A Microdialysis Study of Effects of Gastrodin on Neurochemical Changes in the Ischemic/Reperfused Rat Cerebral Hippocampus

Xianghui ZENG,†,‡ Yun ZHANG,† Shaoming ZHANG,§ and Xiaoxiang ZHENGG,*

†Department of Biomedical Engineering, Key Laboratory for Biomedical Engineering of Ministry of Education of China, Zhejiang University; Hangzhou 310027, P.R. China; and §School of Chemical Science and Technology, Yunnan University; Kunming 650091, P.R.China. Received August 19, 2006; accepted January 17, 2007

Gastrodin is a component extracted from the rhizome of Gastrodia elata, and has been shown to possess protective effects against neuron damage induced by simulated cerebral ischemia in previous studies. But its neurochemical effects on the ischemic brain had not been well studied. The present study aimed at evaluating the effects of gastrodin on the changes of transmitter amino acids in rat hippocampus during cerebral ischemia/reperfusion. Microdialysis sampling was performed during ischemia and early reperfusion periods in rats, and the glutamate and gamma-aminobutyric acid (GABA) in the dialysate were measured using high-performance liquid chromatography (HPLC). Administration of gastrodin (100 mg/kg) before ischemia significantly reduced the ischemia-induced elevation of glutamate levels during the postischemic period, increased the rise of extracellular GABA during the reperfusion periods, thus decreased the glutamate/GABA ratios during ischemia and reperfusion. These results provide insights to explain the neurochemical effects of gastrodin when applied prior to an ischemic event.

Key words microdialysis; amino acid; ischemia/reperfusion; gastrodin; hippocampus

Gastrodin is a primary component of the functional extracts from the rhizome of Gastrodia elata Blume (Orchidaceae). It has been found that gastrodin can reduce the seizure score in seizure-prone gerbils and facilitate learning and memorizing. In addition, some studies found that gastrodin had protective effects against rat cortical neurons and astrocytes damage induced by simulated cerebral ischemia in vitro, and decreased mortality rate resulting from ischemia in mice. However, the mechanism underlying the neuroprotective effects of gastrodin are currently unclear.

Among the amino acids, glutamate and gamma-aminobutyric acid (GABA) are endogenous neurotransmitters in the central nervous system. Though the detrimental cascade of events during cerebral ischemia is complex and not fully understood, it is generally accepted that a pathological release of glutamate from neurons plays a central role in mediating subsequent neuronal cell injury and death. Glutamate activates several types of post-synaptic receptors, increasing intracellular calcium to high toxic levels to brain cells. Blocking the post-synaptic receptors can protect neurons during global and focal ischemia. GABA, the inhibitory amino acid neurotransmitter, has been implied to be involved directly or indirectly in the pathogenesis of neurological and psychiatric disorders. Glutamate release can be blocked by drugs that increase intra-cerebral GABA levels. And drugs that potentiate GABA have been shown to provide significant neuronal protection when used before or after the ischemic insult in global or focal ischemia.

The present study aims to assess the effects of gastrodin on extracellular glutamate and GABA in the hippocampus of rats during transient focal cerebral ischemia, when administrated before ischemic injury.

MATERIALS AND METHODS

Materials Gastrodin (P-hydroxymethyl phenyl-β-D-glucopyranoside, 99.6% pure), was prepared, identified and provided by Kunming Pharmaceutical Co., China. Glutamate and GABA were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of the highest grade available and used without further purification.

Animals Male Sprague-Dawley rats (Zhejiang Academy of Medical Science, Hangzhou, China) weighing 300—350 g were used in this study. All animal handling and surgery were performed in accordance with the Care Standards of the Laboratory Animal (China Ministry of Health publication, 1998).

Microdialysis Procedure The animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed on a stereotaxic frame (ASI SAS-4100, U.S.A.). An intracerebral guide cannula (BAS MD-2251, U.S.A.) was implanted in the hippocampus (coordinates: P = −5.8, L = +5.0 H = −3.0 from Bregma) to guide and secure the probe. At least 5 d were allowed for recovery from surgery before the ischemia experiments were conducted. The microdialysis probes (BAS MD2204, 4 mm membrane, U.S.A.) were perfused with artificial cerebrospinal fluid (ACSF: 125 mM NaCl, 2.5 mM KCl, 0.9 mM NaH₂PO₄·H₂O, 5 mM Na₂HPO₄, 1 mM MgCl₂·6H₂O, 1.2 mM CaCl₂·2H₂O, and pH 7.4—7.6). The recoveries of glutamate and GABA in the probes were approximately 19% and 23% respectively. After a 2 h equilibrium period at a flow rate of 2 µl/min with an infusion micropump (BAS MD-2262, U.S.A.), dialysates were collected in polyethylene vials every 20 or 10 min throughout the experiments. The first sample obtained from every animal before ischemic surgery was used for the baseline. The second sample was collected at the onset of the occlusion of the middle cerebral artery. Seven more samples were collected 30 min afterwards.

Drug Treatment The rats were randomly divided into vehicle group (normal saline), test drug groups (gastrodin), sham groups (control) (n = 6 per group). The doses of drugs used in the present experiment were selected based on our preliminary experiment. At 20 min before MCAO, gastrodin was administered intraperitoneally at a dose of 50 or 100 mg/kg (99.6% pure, dissolved in 0.9% saline) in the gastrodin groups. The vehicle and sham group received only the

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saline (0.9% saline).

Middle Cerebral Artery Occlusion (MCAO) Rats were anesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg). A polyethylene tube was inserted into the right femoral artery for continuously monitoring blood pressure with a computer-assisted system (Medlab-U, Nanjing Medease Science and Technology Co., China). The arterial blood gases and hemoglobin (Hb) were monitored using blood gas analyzer (CIBA850, Corning, U.S.A.) before the surgery, during MCAO and 30 min after reperfusion, respectively. Blood glucose was measured at the same time with one-touch basic blood glucose monitoring system (Lifescan Co., U.S.A.). The rectal temperature was kept at 37±0.5 °C with a heating pad and light during the entire stroke procedure. The MCAO was induced using the intraluminal filament method, as described by Zea-Longa et al. Briefly, a midline incision was made and the right common carotid artery (CCA), the external carotid artery (ECA) and the internal carotid artery (ICA) were exposed. The distal ECA was coagulated completely. The CCA and the ICA were temporarily clamped with microvascular clips. A monofilament nylon filament (diameter 0.234 mm) with a blunted tip was introduced into the ECA lumen. The filament was then gently advanced to the distal ICA until it reached the clipped position. After removing the microvascular clip, the filament was inserted until resistance was felt, which ensured the occlusion of the origin of the middle cerebral artery. The distance between the CCA bifurcation and the resistive point was about 18.5—19.5 mm. The filament was withdrawn from the ICA after 50 min to allow MCA reperfusion. Control rats received sham surgery, in which an identical procedure was followed but inserting the filament 5 mm.

Amino Acids Detection The extracellular concentrations of amino acids glutamate and GABA were measured by OPA-β-mercaptoethanol precolumn derivatization, reversed-phase gradient elution and fluorescence detection. The amino acid in dialysate was first derivatized to its fluorescent isoindoles. Twenty microliters dialysate and 10 μl OPA derivating fluid were allowed to react for 1 min at room temperature. The HPLC employed buffer A: 0.1 m KH₂PO₄ buffer (adjusted to pH 6.6): methanol = 65:35, v/v; and buffer B: 0.1 m KH₂PO₄ buffer (adjusted to pH 6.6): methanol = 10:90, v/v. Buffer A was ultrasonically degassed, buffer B was filtered and degassed through a 0.2 μm nitrocellulose membrane. The above two-buffer HPLC system (Shimadzu-10AVP, Japan) was coupled to a fluorescent detector (RF-10AXL, Shimadzu, Japan). Separation was achieved on a C18 column (Hypersil, BDS, 5 μm). Twenty microliters of the reaction mixture was injected into the column and separated with a gradient from A: B (100:0) to 40% B within 12 min; then eluted with 100% B 5 min to elute other components. The flow rate was set to 1 ml/min. Excitation wavelength: 357 nm; Emission wavelength: 455 nm.

Statistical Analysis All results were expressed as mean±S.E.M. Statistical comparisons between different treatments were performed by one-way ANOVA with Dunnett’s multiple comparison post test. Differences with p value less than 0.05 were considered statistically significant.

RESULTS

There were no significant differences in mean arterial blood pressure, arterial blood gases, glucose, or Hb over time (data not shown). Body temperature was maintained at approximately 38 °C in all the groups. Basal levels of none of the amino acids differed significantly among groups. Moreover, neither the values of glutamate nor those of GABA in the sham group had altered through the ischemic/reperfused periods. For glutamate and GABA, basal concentrations of dialysate were in the 2.5 μM and 0.5 μM respectively. As shown in Fig. 1, during ischemia and early reperfusion glutamate concentrations in the vehicle group peaked almost eleven times higher than the initial values, and glutamate continued to rise within the first 20 min after reperfusion and declined thereafter. However, administration of gastrodin (100 mg/kg) before ischemia significantly reduced the ischemia-induced elevation of glutamate levels during the reperfusion period, with peak values during ischemia were only 7.4 times higher than the initial value. Similar changes in glutamate concentrations were observed in the gastrodin 50 mg/kg group. It was demonstrated that GABA increased 4 times during ischemia and declined after reperfusion (Fig. 2). Compared to the vehicle group, 50 mg/kg gastrodin increased the GABA levels slightly during the ischemic period. But there was a significant increase in the GABA levels during reperfusion in the rats of the 100 mg/kg gastrodin groups.

The glutamate/GABA ratios at the baseline were approximately 5 in preischemic conditions (Fig. 3). In the ischemic period, glutamate/GABA ratios of the vehicle group significantly increased by about 2—3 fold, then declined modestly after reperfusion. Compared with those of the vehicle group, there were statistically significant changes in glutamate/GABA ratios occurred in the gastrodin groups. The ratios in gastrodin groups peaked maximally to a lesser extent and decreased gradually after 20 min of reperfusion. During late ischemia and reperfusion periods the ratios were significantly lower than those in the vehicle group.

Fig. 1. Time Course of Cerebral Ischemia/Reperfusion, Glutamate Concentrations before and after Induction of Focal Ischemia

All the values are expressed as mean±S.E.M. n=6 rats per group. Onset of ischemia is designated as time zero. Solid bar represents a 50-min ischemic episode. *p<0.05, gastrodin (100 mg/kg, H-Gas or 50 mg/kg, L-Gas) vs. vehicle group; **p<0.01 sham group vs. vehicle group.
rate resulting from ischemia in mice. Therefore, the neuroprotective effects of gastrodin during transient focal cerebral ischemia may be partly due to its ability for attenuating extracellular glutamate accumulation.

In addition to glutamate, a large variety of other neurotransmitters and neuroactive substances are also released in brain tissue after ischemia. Of special relevance is the major inhibitory neurotransmitter in mammalian brain, i.e. GABA. Neuroprotection can be achieved with GABAergic drugs acting by various mechanisms: GABA agonists, GABA modulators, GABA transporter inhibitors and GABA transaminase inhibitors. Schwartz et al. found that diazepam microinjected directly into the hippocampus showed neuroprotective effect. Green et al. reported that the GABA modulator chlormethiazole reduced cerebral cortical and striatal infarct size in rats and marmosets when given 1 h after MCAO. The present study showed that gastrodin (100 mg/kg) slowed down declines of the GABA during the periods of reperfusion. This is in accordance with results of a previous study, which demonstrated that gastrodin could reduce immunoreactivities of GABA shunt enzymes in S. gerdhils and thus might cause the elevation of GABA concentration. Based on this, we propose that gastrodin could produce neuroprotection as a GABA transporter inhibitor when administered pre-ischemia. However, it is not clear that whether gastrodin modulates the activities of those enzymes responsible for GABA metabolism, GAD (the predominant pathway of GABA synthesis) or GABA-T (the predominant pathway of GABA degradation) in animals subjected to brain ischemia. Moreover, whether the attenuation of the rise in extracellular glutamate by gastrodin correlates to its effect on GABA remains to be tested.

Although excitotoxic neuronal injury is mediated by multifarious mechanisms, a model that has been previously proposed involves an imbalance in excitatory versus inhibitory transmission. Hence, differences in glutamate versus GABA may be a useful correlate for ischemic excitotoxicity. Using a simplified model of excitatory versus inhibitory imbalance (i.e. glutamate versus GABA), the present data show that there is significant imbalance in the ischemia and early reperfusion period. This finding may be consistent with other studies that significant increases in the excitotoxic index at recirculation as well as ischemia times were found in the striatum. Therefore, in the ischemic stage, glutamate/GABA imbalances can account for the critical collapse of neurotransmitters in the hippocampus. In the present study, however, preschermic administration of gastrodin showed that moderate reductions (60—70% of the vehicle level) were maintained throughout ischemia and reperfusion in the experiment. The results suggest that gastrodin may significantly alter the imbalance of neurotransmitters and then ameliorate the excitotoxicity resulting from brain ischemia.

In conclusion, this study demonstrates different temporal dynamics of extracellular glutamate and GABA in hippocampus of rats during ischemia/reperfusion when gastrodin was administrated prior to ischemic stimuli. However, to explore the specific mechanism of gastrodin and to develop it as an antistroke substance, further studies are needed.

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