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Effects of γ-aminobutyric acid on amino acids and calcium levels in rat brain of acute incomplete global cerebral ischemia

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Abstract: AIM To explore further protection mechanism of GABAergic drugs on cerebral ischemia. **METHODS** The acute incomplete global cerebral ischemia model was induced by ligation of bilateral common carotid arteries. The contents of amino acids were determined by high performance liquid chromatography combined with fluorescent detection, Ca^{2+} level was measured with atom absorption spectrometry. **RESULTS** Cerebral ischemia for 4 h increased glutamate (Glu) and aspartate (Asp) contents in hippocampus and cortex, and GABA content in hippocampus; elevated Ca^{2+} level; increased brain water content. Pretreatment with exogenous GABA (100 $mg \cdot kg^{-1}$, iv, 30 min before ischemia) markedly suppressed ischemia-induced release of Glu and Asp, reduced brain water content in cortex. Moreover, exogenous GABA increased endogenous GABA content in hippocampus. **CONCLUSION** GABA inhibits excessive efflux of excitatory amino acids, increases inhibitory amino acid level, and alleviates brain edema.

Key words: cerebral ischemia; γ-aminobutyric acid; calcium; brain edema; hippocampus; cortex

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Stroke or cerebral ischemia is a leading cause of death and permanent disability, for which there is currently no effective treatment. It has been considered to aggravate the ischemic neuronal damage with the release of excessive excitatory amino acids (EAA) such as glutamate (Glu), aspartate (Asp) during cerebral ischemia and thereby leading to massive activation of EAA receptors, Ca^{2+} build-up and several other excitotoxic damage[1-3]. However, medications that antagonize the effects of Glu at post-synaptic receptors are either ineffective or have serious side-effects. Ca^{2+} channel blockers have shown disappointing results in clinical trials in patients with acute cerebral infarction. Recently, there are increasing interests in the use of agents that increase cerebral inhibitory responses after an ischemic insult. Such agents are shown to be effective when used before, during or up to 4 h after the ischemic insult[4,5].

In central nervous system, there are some inhibitory amino acids, especially γ-aminobutyric acid (GABA), one of the major inhibitory neurotransmitter, which can increase the chloride conductance, blunt depolarization and the opening of calcium channel, decrease ATP consumption and cell apoptosis induced by cerebral ischemia[6,7]. Therefore, exogenously administered GABA or GABAergic drugs may be beneficial to neuronal protection against ischemic damage. But the exact mechanisms of these actions are not clear yet. In the present study, we observed the alteration of endogenous amino acid contents in cortex and hippocampus during rat cerebral ischemia and the effect of administration of exogenous GABA, to explore further protection mechanism of GABAergic drugs.

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1 MATERIALS AND METHODS

1.1 Reagents

o-Phthaldialdehyde, GABA, glycine(Gly), Glu, Asp and internal standard homoserine were purchased from Sigma Chemical Co.. Methanol was chromatography grade. All other chemicals used in these experiments were analytical grade from commercial sources. All water used for reagents were deionized water.

1.2 Animals

Male Wistar rats (Certificate № 97004), weighing 210 – 250 g, were obtained from Center of Experimental Animals, Tongji Medical College.

1.3 Preparation of acute incomplete cerebral ischemia

Cerebral ischemia was produced by bilateral common carotid arteries occlusion according to Ueda, *et al*[8,9] methods. Briefly, rats were anesthetized with urethan (1.0 $g \cdot kg^{-1}$, ip) and breathed room air spontaneously. Bilateral common carotid artery was exposed through a ventral incision in the neck, carefully separated from the vagosympathetic trunks, occluded with suture doubly and incised between the sutures. In the sham group rats common carotid arteries were only separated and stretched but not occluded and incised. In the control group rats operation was not performed. All rats were injected intravenously GABA 100 $mg \cdot kg^{-1}$ or equal volume normal saline 30 min before ligation. During and after the surgery, the rats body temperature was maintained at 37 – 38℃ with a heating lamp. After 4 h ischemia, rats were sacrificed by decapitation, the cortex and hippocampus were separated on ice and put into liquid nitrogen for biochemical detection.

1.4 Amino acids measurement

Brain tissue was mixed with ice-cold 0.1 $mol \cdot L^{-1}$ phosphate buffer, homogenated and centrifuged at 4℃, 10 000 × *g* for 15 min. The supernatent was neutralized with 2.0 $mol \cdot L^{-1}$ $KHCO_3$ and centrifuged again with 0.4 $mol \cdot L^{-1}$ perchloric acid, supernatant was frozen at – 80℃ for amino acids analysis.

Glu, Asp, Gly and GABA analysis was performed with high performance liquid chromatography(HPLC) after precolumn derivatization with *o*-phthalaldehyde as described by Lindroth, *et al*[10]. The *o*-phthalaldehyde derivatives were separated on a reverse phase C_{18} column (Ultrasphere ODS 4.6 mm × 100 mm, 5 μm) at room temperature using gradient elution at a rate of 1.0 $mL \cdot min^{-1}$. The mobile phase consisted of methanol-potassium phosphate (0.1 $mol \cdot L^{-1}$) brought to pH 6.8 by phosphate acid and was run in 2 linear steps from 30% to 90% methanol. Amino acids were measured by a fluorescence detector (Waters 474). The excitation wavelength was set at 330 nm, and the emission wavelength was 450 nm. Amino acids were quantified by means of the internal standard method (homoserine).

1.5 Measurement of the brain water content and Ca^{2+} content

After rats were decapitated, the unilateral cortex was immediately isolated, weighed and dried to a constant weight in an oven at 110℃. The wet-dry weight formula was used to calculate the percentage of brain water content in the brain: water (%) = (wet weight – dry weight)/wet weight × 100%. For determining Ca^{2+} content, dried brain samples were digested with nitric acid, and Ca^{2+} content was determined by WYZ-1200 atomic absorption spectrometry. Data were presented as $\mu mol \cdot g^{-1}$ dry weight.

1.6 Statistics

Results were expressed as $\bar{x} \pm s$. ANOVA was used to test the differences among all groups, and SNK test was used to test the difference between two groups.

2 RESULTS

2.1 Effects of GABA on the releases of endogenous amino acids from hippocampus and cortex in acute incomplete global cerebral ischemic rats

As shown in Tab 1, after 4 h ligation of bilateral common carotid artery, Glu contents in hippocampus and cortex of rats increased by 39%

Tab 1. Effects of GABA on the releases of endogenous amino acids from hippocampus and cortex in acute incomplete global cerebral ischemic rats

Group	Content of amino acids/μmol·g^{-1} wet weight			
	Aspartate	Glutamate	Glycine	GABA
Hippocampus				
Control	2.04 ± 0.39	4.18 ± 0.69	0.48 ± 0.02	12.1 ± 1.7
Sham	2.06 ± 0.72	4.05 ± 0.67	0.53 ± 0.08	12.1 ± 2.3
Ischemia	3.68 ± 0.64*	5.82 ± 0.40*	0.52 ± 0.09	14.7 ± 1.1**
GABA	1.29 ± 0.19$^{\#\#}$	3.29 ± 0.26$^{\#}$	0.62 ± 0.09	17.0 ± 0.6$^{**\#\#}$
Cortex				
Control	2.19 ± 0.31	5.29 ± 0.57	1.02 ± 0.32	8.6 ± 1.5
Sham	2.43 ± 0.22	5.06 ± 0.58	0.73 ± 0.27	9.1 ± 1.7
Ischemia	3.92 ± 0.05*	6.97 ± 0.75*	0.93 ± 0.43	8.4 ± 1.7
GABA	2.89 ± 0.42$^{\#}$	6.05 ± 0.81$^{\#}$	0.87 ± 0.39	8.9 ± 1.1

Rats were iv GABA 100 mg·kg^{-1} 30 min before cerebral ischemia induced by ligation of bilateral common carotid arteries and amino acid contents were measured 4 h after cerebral ischemia. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with ischemia group.

and 32%, respectively; Asp contents in hippocampus and cortex increased by 80% and 79%, respectively; GABA content in hippocampus also resulted by 22%, compared with control group. However, the Gly contents in hippocampus and cortex had no significant change during the acute incomplete global cerebral ischemia ($P > 0.05$), and the operation had little effect on these four amino acids contents ($P > 0.05$, Tab 1). Pretreatment with exogenous GABA (100 mg·kg^{-1}, iv) reversed ischemia-evoked release of Glu and Asp from hippocampus and cortex ($P < 0.05$), compared with ischemia group. Moreover, the exogenous GABA further increased endogenous GABA content from hippocampus.

2.2 Effects of GABA on the Ca^{2+} level of hippocampus and cortex in acute incomplete global cerebral ischemic rats

As shown in Tab 2, Ca^{2+} level of cortex increased significantly at the end of 4 h acute incomplete global cerebral ischemia, which got to 126% of control. Pretreatment with GABA (100 mg·kg^{-1}, iv) 30 min before cerebral ischemia reversed the increase to the level of sham group. The same treatment only induced slight change on Ca^{2+} level from hippocampus.

Tab 2. Effects of GABA on the Ca^{2+} level in hippocampus and cortex of acute incomplete global cerebral ischemic rats

Group	Ca^{2+} level/μmol·g^{-1} dry weight	
	Cortex	Hippocampus
Control	5.7 ± 1.4	4.9 ± 0.9
Sham	6.4 ± 1.4	4.4 ± 0.9
Ischemia	7.2 ± 1.1*	5.4 ± 1.0
GABA	6.5 ± 0.3	4.8 ± 1.0

See Tab 1 for rat treatments. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, compared with control group.

2.3 Effects of GABA on water content in cortex of acute incomplete global cerebral ischemic rats

As shown in Fig 1, 4 h after the bilateral common carotid artery was ligation, the cortex water content increased markedly. Pretreatment with exogenous GABA (100 mg·kg^{-1}, iv) 30 min before cerebral ischemia alleviated the elevation of cortex water content resulted from ischemic damage.

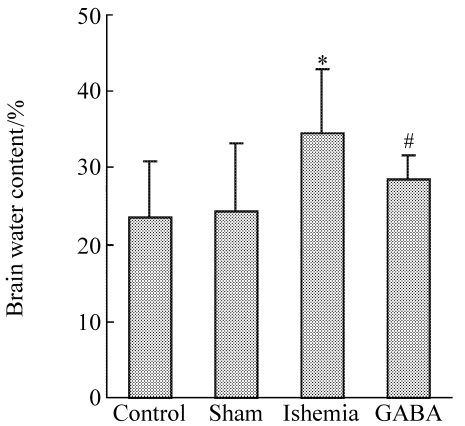

Fig 1. Effects of GABA on water content in cortex of acute incomplete global cerebral ischemic rats. See Tab 1 for rat treatments. $\bar{x} \pm s$, $n = 10$. $^{*}P < 0.05$, compared with control group; $^{\#}P < 0.05$, compared with ischemia group.

3 DISCUSSION

During the bilateral common carotid arteries occlusion, the cerebral blood flow is decreased. A prolonged cerebral hypoperfusion leads to a failure of energy-dependent ion exchange pumps due to a compromised energy supply in the vulnerable regions, such as hippocampus and cortex. This leads to a run-down in transmembrane ion gradients, an anoxic depolarization of cells, a fluid shift from the extracellular to the intracellular compartment, and a massive EAA release, especially Glu and Asp. The increased Glu activates postsynaptic receptors, induces intracellular Ca^{2+} overload, Na^{+} influx increase, and Cl^{-} and water cotransport, further results in cytotoxic brain edema and neuronal damage.

The results in the present study also confirmed that cerebral ischemia induced marked increase in tissue Glu and Asp contents from the cortex and the hippocampus. Therefore, it is significant to decrease EAA content and Ca^{2+} level, for protecting ischemic neuronal damage. A variety of experimental evidences suggest that increased inhibitory neurotransmitter activity by preventing GABA re-uptake and metabolism, or increasing GABA receptor activity with agonists and allosteric modulators, could protect ischemic brain infarction volume[11]. Our results also showed that exogenous GABA (100 $mg \cdot kg^{-1}$, iv) reversed acute incomplete global cerebral ischemia-induced EAA release, reduced brain water content. The protection mechanism may be relevant to GABA induced reduce in ATP consumption and the hyperpolarization of the synaptic membrane.

The increase in GABA content has been demonstrated to be an important self-protective mechanism in cell stress[5]. Our result showed that acute incomplete global cerebral ischemia resulted in an increase in GABA content in hippocampus, which is consistent with this notion, but the same result has not been observed in cortex. The reason is unclear, maybe related to the difference of blood supply and ischemic degree between hippocampus and cortex. Zhu, *et al*[12] documented that acute cerebral ischemia decreased the GABAergic neuron in rat cortex, and this reduce aggravated with the going of cerebral ischemia, leaded to a decrease in GABA synthesis and release. Furthermore, GABA was also lost from brain by penetrating the abnormal blood brain barrier to circulating blood after the ischemic insult[13]. After administration of exogenous GABA, the endogenous GABA content from hippocampus increased significantly, which may be the result of the permeability increase of blood brain barrier during ischemia. Obviously, the increase in GABA content is beneficial in attenuating ischemic brain damage.

In summary, these data in the present study support the potential therapeutic benefit of the exogenous GABA in the rat cerebral ischemia, which attenuates neurotoxicity by inhibiting acute incomplete global cerebral ischemia-induced increase in EAA and brain edema. In addition, exogenous GABA could increase inhibitory amino acid GABA content, maintain diametric effects of excitatory and inhibitory neurotransmitters.

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γ-氨基丁酸对急性不完全性全脑缺血大鼠脑组织氨基酸和钙离子含量的影响

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摘要: 目的 进一步探讨 γ-氨基丁酸(GABA)类药物对脑缺血保护作用的机制。**方法** 结扎双侧颈总动脉制备大鼠不完全性全脑缺血模型, 高效液相法和原子吸收分光光度法分别测定脑组织氨基酸和钙离子含量。**结果** 脑缺血 4 h 显著增加大鼠海马和皮层区的谷氨酸(Glu)和天冬氨酸(Asp)含量及海马 GABA 含量,增高皮层细胞钙离子水平和含水量。脑缺血前 30 min 给予 GABA 100 mg·kg^{-1}, iv,能逆转缺血诱导的 Glu 和 Asp 等兴奋性氨基酸释放增加,减轻脑组织含水量。此外尚能增加内源性 GABA含量。**结论** 外源性给予 GABA 可逆转脑缺血诱导的兴奋性氨基酸释放,升高抑制性氨基酸水平, 减轻脑水肿。

关键词: 脑缺血; γ-氨基丁酸; 钙; 脑水肿; 海马; 皮质

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