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SPINAL ACTIVATION OF ALPHA7-NICOTINIC ACETYLCHOLINE RECEPTOR ATTENUATES POSTTRAUMATIC STRESS DISORDER-RELATED CHRONIC PAIN VIA SUPPRESSION OF GLIAL ACTIVATION

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Abstract—The high prevalence of chronic pain in posttraumatic stress disorder (PTSD) individuals has been widely reported by clinical studies, which emphasized an urgent need to uncover the underlying mechanisms and identify potential therapeutic targets. Recent studies suggested that targeting activated glia and their pro-inflammatory products may provide a novel and effective therapy for the stress-related pain. In this study, we investigated whether activation of alpha-7 nicotinic acetylcholine receptor (α7 nAChR), a novel anti-inflammatory target, could attenuate PTSD-related chronic pain. The experiments were conducted in a rat model of single prolonged stress (SPS), an established model of PTSD-pain comorbidity. We found that SPS exposure produced persistent mechanical allodynia. Immunohistochemical and enzyme-linked immuno sorbent assay analysis showed that SPS also induced elevated activation of glia cells (including microglia and astrocytes) and accumulation of pro-inflammatory cytokines in spinal cord. In another experiment, we found that intrathecal injection of PHA-543613, a selective α7 nAchR agonist, attenuated the SPS-evoked allodynia in a dose dependent manner. However, this anti-hyperalgesic effect was blocked by pretreatment with methyllycaconitine (MLA), a selective α7 nAchR antagonist. Further analyses showed that PHA-543613 suppressed SPS-induced spinal glial activation and SPS-elevated spinal pro-inflammatory cytokines, and these were abolished by MLA. Taken together, the present study showed that spinal activation of α7 nAChR by PHA-543613 attenuated mechanical allodynia induced by PTSD-like stress, and the suppression of spinal glial activation may underlie this anti-hyperalgesic effect. Our study demonstrated the therapeutic potential of targeting α7 nAChR in the treatment of PTSD-related chronic pain.

Keywords: Alpha7-nicotinic acetylcholine receptor, Posttraumatic stress disorder, Stress-induced hyperalgesia, Microglia, Astrocyte, Inflammation.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a stress-related mental disorder that develops after an extremely stressful event, such as physical violence or military combat (Yehuda, 2002). Clinically, PTSD often occurs in comorbidity with other disorders (Cavalcanti-Ribeiro et al., 2012; Sareen, 2014). The frequent co-occurrence of PTSD with chronic pain has been widely reported by clinical studies, with proximately one half to three quarters of PTSD patients being diagnosed with chronic pain (Moeller-Bertram et al., 2012; Sareen, 2014; Brennstuhl et al., 2015). Given the high prevalence of chronic pain in individuals with PTSD, there is an urgent need to uncover the underlying mechanisms and identify potential therapeutic targets.

The phenomenon that exposure to physical or psychological stressors could enhance nociception and pain sensitivity has been observed in many clinical and preclinical studies, which was described as stress-induced hyperalgesia (SIH) (Jennings et al., 2014). Recently, emerging evidence revealed that spinal cord was vulnerable to stress (Quintero et al., 2011; He et al., 2013; Bradesi et al., 2015; Zheng et al., 2015), and stress-induced dysregulation of spinal neuroimmune response was increasingly recognized as an important contributor to the pathogenesis of SIH (Rivat et al., 2010; Golovatscka et al., 2012). As the immunocompetent cells within the central nervous system (CNS), both microglia (Akagi et al., 2014; Yasui et al., 2014; Hong

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Abbreviations: α7 nAChR, alpha-7 nicotinic acetylcholine receptor; AKT, protein kinase B; ANOVA, analysis of variance; CNS, central nervous system; CREB, cAMP response element-binding protein; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immuno sorbent assay; GFAP, glial fibrillary acidic protein; IL-1β, interleukin-1β; IL-6, interleukin-6; JAK2, Janus kinase 2; LPS, lipopolysaccharides; MLA, methyllycaconitine; NF-κB, nuclear factor kappa B; PBS, phosphate buffer solution; PI3K, phosphatidylinositol-3 kinase; PTSD, posttraumatic stress disorder; PWMT, paw withdrawal mechanical threshold; SIH, stress-induced hyperalgesia; SPS, single prolonged stress; TNF-α, tumor necrosis factor α.

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et al., 2015) and astrocytes (Bradesi et al., 2011; Liu et al., 2015) have been demonstrated to function in central sensitization in SIH models by releasing pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) (Golovatscka et al., 2012). Spinal activation of microglia was found in several SIH paradigms, such as repeated cold stress (Akagi et al., 2014), water avoidance stress (Hong et al., 2015), and forced swim stress (Suarez-Roca et al., 2014). Our previous study also found that spinal activation of astrocytes was positively correlated with mechanical allodynia in rats exposed to single prolonged stress (SPS) (Liu et al., 2015), an established PTSD model that has been used to examine the therapeutic responses in somatic pain related to the PTSD-like stress (Zhang et al., 2012; Qi et al., 2014). Together, these evidence indicated that pro-inflammatory activation of spinal glia may be important to the development and maintenance of allodynia and hyperalgesia induced by stress.

An increasing number of studies have investigated the clinical potential of targeting activated glia and their pro-inflammatory products for controlling glially dependent pain (Mika et al., 2013; Grace et al., 2014), among which alpha-7 nicotinic acetylcholine receptor (α7 nAChR) was emerging as a novel potential pharmacological target (Dineley et al., 2015). To date, a total of 17 known subunits of nAChRs were identified (α1-10, β1-4, δ, ε, γ) (Millar 2003). Of these, α7 nAChRs were abundantly expressed in the periphery and within the CNS, in particular on microglia and astrocytes (Deutsch et al., 2015; Kalkman and Feuerbach 2016). Agonism of α7 nAChR was demonstrated to reduce the levels of pro-inflammatory products, and have shown good potential for suppressing numerous inflammatory-mediated pathologies in the periphery, such as ulcerative colitis, asthma, arthritis, and in the CNS, like Parkinson's disease (Bencherif et al., 2011; Quik et al., 2015). Numerous studies also demonstrated the analgesic properties of α7 nAChR agonists in rodent models of inflammatory pain and neuropathic pain (Wang et al., 2005; Rowley et al., 2010; Loram et al., 2012). Further studies indicated that the anti-hyperalgesic effects were attributed, to some extent, to suppression of glially derived pro-inflammatory cytokines (Medhurst et al., 2008; Loram et al., 2010). Considering the view that pro-inflammatory activation of spinal glia may be implicated in the development of hyperalgesia induced by stress, it is assumed that α7 nAChR may play an important role in modulation of SIH via mediating glial activation.

In this study, we evaluated whether spinal activation of α7 nAChR could suppress glial activation and attenuate mechanical allodynia induced by PTSD-like stress. The selective α7 nAChR agonist, PHA-543613, and antagonist, methyllycaconitine (MLA) were used in the experiments. The study would provide novel insights into the spinal mechanisms of the pathogenesis of SIH, and had important implications for the development of novel therapeutic strategies for PTSD-related chronic pain.

EXPERIMENTAL PROCEDURES

Experimental animals

Adult Sprague–Dawley rats, weighting 180–200 g, were used in this study. They were obtained from the Laboratory Animal Center of Nanjing Drum Tower Hospital and pair-housed in a temperature-controlled (21 ± 1 °C) vivarium with 12-h alternating dark/light cycles. The rats were allowed to get food and water *ad libitum*. All experimental procedures were approved by the Policy on the Use of Animals in Nanjing Drum Tower Hospital and in accordance with the ethical guidelines for the use of experimental animals (Zimmermann, 1983).

Drug preparation

The selective α7 nAChR agonist, PHA-543613, and antagonist, methyllycaconitine citrate (MLA) were used in this study and they were purchased from Tocris Biosciences (Minneapolis, USA). PHA-543613 was dissolved in 5% dimethylsulfoxide (DMSO) and prepared at a dosage of 6 μg or 12 μg in a volume of 20 μl. MLA was dissolved in physiologic saline (0.9% sodium chloride) and prepared at a dosage of 10 μg in a volume of 20 μl. The dosages of these drugs were chosen referring to a previous study (Zhang et al., 2015) and based on our preliminary experiment. For vehicle treatment, a same volume of 5% DMSO was used.

SPS procedure

The SPS procedure was carried out as previously described by Liberzon et al. (Liberzon et al., 1997). Briefly, rats were restrained for 2 h inside a plastic animal holder, and then they were forced to swim individually for 20 min in an acrylic water tank (height 60 cm, diameter 25 cm). The tanks were filled with clean water (24 °C) of two-thirds of their heights. After swimming, the rats were allowed to rest for 15 min, followed by inhalation of anesthetic ether until they lost consciousness. Meanwhile, control rats were maintained in an adjacent room without any treatment.

Experimental design

Experiment 1: 16 rats were randomly divided into two groups (n = 8): group Control (rats without any treatment) and group SPS (rats underwent a SPS procedure). Pain behavioral tests were performed on the day before (baseline) and on days 1, 2, 3, 4, 5, 6, 7 after SPS. After the last behavioral testing, the lumbar spinal cord of rats was collected either for immunohistochemistry (n = 3) or for enzyme-linked immuno sorbent assay (ELISA) analysis (n = 5).

Experiment 2: 64 rats were randomly divided into eight groups (n = 8): group Control + vehicle (rats were administered vehicle once daily); group Control + PHA (12 μg) (rats were administered PHA-543613 12 μg once daily); group Control + MLA (rats were administered MLA 10 μg once daily); group SPS + vehicle (rats were

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