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Interleukin-1β promotes the neurogenesis of carotid bodies by stimulating the activation of ERK1/2

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ABSTRACT

The carotid body (CB) is a complex sensory organ that functions to sense homeostatic O_2 in the blood. Previous studies have shown that CBs express interleukin (IL)-1 receptor type I and that the chemosensitivity of CBs is increased following stimulation with pro-inflammatory cytokines. However, the effects of pro-inflammatory cytokines, such as IL-1β, on the neurogenesis of CB are unclear. Thus, in this study, we aimed to assess the effects of IL-1β and intermittent hypobaric hypoxia (IHH) plus IL-1β on the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, tyrosine hydroxylase (TH) and the expression of nestin, a well-established stem cell marker in the nervous system. The results showed that TH, nestin expression and ERK1/2 phosphorylation were increased in the rat CB following intraperitoneal injection of IL-1β. Moreover, IL-1β had additive effects on IHH. These results suggested that the plasticity of CB was increased following treatment with IL-1β and that ERK1/2 may be involved in neurogenic signaling in CBs.

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1. Introduction

Extensive communication exists between the immune and nervous systems; cytokines and other chemical messengers are synthesized and released by immune cells, transmitting immune signals to the brain to induce a series of brain-mediated responses. However, how these cytokines affect brain activity has not been clarified. The afferent route via the vagus nerve has been the subject of many investigations on this topic (Steinman, 2004). Within the vagus nerve, pro-inflammatory cytokines may activate chemoreceptive dopaminergic glomus cells in vagal paraganglia (Goehler et al., 2000) to increase nerve firing for transmission of signals to the brain. Thus, the paraganglia, which are widely distributed along the major branches of sympathetic and parasympathetic nerves, may act as sensory organs for immune information.

The carotid body (CB) is a complex sensory organ that is richly perfused by arterial blood and innervated by a branch of the glossopharyngeal nerve. These structural features make it ideally suited as a chief component of the homeostatic acute O_2-sensing system and for activation of the respiratory center in the brainstem, facilitating the induction of hyperventilation during hypoxemia (Kameda, 2005). Interestingly, the CB is thought to be the largest paraganglion in the body (Nurse and Piskuric, 2013; Pallot, 1987). Our previous studies found that interleukin (IL)-1 receptor type I (IL-1RI) and IL-6 receptor α (IL-6Rα) are mainly expressed in principal cells (glomus cells) of the CB, even under normal conditions (Wang et al., 2002, 2006). Meanwhile, the existence of pro-inflammatory cytokines, such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α, and their corresponding receptors (i.e., IL-1R1, gp130, and TNF receptor type 1 [TNFr1]) has been confirmed in clusters of glomus cells in the rat CB (Lam et al., 2008a, b). In addition, topical application of IL-1β to the CB in anesthetized rats *in vivo* significantly increased the discharge rate in the carotid sinus nerve;

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these effects can be reversed or attenuated by perfusion with an IL-1 receptor antagonist (Shu et al., 2007), and intraperitoneal injection of rmIL-1β could increase IL-1RI and tyrosine hydroxylase (TH) expression in rat CBs (Zhang et al., 2007). These findings suggest that IL-1β may be an essential mediator of the chemosensitivity of CBs.

On the other hand, it is well known that the CB become enlarged and produce new neuron-like glomus cells under hypoxic conditions (Platero-Luengo et al., 2014), and it is considered to be a neurogenic center with a recognizable physiological function in adult humans (Pardal et al., 2007). Previous studies indicate that intermittent hypobaric hypoxia (IHH), which occurs in patients with cardiopulmonary disease and people travel to high altitude, could increase sympathetic activity (Hsieh et al., 2015) and induce morphological and physiological changes in CBs (Jiang and Eyzaguirre, 2006). Moreover, the levels of pro-inflammatory cytokines, such as IL-1β, have been shown to be elevated after exposure to hypoxia (Honda et al., 2014; Lee et al., 2012), and both IL-1β and IHH could activate extracellular signal-regulated kinase (ERK) 1/2, which plays a role for the proliferation (Xiao et al., 2007) and differentiation (Chiou et al., 2006) for the neural progenitor cell. However, whether IL-1β could induce CB neurogenesis and the interaction of IL-1β and IHH on the CB neurogenesis is not clear.

Considering this above, the present study use Sprague-Dawley rats that underwent intraperitoneal (i.p.) injection of IL-1β, intermittent hypobaric hypoxia (IHH), or IHH plus intraperitoneal injection of IL-1β to investigate the effects of IL-1β and IL-1β plus IHH on the expression of nestin (a maker of neural precursor cells) phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 and the differentiation in CBs.

2. Materials and methods

2.1. Animals

The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University. All experiments were performed in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Male Sprague-Dawley rats (nearly 8 weeks old) were housed at four animals per cage in an air-conditioned room with a 12:12 h light/dark cycle and free access to food and water. Animals were allowed to acclimate for at least 10 days before the experiments.

2.2. Experimental

2.2.1. Experiment I

Recombinant murine IL-1β (211-11B; Peprotech, USA) was dissolved in saline. As shown in Fig. 1A, rats were randomly assigned to sham and IL-1β group ($n = 13$ per group). The rats in the sham group received i.p. injection of saline (1 mL/kg daily), while rats in the IL-1β group received IL-1β (750 ng/kg daily) for 3 consecutive days. The dose of IL-1β in the present study was based on the previous study (Zhang et al., 2007). Two hours after the last injection, rats were sacrificed and nestin expression and ERK1/2 phosphorylation in the CB were determined by immunohistochemistry (5 rats per group) and western blot analysis (8 rats per group).

2.2.2. Experiment II

To investigate the cooperative effects between IHH and IL-1β, rats were randomly assigned to sham, IHH1w, IHH2w, IHH3w, IL-1β, IHH1w + IL-1β, IHH2w + IL-1β, and IHH3w + IL-1β groups ($n = 13$ per group). The rats in the sham and IL-1β groups were not exposed to IHH and received i.p. injection of saline (at 07:00, 1 mL/kg daily) or IL-1β (at 07:00, 750 ng/kg daily) for 3 consecutive days. The rats in the IHH1w, IHH2w, and IHH3w groups were exposed to IHH for 1, 2, or 3 weeks (daily from 09:00 to 17:00) and received i.p. injection of saline (at 07:00, 1 mL/kg daily) for the final 3 days. Similar to the IHH groups, rats in the IHH1w + IL-1β, IHH2w + IL-1β, and IHH3w + IL-1β groups were also exposed to IHH but received i.p. injection of IL-1β (at 07:00, 750 ng/kg daily). After the last IHH treatment, rats were sacrificed, and the CB was collected for immunohistochemistry (5 rats per group) or western blot analysis (8 rats per group).

2.3. IHH treatment

To obtain IHH, rats were exposed to a hypobaric chamber at barometric pressure of 430 mmHg, corresponding to an altitude of 4500 m. The temperature of the chamber was kept at 28 °C. The animals were kept at this barometric pressure for 8 h (from 09:00 to 17:00) every day for 1 3 weeks. The control rats stayed in the same environment as the IHH rats with access to food and water *ad libitum*, but breathed normal room air.

2.4. Immunohistochemistry

Rats were sacrificed and perfused with 100 mL of 0.1 M cold phosphate-buffered saline (PBS) and then with 400 mL of 4% cold paraformaldehyde in PBS. All CBs were postfixed for 1 h in paraformaldehyde and stored in 25% sucrose at 4 °C. The sections of the CB (10 μm) were cut with a cryostat and mounted onto gelatinized slides. The slides were washed three times with PBS and then incubated with the primary antibodies, including mouse anti-nestin (1:200; Abcam), mouse anti-tyrosine hydroxylase (TH; 1:500; Sigma–Aldrich, St. Louis, MO, USA), rabbit anti-phospho-ERK1/2 (1:200; Cell Signaling Technology), rabbit anti- protein gene product 9.5 (PGP9.5, 1: 1000, Abcam) and rabbit anti-glial fibrillary acidic protein (GFAP; 1:1000; Sigma–Aldrich, St. Louis, MO, USA) at 4 °C overnight. Sections were then incubated with anti-mouse Alexa Fluor 594-conjugated (1:800) or anti-rabbit Alexa Fluor 488-conjugated (1:500) secondary antibodies for 2 h. The sections were observed under a fluorescence microscope.

2.5. Western blot

CBs of rats from each group were collected and every 4CBs were pooled and lysed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer containing 62.5 mM Tris–HCl, 2% w/v SDS, 10% glycerol, 50 mM DTT, and 0.1% w/v bromophenol blue, and the insoluble materials were separated by centrifugation at 12,000 × *g* for 10 min. The supernatant was heated at 100 °C for 10 min and then cooled on ice for 30 min. Electrophoresis was carried out by SDS-PAGE by using 10% polyacrylamide gels in accordance with routine protocols. The proteins were transferred onto nitrocellulose membranes and blocked in blocking solution containing 5% defatted milk powder and 0.1% Tween-20 in TBS for 1 h at room temperature with gentle shaking. After washing in TBS three times for 8 min each, mouse anti-nestin (1:500; Abcam), rabbit anti-phospho-ERK1/2, rabbit anti-ERK (1:2000; Cell Signaling Technology), and mouse anti β-actin antibodies (1:10000; Sigma–Aldrich) were used for incubation overnight at 4 °C. The membranes were then washed three times in TBS again and incubated with peroxidase-conjugated goat anti-mouse (1:5000) or goat anti-rabbit IgG (1:10000) in TBS containing 0.1% Tween-20 for 1 h. After washing three times in TBS for 8 min each, protein bands on the membrane were detected using a chemiluminescence detection kit (Supersignal West Pico Chemiluminescent Substrate;

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