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Bombesin attenuated ischemia-induced spatial cognitive and synaptic plasticity impairment associated with oxidative damage

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ABSTRACT

The dysfunction of spatial cognition is a character to various neurological disorders and therapeutic strategy. However, it is limited to known risk factors clinically so far. Gastrin releasing peptide (GRP) signaling is a neuropeptide system mediating emotional memory events. However, the effects of GRP agonist on spatial cognition and hippocampal synaptic plasticity are rarely investigated, especially in pathologic condition. This study was designed to investigate the long-term effects of GRPR agonist, bombesin, against cognitive impairment induced by chronic cerebral ischemia in rats and its possible mechanisms. Our results revealed that bombesin administration (30 μg/kg/day, for 14 continuous days) significantly protected the cognitive and synaptic plasticity impairments as assessed by the Morris water maze and long-term potentiation tests. The mechanism studies demonstrated that bombesin significantly alleviated the decreased activity of total superoxide dismutase (T-SOD), catalase (CAT) and altered the increased the content of malondialdehyde (MDA). Besides, the decreased expression of synapse plasticity-related proteins, calcium- calmodulin- dependent protein kinase II (CaMKII) and synaptophysin (SYP) in the hippocampus were increased with drug treatment. In conclusion, bombesin could protect the oxidative stress and expression of proteins, which were important for synaptic plasticity and cognitive function impairment induced by chronic cerebral ischemia. Our study is presented to provide novel insights into the effects of bombesin on spatial learning and memory, which should be further explored as a potential drug in disorders involving deficits in cognitive function.

1. Introduction

The neurological disorders lead directly to physiological, molecular and synaptic modifications in neuronal networks, which in turn affect learning and memory [1]. These disorders can affect various neuromodulation systems, in which neuropeptide systems have been widely recognized as important ones in the modulation of the memory formation, but rarely been investigated [2,3]. Bombesin is a 14 amino acid-containing peptide first isolated from the skin of the frog *Bombina bombina*. Later on, two related mammalian neuropeptides, neuromedin B (NMB) and gastrin-releasing peptide (GRP), which are considered as bombesin-like peptides (BLPs), have been isolated and shown to have a widespread distribution[4,5]. As brain-gut peptides, BLPs play important roles not only in food intake but also in regulating a range of brain function through bombesin receptors (BB1/NMBR or BB2/GRPR) [3,4].

In the central nervous system (CNS), GRPR expression has been characterized in the hippocampus, hypothalamus, amygdala and cortex [6,7]. Increasing evidences indicate that GRPR agonists can prevent the emotional memory impairments in many neurological disorders, such as ischemia, AD or epileptic models [8–10]. But there are still some conflicting results, which showed that they could lead to impairments [11] or no effects at all [8]. Furthermore, few researches focus on the effects of BLPs on spatial learning and memory, hippocampal synaptic plasticity [12], especially on vascular dementia (VD) which has become the second most common causes of cognitive impairment in the elderly population [13]. Therefore, in this study, to test our hypothesis that bombesin could prevent cognitive deficits and synaptic plasticity impairment on a VD model induced by long-term cerebral hypoperfusion, Morris water maze (MWM) test, long-term potentiation (LTP) recording and the expression of synaptic proteins were examined.

In addition, we explore the potential mechanism responsible for the neuroprotective effects of bombesin. Previous studies have reported that bombesin administration protected the jaundiced or colitis model

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rats against oxidative stress, as demonstrated by reduction of intestinal lipid peroxidation, increase of the antioxidant GSH, and additionally reduced protein oxidation [14,15]. Moreover, in the CNS, abnormal changes of reactive oxygen species (ROS) are considered as one of the causal factors in many neurological diseases, including VD [16–18]. Thus, the level of total superoxide dismutase (T-SOD), catalase (CAT) and malondialdehyde (MDA) were examined in hippocampus, which is particularly sensitive to oxidative stress [19] and plays crucial roles in spatial learning and memory. The present study would help to expand therapeutic opportunities for the cognitive impairment.

2. Materials and methods

2.1. Animal model and drug application

We obtained 24 male Wistar rats (6 weeks old, 250 ± 10 g) from the Chinese Academy of Medical Sciences. All experimental protocols were approved by the Committee for Animal Care at Nankai University and were in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals.

Rats were randomly divided into three groups: Sham group (n = 8), 2-VO group (n = 8) and 2-VO + BB group (n = 8). Sustained global cerebral ischemia was induced by permanent bilateral occlusion of the carotid arteries (2-VO) as previously described [20]. At 72 h after surgery, the survival rate of the surgery was about 80%. Rats in the 2-VO + BB group were administered intraperitoneally with bombesin (1 m l, 30 μg/kg/day, purchased from Phoenix Pharmaceuticals, Inc), once a day for 14 continuous days starting from day 15 post-operation. We choose the dose 30 μg/kg/day according to some previous studies, in which bombesin was administered (i.p.) 10 μg/kg (three times a day) [21]or 20–80 μg/kg/day [22,23] ranging from 1 to 3 weeks [24]. The Sham and 2-VO groups were administered intraperitoneally the same volume of dimethyl sulfoxide.

2.2. Morris water maze experiment

Rats were subjected to the MWM test to evaluate spatial learning performance thirty days after the surgery, as described previously [25–27]. The water maze was a 1.5-miter-diameter circular tank divided into four equal imaginary quadrants (I–IV, Fig. 1). The task involve two stages: training and memory test stages. In training period, each rat received two sessions of eight trials every day for 5 days. In each session, subsequent starting positions proceeded in a clockwise manner in trials and rats were located in the same position on each trial at one of four starting quadrant points. Rats were released into the water and allowed to swim freely until they were able to reach and stay on platform. If they failed to locate the platform within 60 s, they were handpicked and placed on it for 10 s. Subsequently, escape latency (the time required to find the platform) and the swimming speed were recorded. The memory test was performed on the sixth day with the platform removed. The rats were released individually into water from quadrant I and allowed to swim for 60 s. Quadrant dwell time (the percentage of time spent in quadrant III) was measured. The swimming traces were collected and analyzed using Ethovision 2.0 software (Noldus, Wagenigen, Netherlands).

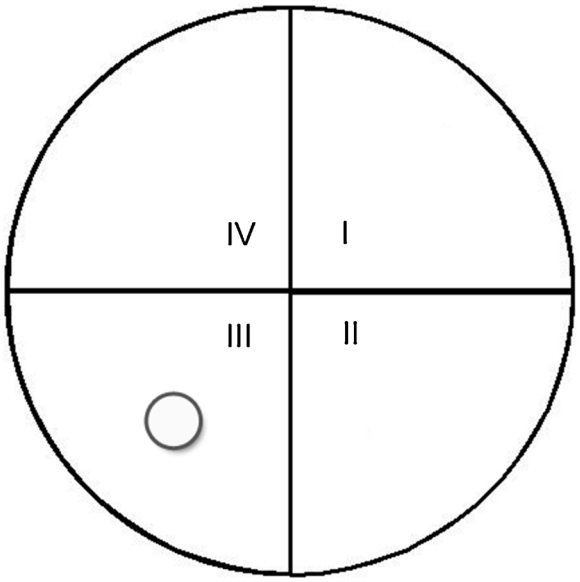

Fig. 1. Scheme of the water maze tank, which shows four quadrants and a circular platform area.

2.3. LTP recording

Synaptic plasticity was examined by long-term potentiation (LTP) recording after the MWM test. As described previously [25–27], the rats were anaesthetized with 30% urethane (1.5 g/kg). The heads were fixed in a stereotaxic apparatus (Narishige, Japan). After skin and skull removal, the tip of the recording electrode was positioned in the stratum radiatum of area CA1 (3.4 mm posterior and 2.5 mm lateral to bregma), and the tip of stimulating electrode was inserted into Schaffer collaterals region (4.2 mm posterior to bregma and 3.8 mm right of the midline). Both the stimulating and recording electrodes were connected to a stimulator and an amplifier (AD instruments Ltd., Australia). The extra-cellular field excitatory post-synaptic potentials (fEPSPs) were evoked by stimulating with a square-wave constant current pulse (0.2–0.4 mA) of 50 ms duration. After the base line was collected, high-frequency stimulus (HFS) was given and the value of slope of fEPSPs was measured every 2 min for 1 h.

2.4. Western blot assay

After the LTP recording, each rat was immediately sacrificed. They were then perfused with 0.1 M phosphate buffer saline (PBS, pH = 7.4) and their hippocampus were removed and stored at −80 °C. For western blot assay, both hippocampi were homogenized in lysis buffer (Beyotime Biotechnology, Haimen, China) and centrifuged at 12,000 rpm for 15 min at 4 °C. The concentration of hippocampal protein was measured by using enhanced BCA protein assay kit (Beyotime Biotechnology, Haimen, China). Then 50-μg proteins from each rat were loaded and separated by SDS-PAGE gel electrophoresis as described previously [26,28]. The primary antibodies were anti-SYP (1:1000), anti-CaMKII (1:1000), anti-NR2B and anti-β-actin (1:2000). Protein band intensities were analyzed by using a Western blot detection system. And the quantitative analysis was performed by Photoshop CS6 and compared to β- actin.

2.5. Measurement of oxidative parameters in the hippocampus

For measuring the levels of total malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) activities in hippocampus, the proteins obtained above were not boiled. And then they were evaluated according to the assay kits (Beyotime Biotechnology, China). Our previous study described this procedure in detail [29].

2.6. Statistical analysis

All data were analyzed by SPSS16.0 software and presented as mean ± S.E.M. Two-way repeated measures ANOVA was applied for analysis of escape latencies and swimming speeds in MWM test. One-way ANOVA was performed to determine the statistical significance of the differences in time in platform zone, EPSP slope, expression of proteins and levels of oxidative parameters. Differences were