Brain Research Bulletin 124 (2016) 190–197

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

$GABA_B$ receptors in the hippocampal dentate gyrus are involved in spatial learning and memory impairment in a rat model of vascular dementia

Guangxie Li[a,b,1], Jing Lv[a,1], Jun Wang[a], Peng Wan[c], Yingshun Li[a], Haiying Jiang[a,*], Qinghua Jin[a,*]

[a] Department of Physiology and Pathophysiology, Yanbian University College of Medicine and Cellular Function Research Center, Yanbian University, Yanji 133002, China
[b] Department of Surgery, Yanbian University Hospital, Yanji 133000, China
[c] Department of Physiology, Jilin Medical University, Jilin 132013, China

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 20 April 2016
Accepted 11 May 2016
Available online 12 May 2016

Keywords:
Vascular dementia
Hippocampal dentate gyrus
GABA
Spatial learning and memory
$GABA_B$ receptors
Rats

ABSTRACT

The roles of γ-aminobutyric acid (GABA) and $GABA_B$ receptors of the hippocampal dentate gyrus (DG) in spatial learning and memory impairment were investigated in a rat model of vascular dementia (VaD) established by permanent bilateral carotid occlusion. The extracellular concentration of GABA in the DG was determined by *in vivo* microdialysis and HPLC, and spatial learning and memory were assessed by the Morris water maze (MWM) test. Next, the possible involvement of $GABA_B$ receptors in spatial learning and memory impairments of VaD rats was examined by microinjection of its antagonist into the DG region. In VaD group rats, the extracellular concentration of GABA in the DG was significantly increased, and during MWM test, the escape latency was increased in place navigation trial and the percentage of time spent in target quadrant and the number of platform crossings were decreased in spatial probe trial, compared with the sham group. In sham-operated rats, the extracellular concentrations of glutamate (Glu) and glycine (Gly) in the DG were significantly increased during place navigation trial of MWM test, and these responses were inhibited in VaD rats. Saclofen (an antagonist of $GABA_B$ receptor) significantly attenuated the spatial learning and memory impairment in VaD rats, and partly reversed the inhibitory effects of VaD in responses of Glu and Gly in the DG during MWM test. Our results suggest that GABA and $GABA_B$ receptors in the hippocampal DG are involved in spatial learning and memory impairment in VaD rats, in part by attenuating the responses of Glu and Gly during spatial learning.

1. Introduction

Vascular dementia (VaD) is generally recognized to be the second most common cause of dementia after Alzheimer's disease (Battistin and Cagnin, 2010). The core symptom of VaD is progressive cognitive dysfunction due to cumulative regional brain tissue injury caused by localized cerebrovascular disruptions (Brundel et al., 2012). The prevention and treatment of VaD are a major medical and social priority in the worldwide aging population. Therefore, elucidating the pathophysiological mechanism responsible for cognitive dysfunction in VaD is necessary.

VaD-associated cognitive dysfunction includes spatial learning and memory deficits (Rockwood, 2002; Tashakori-Sabzevar et al., 2013). Hippocampus is a key structure involved in learning and memory processes, particularly in spatial learning and memory (Aggleton et al., 2000). The mammalian hippocampus is mainly divided into the CA1, CA3, and the dentate gyrus (DG) (Okada and Okaichi, 2009), and each subregion exhibits differing functions in spatial learning and memory (Kesner et al., 2004). Spatial reference and working memory tasks reveal that the formation of spatial representation is impaired in rats with DG lesions (Jeltsch et al., 2001). These results indicate that the DG encodes and processes spatial information upon its entry into the hippocampus, thereby playing an essential role in spatial learning and memory. Focal cerebral ischemia in adult mammals stimulates neuronal regeneration

* Corresponding authors at: Yanbian University, 977 GongYuan Road, Yanji 133002, China.
E-mail addresses: hyjiang@ybu.edu.cn (H. Jiang), yqinghua@ybu.edu.cn, qinghuajin@163.com (Q. Jin).
[1] These authors contributed to the work equally and should be regarded as co-first authors.

http://dx.doi.org/10.1016/j.brainresbull.2016.05.006
0361-9230/

in the DG, thus repairing the structural and functional damage induced by ischemia and improving hippocampal-dependent learning and memory (Epp et al., 2011). Although these reports suggest that the DG plays an important role in spatial learning and memory deficits in VaD, the chemical mediators in the DG responsible for these deficits are incompletely understood.

γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system (CNS) acting at ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors (Bettler et al., 2004). Numerous GABAergic neurons are located in the DG, and $GABA_A$ and $GABA_B$ receptors in this region regulate synaptic plasticity and learning and memory processes (Shahidi et al., 2008). However, the role of the DG-containing GABA and its receptors in spatial learning and memory impairments in VaD has not been reported. It is worth mentioning that, in our pre-experiment, the number of $GABA_B$ receptor-positive cells in the hippcamal DG was decreased in VaD group rats compared to the sham group, whereas the number of $GABA_A$ receptor-positive cells in the DG did not change. Accordingly, this study mainly focuses on the role of $GABA_B$ receptors in spatial learning and memory impairment of VaD. In the present study, we established a rat model of VaD via permanent bilateral carotid occlusion (2-VO), and used in vivo brain microdialysis and HPLC techniques to determine GABA release in the DG. $GABA_B$ receptors antagonist was then used in the DG to examine its effects on spatial learning and memory deficits in VaD rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–300 g) were provided by the Experimental Animal Department of Yanbian University. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and the ethical regulations of Yanbian University. All efforts were made to minimize animal suffering and the number of animals killed.

2.2. Preparation of animal VaD model

The VaD model was prepared via 2-VO. Following 12 h of fasting and 4 h without water, rats were anesthetized with 10% chloral hydrate (300 mg/kg, i.p.) and were placed in the supine position on a rat board. An incision was made along the midline of the neck followed by careful blunt dissection of muscle and connective tissues. The left common carotid artery was isolated and double ligated with silk sutures. The skin and muscle layers were sutured and gentamicin (80,000 units) was applied to the surgical wound by topical spray. The right common carotid artery was also ligated, in the same way, after 7days. The sham-operated rats received the same two-step operation procedure without ligatures. During 4 weeks of recovery, the rats were housed and fed in single cages to prevent infection.

2.3. Experimental procedures

Rats were anesthetized with 10% chloral hydrate (300 mg/kg, i.p.) and placed on a stereotaxic frame (David Kopf, USA). A guide cannula was stereotaxically implanted 1.0 mm above the DG region (coordinates: 3.2 mm posterior to the bregma, 1.6 mm lateral to the midline, and 2.5 mm ventral to the dural surface), and fixed to the skull by dental cement. The animals were individually housed with access to food and water and allowed to recover from surgery for 2 days.

One day before the experiment, the rats were anesthetized using ethyl ether and a microdialysis probe was inserted through the guide cannula into the DG region and stabilized with wax. To reach the DG region, the microdialysis probe was inserted 1.5 mm beyond the guide shaft, and the tip of the probe was covered with a 1.5-mm length of acetate cellulose membrane (o.d., 0.2 mm, cutoff, 5.0×10^4 mol wt; DM-22, Eicom, Japan). The microinjection tube (OD, 0.1 mm) was adhered to the microdialysis probe.

On the day of experiments, the collection of dialysates from the DG region and the behavioral test were carried out under freely-moving conditions. The microdialysis probe was perfused with modified Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl2; pH 6.5) at 1.5 μL/min using a microinfusion pump (ESP-64, Eicom, Japan). After a 60-min stabilization period, the dialysate from the DG region was automatically collected by a fraction collector (EFC-82, Eicom, Japan) every 10 min at 4 °C, and three consecutive dialysate samples were collected to measure the concentrations of amino acids, including GABA, Glu and Gly.

At the end of each experiment, the rats were sacrificed under overdose injection of chloral hydrate, and the brains were fixed in 10% neutral buffer formalin. The implantation site of the dialysis probe was verified histologically in 40 μm coronal sections after cresyl violet staining.

2.4. Measure of amino acids' levels

Concentrations of amino acids (including GABA, Glu and Gly) were measured by HPLC and electrochemical detection (ECD) system (HTEC-500, Eicom, Japan). Before applying the HTEC-500, an 4 mM *o*-phthalaldehyde (OPA) solution was made by adding 1.35 mg of OPA and 1 μL of 2-mercaptoethanol to 2.5 mL of 0.1 M K_2CO_3 buffer (pH 9.5) with 10% ethanol. 12 μL of the standard solution (including 2 μM corresponding amino acid) or the dialysate was mixed with 3 μL of 4 mM OPA solution and allowed to react for 2.5 min at 25 °C incubation. After completing the reaction, 10 μL of the reaction mixture was applied to the SC-5ODS column (2.1 mm ID × 150 mm) in HTEC-500. Detection was accomplished with +600 mV Ag/AgCl electrodes. The elution buffer for Glu and Gly consisted of 0.1 M phosphate buffer, 30% methanol, and 0.5 mM EDTA (pH 6.5), and that for GABA consisted of 0.1 M phosphate buffer, 50% methanol, and 0.5 mM EDTA (pH 3.5). The wave of amino acid in chromatograms was identified by retention time of that in standard solution, and the concentration of amino acid was calculated according to the area of the amino acid wave in standard solution.

2.5. Behavioral tests

Spatial learning and memory abilities of rats were assessed by the Morris water maze (MWM) task, which included the place navigation trial and spatial probe trial. In the place navigation trial, rats were placed in the water facing the pool wall at a fixed location. The time spent finding and standing on the platform (with all limbs attached) was considered as the escape latency. However, if a rat was unable to find the platform within 120 s, it was led to the platform where it stayed for 15 s, and the escape latency was recorded as 120 s. After 15 s on the platform, rats were sent back to the home cages. Training for each rat was conducted once per day, for four consecutive days. The spatial probe trial was performed on the 5th day of the MWM test. Circular platforms were removed from the pool, and animals were placed in the water at a given location to swim freely for 120s. During the probe trial, the proportion of total time spent in each quadrant and the number of platform crossings were recorded.