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Research paper

Effect of delta opioid receptor activation on spatial cognition and neurogenesis in cerebral ischemic rats

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HIGHLIGHTS

- DADLE enhances spatial cognition 3–4 weeks after global ischemia in rats.
- DADLE promotes cell proliferation in the hippocampus 7 days after ischemia.
- DADLE promotes cell differentiation in DG 28 days after ischemia.

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ABSTRACT

This study aimed to investigate whether a selective delta opioid receptor agonist, [D-Ala2, D-Leu5]-Enkephalin (DADLE), regulates neurogenesis in the hippocampus of ischemic rats. Using an intracerebral cannula, rats were subjected to cerebral ischemia using the standard four-vessel occlusion. DADLE (2.5 nmol), DADLE (2.5 nmol) with naltrindole (NAL) (2.5 nmol), or vehicle was administered at the onset of reperfusion. Bromodeoxyuridine (BrdU, 100 mg/kg, intraperitoneal) was used to label newly formed cells from days 1 to 7 after ischemia. Immunohistochemistry was used to evaluate cell proliferation and apoptosis and differentiation 7 days 28 days, respectively, after ischemia. Morris water maze test was conducted to test spatial learning and memory 23–27 days after ischemia. We found that DADLE treatment improved performance in the Morris water maze test, promoted proliferation and differentiation of newly formed neurons, and inhibited differentiation into astrocytes in a rat model of cerebral ischemia. Furthermore, the protective effects of DADLE were significantly reversed by co-administration of NAL ($P < 0.05$), a highly potent and selective delta opioid receptor antagonist. Our findings suggest that DADLE promotes spatial cognitive function recovery and regulates neurogenesis after ischemia, which may provide a promising therapeutic strategy for cerebral ischemia.

1. Introduction

Global ischemic injury following emergency situations, such as stroke and cardiac arrest, can lead to long-term neurologic impairment. Despite obtaining several meaningful findings from animal models of cerebral ischemia [1–3], clinically available therapeutic regimens for achieving neuroprotection and functional recovery from ischemic injury are still rare. Several neuroprotective approaches, such as physical exercise [4], forced limb-use [5], enriched environment [6], and short-term sleep deprivation [7], have been suggested to enhance neuroplasticity and functional repair by stimulating hippocampal neurogenesis in rats.

Studies have shown that transient global ischemia enhances neurogenesis [8], and neurogenesis in the adult involves a prominent endogenous repair mechanism [9] and is strongly correlated with recovery of learning and memory [10,11]. However, it is questionable if ischemia-induced neurogenesis itself can offer clinically effective functional recovery because many newly generated cells die via apoptosis within several weeks after their generation [12]. Therefore, enhancing neurogenesis and preventing death of newly born cells with some pharmacological approaches, such as opioids, may be a very useful clinical strategy.

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The delta opioid receptor (DOR) is involved in neuroprotection against hypoxic/ischemic damages. [D-Ala2, D-Leu5]-Enkephalin (DADLE), a selective DOR agonist, has been shown to possess potent activity in organ and neuronal protection [13]. Our previous studies showed that DADLE significantly increased neuronal survival 3 days after ischemia [14,15] and promoted beneficial activation of astrocytes, thereby reducing detrimental effects of global ischemia on neuronal survival in the hippocampus [16]. In addition, a few studies have demonstrated that DOR is involved in cell proliferation and neurogenesis [17]. Georganta et al. suggested that DOR activation leads to neurite outgrowth and differentiation in Neuro-2A cells under starvation conditions [18].

Despite this data, there are no reports on the long-term effects and outcomes of DADLE administered after the onset of global ischemia. Therefore, the present study was designed to investigate the long-term neurorestorative effects of DADLE treatment after global ischemia, particularly those related to neurogenesis.

2. Material and methods

2.1. Animals

All experimental procedures involving animals were performed in accordance with NIH guidelines and approved by the Animal Care and Use Committee of the Shanghai Jiaotong University. Male Sprague Dawley rats (mean body weight 300 ± 50 g) were purchased from the SLAC Animal Center (Shanghai, China), and housed (two rats per cage) on a 12 h light/dark cycle under constant temperature and humidity. The animals were allowed free access to food and water.

2.2. Intracerebral cannula and induction of transient global ischemia

Rats were anesthetized intraperitoneally with 10% chloral hydrate (300 mg/kg) and implanted with a cannula aimed at the right lateral ventricle as described in our previous study [15], using the coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 3.8 mm ventral to the skull surface derived from the atlas of Paxinos et al. [19]. Following at least 6 days of recovery, rats underwent transient global ischemia in a four-vessel occlusion (4-VO) model [15]. Briefly, rats were anesthetized and both vertebral arteries were electrocauterized through the alar foramens of the first cervical vertebra. Both common carotid arteries were exposed and the ligatures were placed loosely around each artery without interrupting the carotid blood flow. The animals were allowed to recover from anesthesia. On the next day, under light ethyl ether anesthesia, both common carotid arteries were re-exposed. After the animals were fully awake, both carotid arteries were occluded with aneurismal clips for 10 min to induce forebrain ischemia. Ischemia was confirmed by monitoring the loss of righting reflex and bilateral pupil dilation during carotid occlusion. Sham-operated rats had their vertebral arteries coagulated and underwent all other surgical procedures except for carotid artery occlusion. Core body temperatures were monitored with a rectal probe and maintained at 37 °C throughout the experiment using a heating lamp.

2.3. Drug administration and groups

The rats were divided into five groups (n = 16 per group): sham group, DADLE-control group (sham procedure with 2.5 nmol DADLE), ischemia/reperfusion (I/R) group, DADLE-treated group (ischemia procedure with 2.5 nmol DADLE) and DADLE + naltrinode (NAL) group (ischemia procedure with 2.5 nmol DADLE and 2.5 nmol NAL). The dosing regimen for DADLE and NAL were based on our previous publication [15]. For drug delivery, a total volume of 5 μl artificial cerebrospinal fluid (ACSF) or ACSF containing DADLE (Sigma, USA) and NAL (Tocris, USA) was administered into the intracerebroventricular region at the onset of reperfusion at a rate of 5 μl/min with an infusion pump. The needle was left in place for 1 min before retraction.

2.4. Morris water maze test

Morris water maze test was used to test spatial learning and memory from 23 to 27 days after transient global ischemia. The tank was circular, 150 cm diameter and 50 cm height, and filled to a depth of 30 cm with water. The water was made opaque by nontoxic black paint and its temperature was adjusted constantly to 22 ± 1 °C. The surface area of the tank was divided into four equal quadrants. An escape platform (15 cm diameter) was placed in one of the four quadrants submerged 1 cm below the water surface so that it was invisible at water level. In the training session, the rats were trained to find the hidden platform using distal cues available on the curtain (four trials per day). Each trial had a different starting position. Once the rats found the platform, they were permitted to remain on it for 20 s. If the rats did not find the platform within 60 s, they were guided to the platform and also allowed to stay on it for 20 s. During each trial session, escape latency (time spent to find the submerged platform) was measured as a learning score in each trial by an auto-tracking system (Coulburn Instruments, USA). Twenty-four hours after the last training day, a probe test was performed to assess memory. During the probe test, the platform was removed from the tank, and the rats were allowed to swim freely. The time rats spent in each quadrant and the swim path were recorded.

2.5. Bromodeoxyuridine (BrdU) labeling and tissue preparation

Rats were given bromodeoxyuridine (5-bromo-2-deoxyuridine; BrdU) (Sigma, 10 mg/ml freshly prepared in sterile 0.9% NaCl) intraperitoneally at a dose of 100 mg/kg for 7 consecutive days after ischemic insult. Rats were sacrificed 2 h and 21 days after the last injection. Rats were anesthetized with 10% chloral hydrate (300 mg/kg) and transcardially perfused with ice-cold 0.9% NaCl solution, followed by a freshly prepared solution of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer saline (PBS). The brains were removed and immediately fixed in 4% PFA-PBS for 2 h, then immersed in 20% and 30% sucrose-PBS. Subsequently, the brain sections (30 μm in thickness) from each rat were cut from the anterior landmark where the infrapyrmidal and suprapyramidal blade of the dentate gyrus granule cell layer formed (about −2.30 mm from Bregama) on a coronal plane. There were 90 sections from each rat. A total of nine sections were chosen from every tenth section through the whole hippocampus. The sections were mounted on microscope slides and stored at −80 °C until use.

2.6. Immunofluorescence and cell counting

Immunofluorescence was performed as previously described [16]. The slides were incubated with primary antibody for RNA binding protein, fox-1 homolog (NeuN) (rabbit monoclonal, Abcam, 1:300) [20], glial fibrillary acidic protein (GFAP) (rabbit polyclonal, Abcam, 1:1000) [16], BrdU (mouse monoclonal, Millipore, 2 μg/ml) [21] or active caspase-3 (rabbit polyclonal, Abcam, 1:100) [16] overnight at 4 °C. After washing three times in 0.1 M PBS-Tween (T), sections were incubated with Cyanine 3 (Cy3)-conjugated secondary anti-mouse antibodies (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China; 1:1000) or Alexa Fluor 488 goat anti-rabbit IgG (Invtitrogen, Carlsbad, CA, USA; 1:1000) for 2 h at room temperature. Sections were counterstained with 4′,6-