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Efflux transport of [^{3}H]GABA across blood-brain barrier after cerebral ischemia-reperfusion in rats

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KEY WORDS GABA; blood-brain barrier; cerebral ischemia; reperfusion injury; probenecid; Evans blue; parietal lobe; microinjection

ABSTRACT

AIM: To study whether the efflux transport of [^{3}H]GABA across the blood-brain barrier (BBB) would be enhanced after cerebral ischemia-reperfusion. **METHODS**: Brain efflux index (BEI) of [^{3}H]GABA was determined in ischemic-reperfused rats after [^{3}H]GABA or [^{3}H]GABA combined with unlabeled GABA or probenecid (Pro) was microinjected into the parietal cortex area 2 (Par 2), and brain uptake of Evans blue (EB) was assessed after iv EB. **RESULTS**: BEI in rats subjected to 10-min ischemia and 30-min, 2-h, 6-h, or 24-h reperfusion were 67 %, 83 %, 92 %, and 87 %, respectively, which were higher than that in control (58 %). The brain uptake of EB was also considerably increased. Unlabeled GABA or Pro obviously decreased BEI in normal or 6-h reperfused rats, but GABA had no obvious effect on that in 5-min reperfused rats. **CONCLUSION**: The efflux transport of [^{3}H]GABA was markedly enhanced after cerebral ischemia-reperfusion in rats.

INTRODUCTION

Cerebral ischemia induces delayed neuronal death (DND), but the mechanism remains to be elucidated. GABA is a main inhibitory neurotransmitter in mammal CNS. Delayed transneuronal degeneration may be produced by neuronal disinhibition consequent to loss of inhibitory input[1]. Some agents associated with the GABAergic system are neuroprotective in cerebral ischemia[2,3]. Thus, DND may be, at least in part, relevant to the result of loss of GABA. GABA is largely confined to the CNS and it does not penetrate the normal BBB with ease. However, permeability of the BBB is increased after cerebral ischemia-reperfusion. Will GABA be lost from brain by penetrating the abnormal BBB to circulating blood after the ischemic insult? What is the mechanism? The present study aimed at these, which will demonstrate one of the ways through which GABA is lost after the insult and help us to understand the mechanism of DND.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (Certificate No 97004), ♂, weighing 250 - 350 g, were bred by Center of Experimental Animals, China Pharmaceutical University.

Chemicals [4,4-^{3}H]GABA (specific activity, 1.11 PBq·mol^{-1}) was purchased from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. GABA, Evans blue (EB), and HEPES were purchased from Shanghai Branch of Sino-American Biotechnology Co. Probenecid (Pro) was obtained from Sigma. All other chemicals were of analytic grade.

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Rat forebrain ischemia-reperfusion Rats were anesthetized with pentobarbital sodium (40 mg·kg^{-1}, ip). After iv heparin 300 u, the rats were subjected to bilateral common carotid artery occlusion combined with hypotension (6.65 kPa) to induce cerebral ischemia 10 min, followed by reperfusion for 5 min, 30 min, 2 h, 6 h, or 24 h[4].

Effects of cerebral ischemia-reperfusion on BEI of [^{3}H]GABA and brain uptake of EB Rats were divided into 5 groups: ① control (sham); and cerebral ischemia 10 min followed by reperfusion ② 30 min, ③ 2 h, ④ 6 h, and ⑤ 24 h. The rats were injected iv 2 % EB 50 mg·kg^{-1} and then subjected to intracerebral microinjection of [^{3}H]GABA 1 μL (18.5 kBq) (dissolved in physiologic buffer containing NaCl 122, $NaHCO_3$ 25; *D*-glucose 10, KCl 3, $CaCl_2$ 1.4, $MgSO_4$ 1.2, KH_2PO_4 0.4, HEPES 10 mmol·L^{-1}) at the Par 2 (0.2 mm anterior and 5.5 mm lateral to the bregma, and 4.5 mm deep)[5] 30 min and 5 min respectively before the indicated times of the groups. The rats were decapitated when the planned times of reperfusion were up. The left and right cerebral hemispheres were excised. The injected cerebral hemispheres were digested in formic acid 5 mL at 80 ℃ for 3 h. H_2O_2 5 mL was added at 20 – 25 ℃. The solution 1 mL was used to determine radioactivity with a Beckman LS5000TD Liquid Scintillation Counter and BEI was calculated by the formula[5]: BEI (%) = {[drug injected into the brain (Bq)] – [drug retained in the brain (Bq)]}/[drug injected into the brain (Bq)] × 100.

The contralateral cerebral hemispheres were used to determine the brain uptake of EB with the spectrophotometric method to represent the permeability of the BBB. The results were expressed as EB μg·g^{-1} (wet brain weight)[6].

Effects of unlabeled GABA or Pro on BEI of [^{3}H]GABA Rats in normal or 10-min ischemic/5-min or 6-h reperfused groups were subjected to intracerebral injection of physiologic buffer 50 μL or unlabeled GABA 0.2 or Pro 1.4 mmol·L^{-1} (dissolved in the buffer) at the Par 2 30 s before microinjection of 1 μL [^{3}H]GABA (18.5 kBq) at the same location[5]. The rats were decapitated 5 min after [^{3}H]GABA was injected. The measurement of the BEI of [^{3}H]GABA was carried out.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and evaluated by *t*-test.

RESULTS

Effects of cerebral ischemia-reperfusion on BEI of [^{3}H]GABA and brain uptake of EB Rats subjected to 10-min cerebral ischemia followed by reperfusion for 30 min, 2 h, 6 h, or 24 h had much higher BEI of [^{3}H]GABA than control rats, especially in 6-h reperfusion group ($P < 0.01$). The brain uptake of EB was also increased after cerebral ischemia-reperfusion ($P < 0.01$) (Tab 1).

Tab 1. BEI of [^{3}H]GABA 5 min after intracerebral microinjection of [^{3}H]GABA and brain uptake of EB 30 min after iv EB in ischemic-reperfused rats. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* control.

Group	*n*	BEI/%	EB/μg·g^{-1} (wet brain)
Control	5	58 ± 5	11.8 ± 1.5
10-min ischemia			
30-min reperfusion	6	67 ± 6^b	17.8 ± 1.5^b
2-h reperfusion	5	83 ± 3^c	25.9 ± 0.8^c
6-h reperfusion	6	91 ± 1^c	32.3 ± 1.2^c
24-h reperfusion	6	87 ± 3^c	28.1 ± 1.8^c

Effects of unlabeled GABA or Pro on BEI of [^{3}H]GABA GABA 0.2 mmol·L^{-1} or Pro 1.4 mmol·L^{-1} reduced the BEI of [^{3}H]GABA in normal or 6-h reperfused rats ($P < 0.01$). In contrast, GABA 0.2 mmol·L^{-1} had no obvious effect on the BEI in 5-min

reperfused rats ($P > 0.05$, Tab 2).

Tab 2. BEI of [^{3}H]GABA 5 min after intracerebral microinjection of [^{3}H]GABA plus GABA or Pro in rats. $\bar{x} \pm s$. $^aP > 0.05$ *vs* ④. $^fP < 0.01$ *vs* ①. $^iP < 0.01$ *vs* ⑥.

Group	*n*	BEI/%
Normal		
①Buffer + [^{3}H]GABA	6	55 ± 2
②Pro + [^{3}H]GABA	6	37 ± 13^f
③GABA + [^{3}H]GABA	6	34 ± 13^f
10-min ischemia		
5-min reperfusion		
④ Buffer + [^{3}H]GABA	5	61 ± 2
⑤ GABA + [^{3}H]GABA	5	53 ± 8^a
6-h reperfusion		
⑥ Buffer + [^{3}H]GABA	6	91 ± 2
⑦ GABA + [^{3}H]GABA	6	62 ± 7^i

DISCUSSION

The present study showed that rats subjected to ischemia followed by 30-min – 24-h reperfusion had much higher BEI of [^{3}H]GABA than rats in control group, indicating the enhanced efflux transport of [^{3}H]GABA after cerebral ischemia-reperfusion. It has been known that DND occurred 2 to 4 d after reperfusion. It is difficult to understand the mechanism. The present results suggested that the loss of GABA from brain because of enhanced efflux transport of GABA after cerebral ischemia-reperfusion might play a pathogenic role in DND.

The brain uptake of EB was also significantly increased, which indicated that permeability of the BBB was increased after the insult. The time-course of the changes in brain uptake of EB was almost parallel to that in BEI of [^{3}H]GABA. Thus, the enhanced efflux transport of [^{3}H]GABA may be related to the increased permeability of the BBB to a certain extent.

In normal rats, unlabeled GABA reduced the BEI of [^{3}H]GABA significantly. It suggested that there might be specific efflux transporters of GABA at the BBB. It was also found that Pro reduced the BEI of [^{3}H]GABA. It has been known that there are Pro-sensitive efflux transporters at the BBB, which mediate the efflux transport of many kinds of organic anion and cation from brain[7,8]. However, it is not clear whether the specific or Pro-sensitive efflux transporters here are just the same one. Thus, under normal conditions the efflux transport of [^{3}H]GABA may be mediated by the specific and/or Pro-sensitive efflux transporters. This may explain the reason why [^{3}H]GABA is relatively easy to penetrate the normal BBB to circulating blood with a BEI 58 % in 5 min.

Efflux transport of [^{3}H]GABA across the BBB after the ischemic insult may involve saturable (transporter) or/and non-saturable (most likely, diffusion via pores) mechanism. The present results showed that unlabeled GABA had no obvious effect on the BEI of [^{3}H]GABA in rats subjected to ischemia/5-min reperfusion, which suggested that non-saturable mechanism might play an important role in the efflux transport of [^{3}H]GABA at the moment. Suzuki *et al*[9] has shown that cerebral ischemia decreased the affinity of the BBB glucose transporters to glucose, suggesting the down-regulated glucose transporters. The efflux transporters of GABA here may be also down-regulated after the ischemic insult followed by the limited reperfusion period. In contrast, unlabeled GABA significantly reduced the BEI of [^{3}H]GABA in rats subjected to ischemia/6-h reperfusion. It suggested that saturable mechanism might play a part in the efflux transport of [^{3}H]GABA after the ischemic insult followed by the relatively long reperfusion period. These results are reminiscent of the investigation of Hauptman *et al*[10], which has demonstrated that *in vitro* anoxia caused a decrease in the uptake function of GABA transporters in synaptosomes, however, reintroduction of oxygen recoverd the

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function. In addition, permeability of the BBB was greatly increased after 6-h reperfusion. Thus, the efflux transport of [^{3}H]GABA may involve both saturable and non-saturable mechanisms at the moment, which may account for the highest BEI of [^{3}H]GABA.

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脑缺血再灌注后[^{3}H]GABA 通过大鼠血脑屏障的外排转运

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关键词 γ-氨基丁酸; 血脑屏障; 脑缺血; 再灌注损伤; 丙磺舒; 伊文思蓝; 顶叶; 微量注射

目的: 研究脑缺血/再灌后[^{3}H]GABA 通过大鼠血脑屏障的外排转运是否增强及其机制. **方法**: 将[^{3}H]GABA 或 GABA(或丙磺舒)与其联合注射到缺血/再灌大鼠大脑皮层顶二区后, 测定[^{3}H]GABA 的脑外排指数(BEI)及 iv 依文思蓝(EB)后 EB 的脑摄取量. **结果**: 10 min 缺血/再灌 30 min、2 h、6 h 和 24 h 大鼠的 BEI 分别为 67 %, 83 %, 92 %和 87 %, 显著高于对照值(58 %). EB 脑摄取量也显著增加; GABA 或丙磺舒明显降低正常及再灌 6 h 大鼠的 BEI, 但对再灌 5 min 大鼠的 BEI 无明显影响. **结论**: 大鼠脑缺血/再灌后[^{3}H]GABA 通过血脑屏障的外排转运显著增强.

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