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Hypoxia-ischemia altered expression of glutamate transporter EAAT1 in neonatal rat brain[1]

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KEY WORDS anoxia; cerebral ischemia; glutamates; carrier proteins; immunohistochemistry; cerebral cortex

ABSTRACT

AIM: To observe altered expression of glutamate transporter EAAT1 after hypoxia-ischemia (H-I) in newborn rat brain. **METHODS**: Expression levels of EAAT1 were detected with immunohistochemistry method. **RESULTS**: EAAT1 was a little expressed in cerebral cortex at sham-operated group [(36 ± 10) cells/slice]. Its expression in cerebral cortex increased at 24 h and 48 h following H-I [(314 ± 162) cells/slice and (431 ± 149) cells/slice, respectively], and recovered to control level at 72 h following H-I [(52 ± 8) cells/slice]. The expression of EAAT1 in the ipsilateral cortex to common carotid artery (CCA) ligation was higher than that in the contralateral cortex. **CONCLUSION**: After H-I, the expression of EAAT1 had a temporal change in cerebral cortex of newborn rat, and was mainly located in the ipsilateral cortex to CCA ligation.

INTRODUCTION

Glutamate is a major neurotransmitter mediating the fast excitatory transmission at central synapses, and it plays critical roles in synaptic plasticity and development[1]. The extracellular glutamate concentration is kept low enough to avoid neurons from glutamate excitotoxicity[2]. Excitotoxicity has been implicated as a pathogenic event in hypoxia-ischemia (H-I) infant brain[3], possibly through transient abnormalities in glutamate uptake[4] and marked increases in extracellular glutamate[5]. The low extracellular concentration is attributable to the action of high-affinity, sodium-dependent glutamate transporters. Molecular biological studies have identified four subtypes of the glutamate transporter with distinct structures, functions, and distributions, and they were named GLAST (GluT-1 or EAAT1), GLT1 (EAAT2), EAAC1 (EAAT3), and EAAT4[6]. EAAT1 is detected in neurons and glial cells with a higher K_m value compared with other glutamate transporters, and may be functionally associated with limiting the excess rise of glutamate levels and inhibiting the expansion of the lesion in some pathologic conditions[7]. However, the expression of EAAT1 after H-I in newborn rat brain remained unclear. The present study was to observe altered expression of EAAT1 in newborn rat brain following H-I.

MATERIALS AND METHODS

Perinatal H-I rats Cerebral H-I was induced in postnatal d 7 rats[8] with minor modifications. Sprague-Dawley d 7 rats of either sex, weighing 17 g ± *s* 4 g (10 – 20 g), were supplied by the Experimental Animal Center, Shanghai Medical University (Grade Ⅱ, Certifi-

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cate № 02-22-2). The rats were lightly anesthetized with ether. The right common carotid artery (CCA) was ligated with 4 - 0 surgical silk. The wound was sutured. Upon recovery from anesthesia, the rats were returned to their dams for 3 h. Groups of 8 rats were placed in 25 cm × 15 cm × 15 cm air-tight box made of plexiglass partially submerged in a 37 ℃ water bath. A gas mixture of 8 % O_2 + 92 % N_2 was delivered for 2 h. Then they were maintained in the box with natural ventilation for 15 min. The pups were returned to their dams and killed at 24, 48, and 72 h following H-I. Since brain damage did not occur in rat pups exposed to either CCA ligation or hypoxia alone[8], control littermates underwent neither arterial ligation nor hypoxia.

Tissue preparation Pup was anesthetized with ether and transcardially perfused with 0.9 % saline solution followed by 50 mL fixative (4 % paraformaldehyde in phosphate buffer 0.1 $mol \cdot L^{-1}$, pH 7.4) for 30 min. The brain was postfixed in the same fixative for about 12 h, then transferred to 20 % and 30 % sucrose solutions in phosphate buffer 0.1 $mol \cdot L^{-1}$ (pH 7.4) until sinking. Coronal 30 μm slices were cut on a freezing microtome at the interaural line level from A 6.2 mm to A 1.2 mm[9] and stored at - 20 ℃ in a cryoprotectant solution.

Immunohistochemistry The avidin-biotin peroxidase complex method[10] was used to detect glutamate transporter EAAT1 immunoreactivities. Two slices (A 5.3 and A 2.3) were incubated with a monoclonal antibody to EAAT1 (Novocastra Laboratory) at a 1 : 80 dilution as primary antibody, then with affinity-purified horse anti-mouse IgG (ABC kits, Vector Laboratory) at a 1 : 200 dilution as secondary antibody, and with avidin-biotin peroxidase complex (ABC kits, Vector Laboratory) at a 1 : 200 dilution. Immunoreactivity was visualized with 0.05 % diaminobenzidene (Sigma) as chromogen. Negative control slices received antibody-diluting solution for replacing the primary antibody.

Data analysis The expression of EAAT1 in cerebral cortex (sum of each layer) was quantified by positive cell number using a microscopical micrometer. Two slices (A 5.3 and A 2.3) from each brain were taken for quantitation. Data were expressed as $\bar{x} \pm s$ and compared with one-way ANOVA followed by Student-Newman-Keuls method and paired *t*-test.

RESULTS

Altered expression of EAAT1 after H-I in newborn rat brain EAAT1 was a little expressed in cerebral cortex at sham-operated rat (control group). Following H-I, the expression of EAAT1 markedly increased at 24 h and 48 h, which was mainly located in cerebral cortex (Fig 1), a few in hippocampus, striatum, and thalamus.

Compared with control, the expression of EAAT1 in cerebral cortex increased at 24 h and 48 h ($P < 0.05$) and recovered to control level at 72 h ($P > 0.05$) following H-I (Tab 1).

Tab 1. Expression of EAAT1 in cerebral cortex. $n = 3$ rats (control and 72 h) or $n = 4$ rats (24 h and 48 h). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* contralateral.

Treatment	Positive cell number in cortex per slice		
	Sum	Ipsilateral	Contralateral
Control	36 + 10		
24 h	314 ± 162^b	252 ± 136^e	62 ± 43
48 h	431 ± 149^b	303 ± 106^e	128 ± 59
72 h	52 ± 8^a	52 ± 8^f	0

The distribution of EAAT1 in cerebral cortex was in the form of cluster, and mainly located in layers Ⅱ, Ⅲ, Ⅴ, and Ⅵ. According to phenotype of the positive staining cells, they

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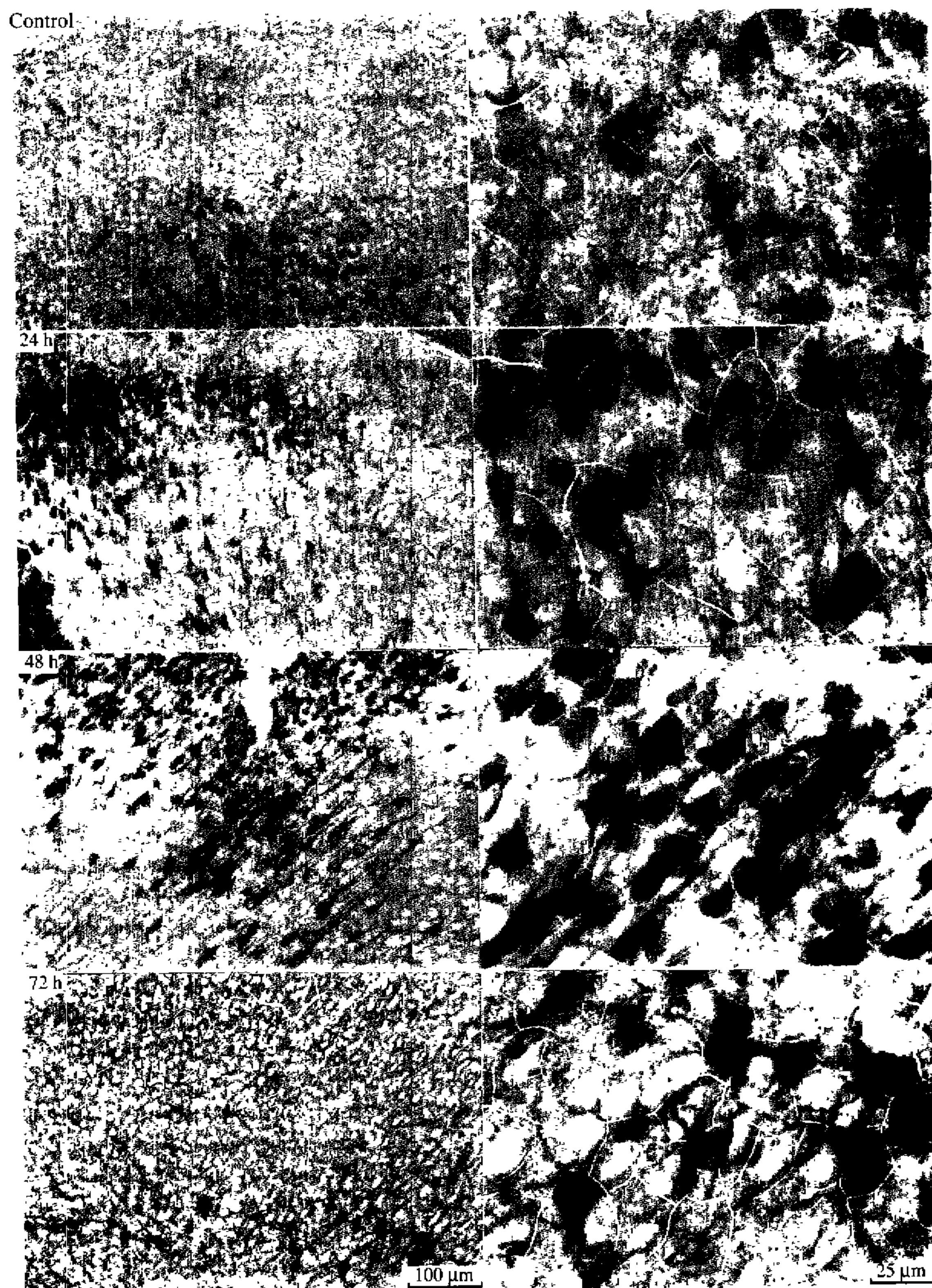

Fig 1. EAAT1 expression in cerebral cortex after hypoxia-ischemia (immunohistochemical stain). Left column × 100; right column × 400.

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were predominantly in glial cells in control group, and both in neuronal and glial cells in H-I group (Fig 1).

Different expression of EAAT1 in cerebral cortex EAAT1 was mainly expressed in the ipsilateral cortex to CCA ligation after H-I. Differences of EAAT1 expressions between ipsilateral cortex and contralateral cortex to CCA ligation were significant (24 h and 48 h: $P < 0.05$; 72 h: $P < 0.01$, Tab 1).

DISCUSSION

GLAST (EAAT1) can be consistently expressed in rat neocortex between d 1 and d 10 postnatal[11]. In the present study, postnatal d 7 rats were chosen to observe EAAT1 expression in cerebral cortex following H-I. We observed that EAAT1 expressed in neocortex in control group, which further conformed the previous report. Furthermore, the expression of EAAT1 in cerebral cortex markedly increased at 24 h and 48 h following H-I. Such delayed response was consistent with the results from adult rats[12-14]. Besides, the pattern of ischemia-induced EAAT1 expression was the same as that of GLAST mRNA expression[12-14] and of the transporter activity change following the cerebral ischemia[15]. On the other hand, EAAT1 expression following H-I was more obvious in the ipsilateral cortex to CCA ligation than in the contralateral cortex, which may reflect neuronal self-defence mechanisms to ischemic injury. In our results, EAAT1 did not express in contralateral cortex to CCA ligation at 72 h following H-I. It probably resulted from time course change of EAAT1 expression after H-I because in this H-I model contralateral cortex also suffered damage to a certain extent. In addition, our laboratory recently observed that *L*-trans-2,4-PDC, a glutamate transporter inhibitor, dose-dependently increased the infarction volume induced by cerebral ischemia[12,13]. Putting together, it may be postulated that the increase of EAAT1 expression at 24 h and 48 h following H-I may reflect a compensative mechanism, which probably contributes to the clearing of excessive extracellular glutamate after H-I.

In conclusion, after H-I, the expression of EAAT1 had a temporal change in cerebral cortex of newborn rats, and was mainly located in the ipsilateral cortex to CCA ligation.

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缺氧缺血改变了新生大鼠脑内谷氨酸载体 EAAT1 的表达[1]

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关键词 缺氧症; 脑缺血; 谷氨酸盐类; 载体蛋白; 免疫组织化学; 大脑皮质

目的: 观察缺氧缺血后新生大鼠脑内谷氨酸载体 EAAT 表达的改变. **方法**: 用免疫组织化学方法检测 EAAT 的表达水平. **结果**: 在假手术对照组, EAAT1 仅少量表达在大脑皮层[每脑片(36 ± 10)个细胞]; 缺氧缺血后 24 h 和 48 h, EAAT1 在大脑皮层的表达明显增加, 分别为每脑片(314 ± 162)个细胞和(431 ± 149)个细胞; 缺氧缺血后 72 h, EAAT1 在大脑皮层的表达恢复到对照组水平[每脑片(52 ± 8)个细胞]. 缺氧缺血后 EAAT1 在颈总动脉结扎同侧大脑皮层的表达明显高于对侧皮层. **结论**: 缺氧缺血后 EAAT1 在新生大鼠大脑皮层的表达随时间而改变; 且主要定位于颈总动脉结扎同侧皮层上.

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