoglutarate, 0.2 M D,L-alanine for AST) was incubated with 0.2 ml enzyme source for 30 min (for AST) or 60 min (for ALT) followed by addition of 1 ml of 1 mM DNPH. After 20 min, 10 ml 0.4N NaOH was added to each tube. The absorbance of the solution was measured at 505 nm after 30 min.

Protein was measured by the method of Lowry et al. [17] using bovine serum albumin as standard. All values represented in histograms are expressed as mean ± standard deviation for number of animals indicated. Significance of differences was determined by an unpaired Student's *t*-test with the help of Analyse-it Program for General and Clinical statistics version 1.73.

Rats, after 2 h of compression and different decompression periods, showed significant increase in glutamate levels of CSF by 0.8-fold ($P < 0.05$) in both Groups II and III, in comparison to Group I/control (Fig. 1). Changes in glutamate levels of olfactory lobes, cerebral cortex, cerebellum and medulla oblongata are shown in Fig. 1.

Cytosolic AST enzyme activities of olfactory lobes, cerebral cortex and cerebellum showed reduction by 31.3% ($P < 0.001$), 26.1% ($P < 0.001$) and 19.4% ($P < 0.001$), respectively, after exposure to 2 h of compression in comparison to control Group I, except for medulla oblongata. Following 48 h of decompression period, the enzyme activities reduced by 22.4% ($P < 0.01$), 51.0% ($P < 0.001$), 62.5% ($P < 0.001$) and 71.9% ($P < 0.001$) in olfactory lobes, cerebral cortex, cerebellum and medulla oblongata, respectively, in comparison to Group II (Fig. 2A). Mitochondrial AST activities of olfactory lobes, cerebellum and medulla oblongata of Group II reduced by 36.7% ($P < 0.01$), 25.8% ($P < 0.001$) and 17.26% ($P < 0.001$), respectively in comparison to control/Group I except for cerebral cortex. Following 48 h of decompression, mitochondrial activities reduced [by 25.3% ($P < 0.001$) in olfactory lobes, 63.2% ($P < 0.001$) in cerebral cortex, 56.1% ($P < 0.001$) in cerebellum and 73.2% ($P < 0.001$) in medulla oblongata] in comparison to Group II rats (Fig. 2B). Cytosolic ALT activities of Group II rats showed reduction of enzyme activities by 51.1% ($P < 0.001$) in olfactory lobes, 52.4% ($P < 0.001$) in cerebral cortex, 47.4% ($P < 0.001$) in cerebellum and 37% ($P < 0.001$) in medulla oblongata when compared to Group I rats. Following 48 h of decompression there was increase of cytosolic ALT activity by 29.9% ($P < 0.01$), 55.3% ($P < 0.001$) and 46.4% ($P < 0.01$) in olfactory lobes, cerebral cortex and medulla oblongata, respectively when compared to Group II rats (Fig. 2C). Mitochondrial ALT activities of Group II rats' brain (olfactory lobes, cerebral cortex, cerebellum and medulla oblongata) showed reduction in comparison to control Group I by 60.3% ($P < 0.001$) in olfactory lobes, 90% ($P < 0.001$) in cerebral cortex, 89.1% ($P < 0.001$) in cerebellum and 59.7% ($P < 0.001$) in medulla oblongata. Following 48 h of decompression there was further reduction of enzyme activity by 82.5% ($P < 0.001$) in olfactory lobes, 75.7% ($P < 0.001$) in cerebral cortex, 89.1% ($P < 0.001$) in cerebellum and 96.8% ($P < 0.001$) in medulla oblongata in comparison to Group II rats (Fig. 2D).

It is well known that biological activity of brain varies with respect to many factors and one of them is region specificity. Mature normal brain is said to have obligatory dependence on glucose except in the case of long-term starvation [16]. Brain also shows

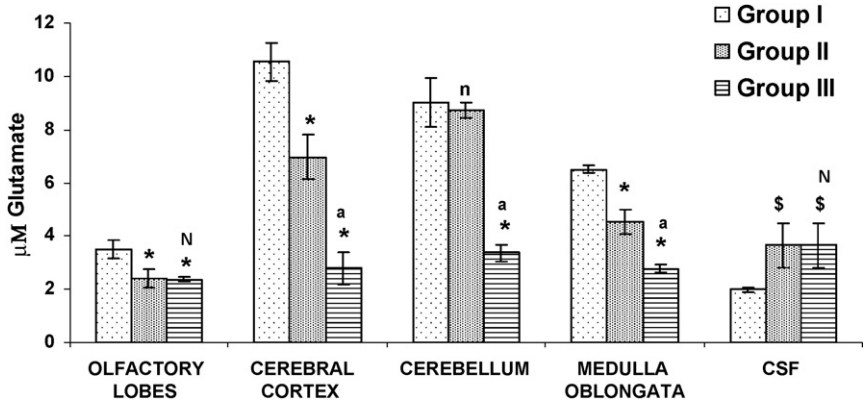

Fig. 1. Changes in glutamate levels of olfactory lobes, cerebral cortex, cerebellum, medulla oblongata and CSF of rat brain during 2 h compression and 48 h of decompression. Data are mean ± S.D. for five independent experiments performed. *Different from control (Group I), $P < 0.001$; $^{\$}P < 0.01$; n, insignificant. a, different from Group II, $P < 0.001$; N, insignificant (Student's *t*-test).

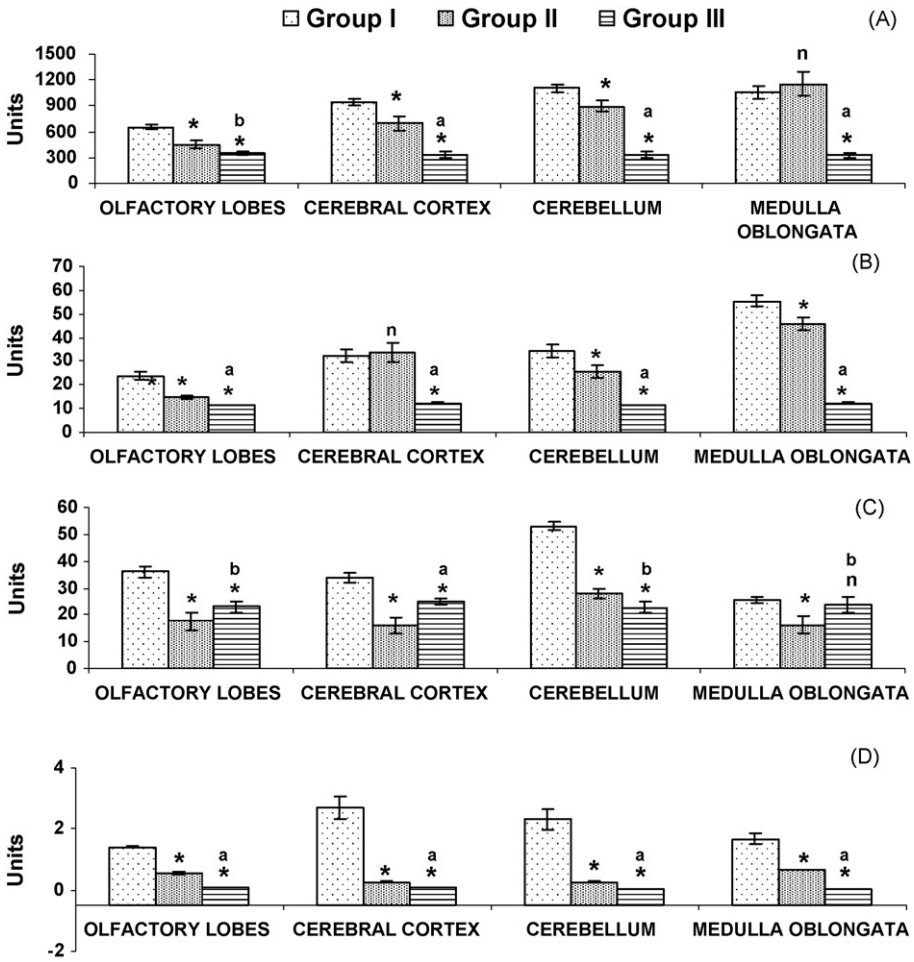

Fig. 2. Changes in cytosolic AST [A]; mitochondrial AST [B]; cytosolic ALT [C]; mitochondrial ALT [D] activities of olfactory lobes, cerebral cortex, cerebellum and medulla oblongata of rat brain during 2 h compression and 48 h of decompression. Data are mean ± standard deviation for five independent experiments performed. *Different from control (Group I), $P < 0.001$; n, insignificant. a, different from Group II, $P < 0.001$; b, $P < 0.01$; N, insignificant (Student's *t*-test). One unit equals 1 mM pyruvate/mg protein/min.

variability in regional energy requirements and particularly on the release of enzymes associated with their requirements. In general, relatively less aminotransferase activities, observed in mitochondria in comparison to cytosolic enzyme activities, indicate lesser intramitochondrial glutamate availability and presence of relatively less pyruvate conversion to alanine in brain mitochondria [4].

In general, all four brain regions experienced hypoaminotransferasemia, with respect to cytosolic as well as mitochondrial, AST and ALT activities, after 2 h of compression and during late hours of decompression, with some exceptions. In tune with low AST and ALT activities all four regions of brain exhibited low glutamate concentrations. However, CSF glutamate levels were elevated in comparison to control. Cytosolic AST activity of medulla oblongata and mitochondrial AST activity of cerebral cortex remained unaltered after exposure of rats to 2 h of compression indicating normalcy of malate–aspartate shuttle, saccharide metabolism as well as normal synthesis of glutamate by glutamatergic neurons during compression. But following decompression of 48 h their activity reduced significantly ($P < 0.001$) which may cause disturbed malate–aspartate shuttle, saccharide metabolism and reduced glutamate synthesis.

Cytosolic AST activity of cerebellum and medulla oblongata reduced maximally following 48 h decompression (Group III rats), while mitochondrial AST activity of medulla oblongata of Group III rats reduced sharply following 48 h of decompression.

Of all brain regions, cerebellar cytosolic ALT activity reduced maximally in Groups II and III rats in comparison to Group I/control ($P < 0.001$). Mitochondrial ALT activities reduced maximum in cerebral cortex of Groups II and III rats in comparison to control Group I ($P < 0.001$).

It is known that during crush syndrome, compression causes stress and shock. Such stressful conditions result in the secretion of glucocorticoids, from the adrenal cortex [1], which is known to inhibit glutamate uptake by astrocytes in the hippocampus [28]. There are also reports on decrease in glutamate uptake by brain cerebral cortex and hypothalamus slices in restricted movement [2] and during crush syndrome [14]. Crush syndrome pathogenesis leads to excess release of amino acids and failure of kidney to eliminate urea, which leads to accumulation of ammonia in the blood. This increased level of ammonia readily traverses through blood brain barrier and in the brain it is converted to glutamate by glutamate dehydrogenase, thereby actually elevating the brain glutamate levels. Such conditions bring about reduction of α-ketoglutarate and oxaloacetate levels in brain which halts TCA cycle thus affecting the energy production which is known to cause irreparable cell damage [20]. Besides, accumulated high levels of glutamate, including that at synapses, due to failure of its normal reuptake, promotes its passage to CSF [21], and some of it is also converted to glutamine, thereby reducing the glutamate of brain, during such pathogenesis as observed in present study. Thus, reduction of brain glutamate levels observed in Group III rats could be

attributed to: (a) its passage to CSF, (b) reduction in AST and ALT mediated synthesis by glutamatergic neurons and (c) probable conversion of glutamate to glutamine. But at the same time decreased levels of glutamate affects the synthesis of GABA thereby interfering the normal neurotransmission.

Under normal condition, alanine and aspartate links glucose, tricarboxylic acid (TCA) cycle and amino acid metabolism via its reaction with α-ketoglutarate to form pyruvate and glutamate, when catalyzed by ALT and AST. Therefore, aminotransferases serve as a strategic link between carbohydrate and protein metabolism under pathophysiological stress [19], and any disturbance in the activities of aminotransferases would disturb this strategic link between carbohydrate and protein metabolism. There are reports of less utilization of glucose in brain during crush syndrome [13]. Thus, reductions of AST and ALT activities at cytosolic and mitochondrial level in these four brain regions during crush syndrome would lead to disturbances in the linkages between carbohydrate and protein metabolism, causing energy crisis as well as glutamate metabolism as observed in this study. This leads to disturbed neuronal functions of olfactory lobes, cerebral cortex, cerebellum and medulla oblongata, which may impair sense of smell, sensory and locomotory responses, balancing and coordination of respiratory and circulatory activities, during crush syndrome.

References

[1] J. Axelrod, T.D. Reisine, Stress hormones: their interaction and regulation, Science 224 (1994) 452–459.
[2] S.D. Barsegyan, A.S. Kanayan, V.A. Knaryan, L.N. Arakelyan, Influence of hypokinesia on the uptake of neuromediator amino acids in rat brain cortex and hypothalamus slices, Med. Sci. Armenia 38 (1998) 51–60.
[3] M. Benuck, A. Lajtha, Aminotransferase activity in brain, Int. Rev. Neurobiol. 17 (1975) 85–129.
[4] M. Benuck, F. Stein, A. Lajtha, Regional and subcellular distribution of aminotransferases in rat brain, J. Neurochem. 19 (1972) 949–957.
[5] O.S. Better, Z. Abassi, I. Rubinstein, Z. Abassi, S. Marom, J. Winaver, M. Silbermann, The mechanism of muscle injury in the crush syndrome: ischemic versus pressure-stretch myopathy, Miner. Electrolyte Metab. 16 (1990) 181–184.
[6] S. Chakrabarti, M.K. Poddar, Long-term electropuncture-induced changes in mammalian central GABA system, Biog. Amines 6 (1989) 417–426.
[7] G.A. Chernysheva, M.B. Plotnikov, V.I. Smol'yakova, A.D. Avdoshin, A.S. Saratikov, T.G. Sutormina, Relationship between rheological and hemodynamic changes in rats with crush syndrome, Bull. Exp. Biol. Med. 130 (2000) 1048–1050.
[8] A. Chiaretti, M. Piastra, E. Caresta, L. Nanni, L. Aloe, Improving ischaemic skin revascularisation by nerve growth factor in a child with crush syndrome, Arch. Dis. Child. 87 (2002) 446–448.
[9] S.N. Desai, P.V. Desai, The study of Na^+, K^+-ATPase activity of rat brain during crush syndrome, Neurochem. Res. 32 (2007) 1843–1848.
[10] F. Fonnum, Regulation of the synthesis of the transmitter glutamate pool, Prog. Biophys. Mol. Biol. 60 (1993) 47–57.
[11] H.L. Hayrapetyan, H.F. Khachatryan, S.S. Mardanyan, S.S. Mardanyan, Y.G. Sargisova, G.A. Kevorkyan, Activity of enzymes of adenyline compounds metabolism during crush and decompression of muscle tissue. Part II. Adenosine deaminase activity at experimental crush syndrome, Med. Sci. Monit. 6 (2000) 1068–1076.
[12] H.H. Hendrickx, P. Safar, B.P. Baer, R.E. Basford, Brain lactate and alanine–glutamate ratios during and after asphyxia in rats, Neurochem. Res. 12 (1984) 129–140.
[13] G.A. Kevorkian, G.L. Marukhyan, L.N. Arakelyan, A.G. Guevorkian, A.A. galoyan, Influence of hypothalamic praline-rich peptide on the level of [14C]glucose utilization during crush syndrome, Neurochem. Res. 26 (2001) 829–832.
[14] V.H. Knaryan, L.N. Arakelyan, G.L. Marouchyan, G.A. Kevorkian, High affinity glutamate uptake in rat brain slices in experimental crush syndrome, Med. Sci. Monit. 8 (2002) 75–79.
[15] P. Kugler, Enzymes involved in glutamatergic and GABAergic neurotransmission, Int. Rev. Cytol. 147 (1993) 285–336.
[16] S.F. Leong, J.B. Clark, Regional enzyme development in rat brain. Enzymes of energy metabolism, Biochem. J. 218 (1984) 139–145.
[17] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[18] D.J. Malinoski, M.S. Slater, R.J. Mullins, Crush injury and rhabdomyolysis, Crit. Care Clin. 20 (2004) 171–192.
[19] M. Netopilová, R. Haugvicová, H. Kubová, J. Daršata, P. Mareš, Influence of convolusants on rat brain activities of alanine aminotransferases and aspartate aminotransferases, Neurochem. Res. 26 (2001) 1285–1291.
[20] M.D. Norenberg, K.V. Rama Rao, A.R. Jayakumar, Ammonia neurotoxicity and the mitochondrial permeability transition, J. Bioenerg. Biomembr. 30 (2004) 303–307.
[21] M. Palmada, J.J. Centelles, Excitatory amino acid neurotransmission Pathways for metabolism, storage and reuptake of glutamate in brain, Front. Biosci. 20 (1998) 701–718.
[22] K.W. Peng, S.F. Chew, C.B. Lim, S.S.L. Kuahl, W.K. Kok, Y.K. Ip, The mudskippers *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti* can tolerate environmental NH_3 concentrations of 446 and 36 μM, respectively, Fish Physiol. Biochem. 19 (1998) 59–69.
[23] B. Pulford, D. Ischii, Uptake of circulating insulin by growth factors in CSF appears to be independent of IgF receptors as well as IgF binding probe, Endocrinology 142 (2001) 213–220.
[24] R.N. Reddix, R.A. Probe, Crush syndrome presenting three days after injury, Injury Extra 35 (2004) 73–75.
[25] N.D. Reis, M. Michaelson, Crush injury to the lower limbs: treatment of the local injury, J. Bone Joint Surg. Am. 68 (1986) 414–418.
[26] S. Reitman, S. Frankel, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol. 28 (1957) 56–63.
[27] I. Rubinstein, Z. Abassi, R. Coleman, F. Milman, J. Winaver, O.S. Better, Involvement of nitric oxide system in experimental muscle crush injury, J. Clin. Invest. 101 (1998) 1325–1333.
[28] C.E. Virgin, T.P-T. Ha, D.R. Packan, G.C. Tombaugh, S.H. Yang, H.C. Horner, R.M. Salpolsky, Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity, J. Neurochem. 57 (1991) 1422–1428.