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Alterations of amino acid levels from striatum, hippocampus, and cerebral cortex induced by global cerebral ischemia in gerbil[1]

TANG Xing-Chun[2], RAO Man-Ren[2], HU Gang[2,3], WANG Hai[4]
([2]*Department of Physiology and Pharmacology, Nanjing Medical University, Nanjing 210029, China*;
[4]*Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China*)

KEY WORDS brain ischemia; gerbillinae; hippocampus; corpus striatum; cerebral cortex; glutamates; aspartate; GABA; ketamine; excitatory amino acids

ABSTRACT

AIM: To investigate global cerebral ischemia-induced alterations in the levels of glutamate, aspartate, γ-aminobutyric acid (GABA), glutamine, glycine, and taurine from hippocampus, striatum, and cerebral cortex in gerbils. **METHODS**: The gerbil global cerebral ischemia model was prepared by bilateral carotid artery occlusion; the contents of amino acids were assayed using high performance liquid chromatography (HPLC) combined with fluorescent detection after precolumn derivatization. **RESULTS**: After the ligation of bilateral carotid artery for 5 min and reperfusion for 60 min, the contents of glutamate from hippocampus, striatum, and cortex in gerbils were increased by 40 %, 49 %, and 67 %, respectively. Similarly, the global cerebral ischemia resulted in increase by 80 %, 69 %, and 83 % of aspartate contents in hippocampus, striatum, and cortex, respectively. Moreover, the same treatment also induced significant increases in the contents of GABA, glutamine, glycine, and taurine from various brain regions in gerbils. Furthermore, pretreatment with ketamine (120 mg/kg, ip) reversed ischemia-evoked increases of glutamate, aspartate, glycine, and glutamine in hippocampus, striatum, and cortex of gerbils. However, administration of ketamine (120 mg/kg, ip) markedly suppressed but not abolished the ischemia-induced increases of taurine and GABA from hippocampus, striatum, and cortex in gerbils. **CONCLUSION**: The increases of glutamate, aspartate, glycine, and glutamine induced by acute global cerebral ischemia may constitute the biochemical basis of ischemic brain damage. Correspondingly, the release of GABA and taurine may be an important self-protective mechanism. Ketamine may protect neurons against ischemic insult by inhibiting global cerebral ischemia-evoked increase of glutamate, glycine, and aspartate.

INTRODUCTION

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, aspartate, and glutamine during cerebral ischemia and thereby leading to massive activation of EAA receptors[1-3]. Blockade of EAA receptors has been reported to alleviate ischemic neuronal damage[2]. On the other hand, some inhibitory amino acids are suggested to be important for the neuronal protection against ischemic brain damage[2]. However, there are few studies available to confirm directly the changes in the levels of all above amino acids induced by global cerebral ischemia. Ketamine, a non-competitive *N*-methyl-*D*-aspartate (NMDA) receptor antagonist, has been demonstrated to possess a protective effect on cerebral ischemia-induced brain insult by abundant pharmacological, electrophysiological, pathological, and behavioral studies[4,5]. In the present study, gerbil global cerebral ischemia model and HPLC-fluorescent detector system were employed to investigate the alterations in the contents of glutamate, aspartate, GABA, glutamine, glycine, and taurine induced by global cerebral ischemia from hippocampus, striatum, and cortex in gerbils. The possible mechanism whereby ketamine produces the neuroprotective effect on ischemia-induced neuronal damage was studied.

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[3] Correspondence to Prof HU Gang.
Phn 86-25-666-3108. Fax 86-25-650-8960
E-mail ghu@njmu.edu.cn.
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MATERIALS AND METHODS

Chemicals and reagents Ketamine, β-mercaptoethanol, and tetrahydrofuran were purchased from RBI (Natick, MA, USA), Fluka (Milwaukee, WI, USA), and BDH Chemicals Ltd (Dorset, England), respectively. Glutamate, aspartate, glutamine, glycine, taurine, GABA, and *o*-phthaldialdehyde were purchased from Sigma Chemicals Co, St Louis, MO, USA. All other chemicals used in the present study were AR.

Animals Mongolian gerbils (60 g ± *s* 5 g, grade Ⅱ, certificate №98011) of either sex were supplied by Experimental Animal Center of Zhejiang Medical University.

Preparation of global ischemia model of gerbil The global ischemia model of gerbil was induced by bilateral carotid artery ligation for 5 min, with successful induction of ischemia determined by rapid isoelectric electroencephalogram records[6]. Following recovery of normal righting reflexes and locomotion, the gerbils had free access to food and water and were housed individually under diurnal light condition (lights on at 08:00 and off at 20:00).

Medication and sampling Ketamine (120 mg/kg, ip) and saline were administered 40 min prior to carotid artery ligation. After recirculation for 60 min, gerbils were decapitated, the hippocampus, striatum, and cortex were removed and then homogenized in ice-cold homogenizing medium ($HClO_4$ 0.4 mol/L). Sham-operated gerbils were sacrificed just after exposing the carotid artery without clamping the vessels. In non-operated gerbil, ketamine and saline were administered 40 min prior to decapitation. Ischemic gerbils treated with ketamine up to d 7 were decapitated 40 min after the last medication. The homogenate was centrifuged (10 000 × *g*, 4 ℃ for 15 min. The supernatant (S_1) was neutralized with 0.75 volumes of $KHCO_3$(2.0 mol/L). After recentrifuged at 3 000 × *g* at 4 ℃ for 5 min, supernatant (S_2) was frozen at −40 ℃ for later HPLC analysis.

HPLC-fluorescent detector[7] HPLC-fluorescent detector system consisted of Varian Series 5060 HPLC, a reverse phase C_{18} column (Ultrasphere ODS 4.6 mm × 250 mm, 5 μm, Beckman), 157 fluorescent detector and SP4270 digital integraph (Varian). Samples were separated at 37 ℃ with gradient elution using flow rate of 0.8 mL/min which produced a back pressure of 12 160 kPa (91 200 mmHg). The mobile phase A consisted of KH_2PO_4 67 mmol/L, tetrahydrofuran 2 %, methanol 35 % (pH 6.0). The mobile phase B was 90 % methanol. The mobile phase B increased from 0 to 40 % within 12 min. Then phase B was used to elute for 5 min prior to balancing for 5 min with the phase A.

Assay of amino acids All amino acids original fluids were confected with distilled water and diluted with artificial cerebrospinal fluid (mmol/L): NaCl 130, KCl 2.99, $NaH_2PO_4 \cdot H_2O$ 0.52, $Na_2HPO_4 \cdot 12\ H_2O$ 0.04, $NaHCO_3$ 25, $MgCl_2 \cdot 6\ H_2O$ 0.8, $CaCl_2$ 1.3, pH 7.4 just before using. Equivalent volume of sample and derivative fluid containing *o*-phthaldialdehyde 20 mmol/L, β-mercaptoethanol 2 mmol/L, tetraborate 25 mmol/L, and methanol 50 % (pH 9.6) were mixed to react for 2 min. The reaction solutions were used to elute and assay the amino acids with HPLC combined with fluorescent detection.

Statistical analysis of data Results are given as $\bar{x} \pm s$. All data were analyzed with unpaired *t*-test.

RESULTS

Effects of acute ketamine pretreatment on the contents of amino acids from hippocampus, striatum, and cortex in non-operated gerbils The contents of glutamate, aspartate, GABA, glutamine, glycine, and taurine in hippocampus, striatum, and cortex of gerbils were assayed in the normal (non-operated) gerbils. Acute administration of ketamine 120 mg/kg (ip), a dose reportedly known to protect neurons against global cerebral ischemia, failed to change the levels of all above amino acids (results not shown). Moreover, repeated administration of the same dose of ketamine for 7 d did not change the contents of glutamate, aspartate, GABA, glutamine, glycine, and taurine in all above mentioned brain regions of gerbils (Tab 1).

Effects of acute global cerebral ischemia and ketamine pretreatment on the levels of amino acids from hippocampus, striatum, and cerebral cortex in gerbils After the ligation of bilateral carotid artery for 5 min and reperfusion for 60 min, the contents of glutamate in hippocampus, striatum, and cortex of gerbils were increased by 40 %, 49 %, and 67 %, respectively (Tab 2). Similarly, the global cerebral ischemia resulted in an increase by 80 %, 69 %, and 83 % in aspartate contents in hippocampus, striatum, and cortex, respectively (Tab 2). Moreover, the same treatment also induced significant increases in the contents of GABA, glutamine, glycine, and taurine from hippocampus, striatum, and cortex ($P < 0.01$) (Tab 2).

Furthermore, pretreatment with ketamine (120 mg/kg, ip) reversed ischemia-evoked increases of glutamate, aspartate, glycine, and glutamine from hippocampus, striatum, and cortex (Tab 2). However, administration of ketamine (120 mg/kg, ip) markedly suppressed but not abolished the ischemia-induced increases of taurine and GABA from hippocampus, striatum, and cortex (Tab 2).

Effect of repeated administration of ket amine on the contents of amino acids from hippocampus, striatum, and cortex in gerbils with global cerebral ischemia Surprisingly, the levels of glutamate, aspartate, GABA, glutamine, glycine, and taurine from hippocampus, striatum and cortex in gerbils with global cerebral ischemia had no significant change on d 7 after ischemic-treatment (Tab 3). Moreover, repeated administration of ketamine (120 mg/kg, ip) did not affect the levels of all above mentioned amino acids (Tab 3).

DISCUSSION

Substantial evidence from behavioral, electrophysiological, and pathological studies implies that ischemia-induced cellular and neurological damage may result from an increase in extracellular excitatory amino acids (eg, glutamate, aspartate, and glutamine)[8,9]. The results in the present study confirm that acute global cerebral ischemia induced significant increase in extracellular glutamate, aspartate, and glutamine from hippocampus, striatum, and cortex in gerbils, supporting the excitotoxic hypothesis. Our research results also show that ketamine (120 mg/kg, ip) protected against global cerebral ischemia-induced neuronal apoptosis of some brain regions

Tab 1. Contents of amino acids from hippocampus, striatum, and cerebral cortex in non-operated gerbils following repeated treatment with ketamine for 7 d. $n=6$, $\bar{x}\pm s$. $^aP>0.05$ *vs* control group.

Groups	Contents of amino acid (μmol/g wet weight)					
	Glutamate	Aspartate	GABA	Glutamine	Glycine	Taurine
Hippocampus						
Control	15.4±1.6	3.2±0.6	2.5±0.4	8.0±1.1	1.9±0.3	7.4±1.0
Ketamine	14.7±1.4[a]	3.0±0.3[a]	2.36±0.24[a]	7.9±1.3[a]	1.9±0.6[a]	7.3±0.8[a]
Striatum						
Control	16.7±1.4	3.5±0.4	2.34±0.26	8.7±1.0	0.94±0.28	10.7±1.0
Ketamine	15.7±1.7[a]	3.1±0.4[a]	2.4±0.3[a]	8.9±1.0[a]	0.73±0.16[a]	10.1±1.2[a]
Cerebral cortex						
Control	15.6±1.6	3.8±0.4	1.62±0.24	6.7±1.2	0.90±0.21	8.4±1.0
Ketamine	15.8±1.3[a]	3.6±0.5[a]	1.61±0.24[a]	7.0±1.3[a]	0.78±0.25[a]	8.7±0.8[a]

Tab 2. Effects of acute global cerebral ischemia and pretreatment with ketamine on the contents of amino acids from hippocampus, striatum, and cortex in gerbils. $n=7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* sham group $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* ischemia group.

Groups	Contents of amino acid (μmol/g wet weight)					
	Glutamate	Aspartate	GABA	Glutamine	Glycine	Taurine
Hippocampus						
Sham	10.6±1.3	1.5±0.4	1.48±0.22	6.7±1.0	1.3±0.4	6.4±0.8
Ischemia	14.8±1.4[c]	2.7±0.3[c]	2.48±0.29[c]	11.0±1.2[c]	1.9±0.4[b]	8.9±0.9[c]
Ketamine	11.0±1.5[af]	1.5±0.3[af]	1.9±0.4[bf]	7.1±0.8[af]	1.48±0.26[ae]	7.4±1.0[af]
Striatum						
Sham	10.6±1.2	1.55±0.26	1.6±0.4	9.6±1.0	1.4± 0.4	8.0±1.5
Ischemia	15.8±1.6[c]	2.6±0.3[c]	3.0±0.5[c]	14.7±1.5[c]	2.3±0.5[c]	15.4±1.5[c]
Ketamine	11.2±1.3[af]	1.65±0.29[af]	2.12±0.24[bf]	9.9±1.4[af]	1.5±0.4[af]	10.8±1.1[cf]
Cortex						
Sham	9.8±1.1	2.9±0.4	0.93±0.13	6.0±0.8	1.08±0.24	5.8±0.6
Ischemia	16.4±1.6[c]	5.3±0.5[c]	1.85±0.20[c]	11.5±1.2[c]	1.8±0.6[c]	9.4±1.1[c]
Ketamine	10.3±1.2[af]	2.9±0.3[af]	1.30±0.22[cf]	6.3±0.9[af]	1.17±0.26[ae]	7.0±0.7[cf]

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Tab 3. Effects of continuous administration of ketamine for 7 d on the contents of amino acids from hippocampus, striatum, and cortex in gerbils with global cerebral ischemia. $n = 7$, $\bar{x} \pm s$. [a]$P > 0.05$ *vs* sham group. [d]$P > 0.05$ *vs* ischemia group.

Groups	Contents of amino acid (μmol/g wet weight)					
	Glutamate	Aspartate	GABA	Glutamine	Glycine	Taurine
Hippocampus						
Sham	16.2±1.3	2.35±0.26	1.84±0.27	7.9±0.6	1.6±0.3	8.0±1.0
Ischemia	16.1±1.7[a]	2.4±0.5[a]	1.81±0.3[a]	7.3±1.4[a]	1.7±0.4[a]	7.3±0.9[a]
Ketamine	15.3±1.7[ad]	2.3±0.6[ad]	1.96±0.3[ad]	8.2±1.1[ad]	1.7±0.3[ad]	7.6±0.8[ad]
Striatum						
Sham	11.8±1.3	2.6±0.4	2.64±0.23	9.1±1.5	2.6±0.4	10.5±0.9
Ischemia	12.1±1.2[a]	2.6±0.5[a]	2.6±0.4[a]	10.0±1.2[a]	2.7±0.5[a]	10.2±1.0[a]
Ketamine	11.2±1.4[ad]	2.5±0.3[ad]	2.8±0.3[ad]	9.0±1.0[ad]	2.5±0.4[ad]	10.4±1.2[ad]
Cortex						
Sham	14.8±1.8	4.3±0.5	2.9±0.3	6.9±1.4	2.2±0.4	8.1±1.0
Ischemia	15.7±1.5[a]	4.5±0.5[a]	3.0±0.4[a]	7.1±0.8[a]	2.1±0.3[a]	8.7±0.9[a]
Ketamine	15.0±1.5[ad]	4.3±0.6[ad]	3.1±0.3[ad]	7.2±0.7[ad]	2.1±0.3[ad]	8.8±1.0[ad]

including hippocampus, striatum, and cortex of gerbils. Taken together, the neuroprotective role of ketamine may thus result from its ability to reverse the increase in glutamate and aspartate levels-induced by global cerebral ischemia. Of course, ketamine may also play a protective role against ischemic neuronal damage by blockade of NMDA receptors[10]. Glycine is in itself an inhibitory amino acid, but there are modulatory sites of glycine on the NMDA receptor. It has been put forward that the ischemia-induced increase of glycine may aggravate ischemic neuronal injury[2]. Theoretically, suppression of glycine outflow should be beneficial to neuronal protection. However, there is few experimental evidence to support the theory that free glycine plays a role in protecting tissues against insults such as ischemia, hypoxia, and reperfusion. Thus, the significance of the change in glycine levels induced by ischemic cerebral damage remains to be further evaluated.

GABA is an important inhibitory transmitter in central nervous system. The release of GABA during ischemia has been demonstrated to be an important self-protective mechanism[11]. The result that global cerebral ischemia resulted in an increase of GABA in hippocampus, striatum, and cortex of gerbils in our study is consistent with this view. GABA is present in virtually all regions of the brain and is synaptically active at approximately 40 % of brain receptors[2]. GABAergic medications have potent neuroprotective effects when used for up to 4 h after ischemic insult[12]. Obviously, the increase of GABA during ischemia is beneficial in attenuating ischemic brain damage[2,13,14]. Notably, the ischemia-induced increase of GABA was not completely reversed by ketamine. This rapid responsive increase of GABA may be induced by other mechanisms than NMDA receptor modulation.

Taurine, a neuronal modulator, may modulate Ca^{2+} entry into hypoxic cells[2,13,15]. Taurine is reported to possess protective effects in cardiac ischemic conditions by preventing the loss of sarcolemmal ATPase activity and by decreasing lipid peroxidation related cell damage[2,13]. However, no studies of taurine as a neuroprotective agent have been published to date. The present study indicates that ischemia-evoked dramatic increase of extracellular taurine from various regions in gerbil brain may be a compensatory response to the release of glutamate or local edema, indicating that taurine may play a protective role in ischemia-induced pathophysiological events.

We also found that the levels of glutamate, aspartate, GABA, glutamine, glycine, and taurine from hippocampus, striatum, and cortex in gerbils with global cerebral ischemia recovered to the normal levels on d 7 after ischemic-treatment. The results seem to be consistent with the idea that there are few biochemical changes observed more than 7 d following ischemia and reperfusion while some functional deficits exist then[15]. The results show that ketamine failed to change the normal levels of all the amino acids observed in the present study.

In conclusion, the results shown in the present study suggest that increases of some EAA such as glutamate, aspartate, and glutamine induced by acute global

cerebral ischemia may constitute the biochemical basis of ischemic brain damage. Correspondingly, the release of some inhibitory transmitters such as GABA and taurine may act as an important self-protective mechanism by which cerebral ischemia-evoked neuronal damage is reduced. Further elucidation of the mechanisms for the release of inhibitory amino acids evoked by cerebral ischemia would provide a therapeutic implication for ischemic neuronal damage. Moreover, ketamine attenuates neurotoxicity against ischemic neuronal insult by inhibiting global cerebral ischemia-evoked increase of glutamate, aspartate, and glutamine. Furthermore, ketamine could reverse ischemia-evoked increases of glutamate, aspartate, and glutamine but not influence the normal levels of all the amino acids in gerbil brains. This characteristic effect of ketamine on the above amino acids reveals its potential therapeutic value for protecting neurons against ischemic insult.

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全脑缺血诱导沙土鼠纹状体，海马和皮层氨基酸水平的改变[1]

唐性春[2]，饶曼人[2]，胡 刚[2,3]，汪 海[4]
([2]南京医科大学生理学与药理学系，南京 210029，中国；[4]军事医学科学院毒物药物研究所，北京 100850，中国)

关键词 脑缺血；沙土鼠；海马；纹状体；大脑皮质；谷氨酸类；天冬氨酸；GABA；氯胺酮；兴奋性氨基酸

目的：研究全脑缺血时沙土鼠海马、纹状体和皮层谷氨酸、天冬氨酸、γ-氨基丁酸（GABA）、谷氨酰胺、甘氨酸和牛磺酸含量的变化及氯胺酮对上述氨基酸含量的影响. **方法**：采用结扎双侧颈总动脉的方法制备沙土鼠全脑缺血模型，应用 HPLC 和荧光检测器联用测定氨基酸的含量. **结果**：全脑缺血显著增加沙土鼠海马，纹状体和皮层的谷氨酸，天冬氨酸，谷氨酰胺，GABA，甘氨酸和牛磺酸含量；氯胺酮（120 mg/kg，ip）预处理能完全逆转缺血诱导的谷氨酸、天冬氨酸、甘氨酸和谷氨酰胺释放的增加，但不能完全逆转缺血诱导的 GABA 和牛磺酸释放增加. **结论**：脑缺血诱发的神经元损伤可能与其增加谷氨酸、天冬氨酸、甘氨酸、谷氨酰胺含量有关，而抑制性氨基酸 GABA 和牛磺酸释放增加则可能是机体一种重要的自身脑保护机制. 氯胺酮逆转脑缺血诱导的兴奋性氨基酸释放增加可能是其抗兴奋性神经毒的生化基础.

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