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L-3-*n*-butylphthalide improves cognitive deficits in rats with chronic cerebral ischemia

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ABSTRACT

3-*n*-Butylphthalide (NBP) has been shown to have protective effects against ischemic stroke. In the present study, we investigated effects of l-3-*n*-butylphthalide (l-NBP) on the learning and memory impairment induced by chronic cerebral ischemia in rats. Male Wistar rats were administered 20 mg/kg l-NBP by gavage daily for 30 days after the bilateral common carotid artery clamping (two-vessel occlusion, 2-VO). Results showed that daily treatments of 20 mg/kg l-NBP significantly attenuated spatial learning deficits in Morris water maze (MWM) task. Results of long-term potentiation (LTP) indicated that treatment with 20 mg/kg l-NBP attenuated the inhibition of LTP in rat model of 2-VO. Moreover, l-NBP reduced glial fibrillary acidic protein (GFAP)-positive astrocytes induced by chronic cerebral ischemia. The present findings demonstrate the protective effect of l-NBP on chronic cerebral ischemia-induced hippocampus injury, which supports using l-NBP for therapy of cerebral ischemia in the future.

1. Introduction

Senile dementia, a progressive aging-related disease, has become an important medical and social problem due to the increase in the number of elderly. Vascular dementia (VaD), as the second most common form of dementia in the elderly (Giacobini, 2004), has gained much attention in recent years. Repeated focal infarct is the most common reason of human VaD. With pathological changes in cardiovascular system, many old people suffer from repeated focal infarct and chronic cerebral ischemia, which may lead to dementia. VaD is characterized by a progressive cognitive and behavioral deterioration induced by loss of blood supply in various areas of the brain (Kalaria et al., 2004). Reduction in cerebral blood flow that arising in chronic cerebral ischemia can lead to selective neuronal injuries in vulnerable regions of the brain, especially the hippocampus (McBean and Kelly, 1998; Pulsinelli and Brierley, 1979). Spatial learning and memory are dependent on the integrity of the hippocampus. The hippocampal formation is centrally involved in the initial phase of memory retention processes. Thus this injury is accompanied by a progressive cognitive decline (Alagona et al., 2004). At present, no specific drug exists to prevent, delay, or cure VaD. Therefore, more and more studies have focused on finding new drugs to improve cognitive deficits caused by VaD.

Previous studies showed that 3-*n*-butylphthalide (NBP) had the ability to decrease the area of cerebral infarct in focal cerebral ischemic rats (Liu and Feng, 1995). It can also improve energy metabolism in mice with complete brain ischemia (Feng et al., 1995). NBP is a chiral compound, which contains both L- and D-isomers. Peng et al found that l-3-*n*-butylphthalide (l-NBP) attenuated learning and memory deficits induced by chronic cerebral hypoperfusion in rats (Peng et al., 2007). Moreover, l-NBP showed potent neuroprotective effects by decreasing oxidative damage (Dong and Feng, 2002), reducing neuronal apoptosis (Chang and Wang, 2003) and inhibiting inflammatory responses (Xu and Feng, 2000) in middle cerebral artery occlusion rat models. The positive effects of NBP and l-NBP on cerebral ischemia and cerebral infarct have been verified in ischemic patients and animal models, however little is known about the effect of l-NBP on chronic cerebral ischemia, especially the electrophysiological behavior. Therefore, we set out to examine the functions of l-NBP as well as to investigate whether l-NBP has the ability to protect rat brains from chronic cerebral ischemia.

To test the hypothesis, a chronic cerebral ischemia rat model-the bilateral common carotid artery clamping (two-vessel occlusion, 2-VO), which can reduce the blood flow in the brain to one-third of its normal value was set up (Farkas et al., 2007). As the hypoperfusion also affects the hippocampus (Todd et al., 1984), it

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may exert effects on various neuronal properties, including the neuronal cell viability or its electrophysiological behavior. The best-studied cellular model for hippocampal learning and memory is the detection of long-term potentiation (LTP), which is a long lasting increase in the synaptic transmission efficiency induced by the high frequency stimulation (Bliss and Lomo, 1973). The Morris water maze (MWM) test is a memory test based on the capacity of animals to rescue themselves by reaching a hidden goal platform in a pool of water (Morris et al., 1982). Animals with a damaged hippocampus display spatial navigation impairments and perform poorly in the MWM test (Morris et al., 1982).

In the present study, we investigated the effects of l-NBP on improving cognitive deficits in rat model induced by chronic cerebral ischemia. In brief, MWM test, LTP in hippocampus and brain histology analyses were used to evaluate the effects of l-NBP on rat brain function after permanent bilateral occlusion of the carotid arteries, which may provide an interesting view of the potential application of l-NBP for VaD therapy in the future.

2. Materials and methods

2.1. Chemicals and materials

l-NBP was provided by CSPC, the Institute of Pharmaceutical Research, Shijiazhuang, China. It was diluted with vegetable oil (Peng et al., 2007).

2.2. Animals

Male Wistar rats (270–300 g) were subjected to surgery. Animals were group-housed with free access to water and food in an established animal house having a 12 h light: 12 h darkness cycle and a thermo regulated environment. The animal care and experimental protocol were approved by the Ethical Commission at Nankai University, China.

2.3. Surgery

Rats were randomly divided into three groups: sham group, vehicle group and l-NBP group. Rats were anesthetized using 4% chloral-hydrate (intraperitoneal). The common carotid artery was isolated and double ligated with 5–0 silk suture in rats of vehicle group and l-NBP group. As sham-operated controls, rats of another group received the same operation without ligation.

2.4. Drug administration and experimental design

After 2-VO surgery, rats were randomly divided into two groups, both of which consisted of 10 animals with identical mean body weights. The daily administration of l-NBP (20 mg/kg) or vehicle (vegetable oil) by gavage started from the surgery day, and lasted for 30 days. Then spatial learning and memory were assessed in all rats. The experimental design is shown in Fig. 1.

2.5. MWM task

The day after the last time of drug treatments, all rats were trained and tested in MWM (RB-100A type, Beijing, China) to monitor their spatial learning and memory. The water maze consists of a large circular pool (150 cm in diameter, 60 cm in height, filled to a depth of 45 cm with water at 23 °C ± 1 °C). The water maze was divided into 4 equal quadrants (I–IV) by two imaginary perpendicular lines crossing in the center of the tank. There was a 10-cm diameter platform submerged 2 cm below the water surface in the center of quadrant III. The water was made opaque using nontoxic black ink. Each rat received two trials every day and the test lasted for 5 days. The escape latency (swimming time to locate the hidden platform) was used to assess performance of learning and memory of the animals. The retention of spatial memory was assessed on day 6 by the spatial probe test with the platform removed. The evaluator conducting the MWM was blinded to the treatment groups.

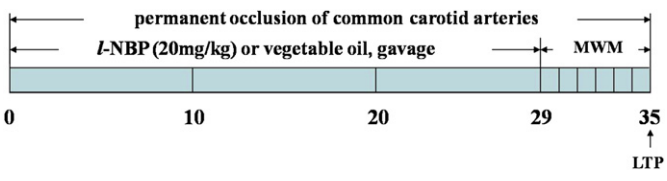

Fig. 1. Experimental design.

2.6. Electrophysiological recordings

The day after finishing MWM test, rats were given LTP test. Rats were anesthetized with 30% urethane (0.4 ml/kg), and then were placed in a stereotaxic frame (Narishige, Japan). Small holes were drilled in the skull for inserting stimulating and recording electrodes (Advent Co., UK). The tip of the recording electrode was positioned in the stratum radiatum of area CA1 (3.5 mm posterior to bregma and 2.5 mm lateral to the midline). The stimulating electrode was inserted into the CA3 region (4.2 mm posterior to bregma and 3.5 mm to the midline). The test stimuli were delivered to the CA3 region every 30 s at an intensity that evoked a response of 50% of its maximum. After every 20 s for 20 min stable baseline recording, a high frequency stimulation (HFS) consisted of 10 trains of 10 stimuli at 100 Hz with 2 s intertrain interval was given in three groups. The field excitatory postsynaptic potential (fEPSP) was then recorded at 40 kHz (Scope Software, Powlab, ADInstruments, Australia) every 20 s for 60 min. The fEPSP slope (20–80% level of the falling phase) was used to measure synaptic efficacy.

2.7. Histology

After the electrophysiology experiment, rats were deeply anesthetized and were perfused through the left cardiac ventricle with phosphate buffered sodium (PBS, pH 7.2) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The brain of each rat was removed and fixed by immersion in the same solution. Fixed tissues were embedded in paraffin. Brains were serially sectioned at 5 micron thick. Sections for hematoxylineosin (HE)-staining were placed onto uncoated slides. Sections intended for use in immunohistochemistry assays were placed onto coated slides (ZSJQ-Bio, China). Sections were routinely HE stained for histomorphological assessment. The experimenter evaluating the histology was blinded to the treatment group of the rats.

2.8. Immunohistochemical procedures for glial fibrillary acidic protein (GFAP)

Sections for immunoreactivity assays were placed onto coated slides (Zhongsan Goldenbridge Biotechonology, China). The labeled dextran polymer (LDP) immunohistochemistry was used to detect expressions of GFAP in CA1 subfield. The deparaffinized sections were boiled in citrate buffer in microwave oven for antigen retrieval. After that, peroxidase activity was inactivated by incubation with 3% H_2O_2 solution for 30 min at room temperature. Then the sections were incubated with rabbit polyclonal anti-GFAP antibody (diluted 1:100 ZSJQ-Bio, China) in a moist chamber at 4 °C overnight. Negative controls were conducted by exchange of primary antibody for PBS. Sections were then incubated with En Vision-Systems polymer-conjugated secondary antibody PV-9000 (GBI, USA), and finally incubated with diaminobenzidin (DAB) at room temperature for 3 min. The slides were washed twice in PBS between steps. Sections were observed and photographed using an OLYUMPUS YS100 microscope with a CCD camera (JVC, Japan). The experimenter evaluating the histology was blinded to the treatment group of the rats.

2.9. Statistics

All data were presented as mean ± SEM. Escape latencies in place navigation were compared using repeated measure ANOVA. Data for spatial probe and LTP recording were compared using one-way ANOVA. Significance levels were established at a level of $P < 0.05$. The analyses were performed using SPSS 16.0 software.

3. Results

3.1. MWM performance

In order to determine whether the rats' cognitive function was affected after the oral administration of l-NBP, the rats' abilities of spatial learning acquisition and memory retention were tested by using MWM test (Morris et al., 1982). In the MWM, animals from all groups became more efficient at locating the platform on successive trials. The escape latencies were progressively shorter in all groups in a day-dependent manner. Two-way repeated measures ANOVA conducted on the escape latency for three groups confirmed statistical difference of day ($F = 139.34$, $P < 0.001$) and group ($F = 7.598$, $P < 0.01$). There was no significant difference in day × group interaction ($F = 2.418$, $P = 0.065$). On day 1, group comparisons revealed that animals in the sham group displayed a shorter latency in finding the platform when compared with that of vehicle group (sham: 26.25 ± 1.58 s; vehicle: 44.27 ± 5.05 s, $P < 0.01$), and similarly on day 3 (sham: 7.52 ± 0.94 s; vehicle: 14.50 ± 2.26 s, $P < 0.05$), day 4 (sham: 5.84 ± 0.94 s; vehicle: