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Research Report

Effects of dexmedetomidine on P2X4Rs, p38-MAPK and BDNF in spinal microglia in rats with spared nerve injury

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ABSTRACT

Microglia in the spinal cord is evidenced to play a crucial role in neuropathic pain. Spinal P2X4 receptors (P2X4Rs), which are mainly expressed in microglia, have been investigated for their roles in neuropathic pain. Dexmedetomidine (DEX), a highly selective agonist of α_2-adrenergic receptors, is clinically applied to sedation and analgesia. Despite the proposed mechanisms underlying DEX-induced analgesia, the possible interactions between DEX and P2X4Rs at a molecular level have not been elucidated. We designated the spared nerve injury (SNI) to establish the neuropathic pain model. Mechanical paw withdrawal threshold (MWT) was measured to evaluate the sensitivity of neuropathic pain in rats. MWT was significantly decreased in SNI rats versus control rats. Expressions of spinal P2X4Rs, phosphorylated p38-mitogen-activated protein kinase (p-p38-MAPK) and brain-derived neurotrophic factor (BDNF) were upregulated in SNI rats. Immunofluorescence assay indicated higher densities of microglia and P2X4Rs, which appeared yellow in colour, suggesting they were co-labelled. Intraperitoneal injections of DEX 40 μg/kg for 14 consecutive days markedly reversed the SNI-induced decline of MWT; the activation of microglia was markedly inhibited; in addition, the protein expressions of P2X4Rs, p-p38-MAPK and BDNF were significantly downregulated. Thus, DEX could attenuate the neuropathic pain in SNI rats, of which the mechanism might be related to the down-expressed P2X4Rs, p-p38 and BDNF in microglia of spinal dorsal horn.

Abbreviations: BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DEX, dexmedetomidine; Iba-1, ionised calcium binding adaptor molecule 1; IP, intraperitoneal; MWT, mechanical paw withdrawal threshold; P2X4Rs, P2X4 receptors; p38-MAPK, p38-mitogen-activated protein kinase; *p*-p38-MAPK, phosphorylation of p38-mitogen-activated protein kinase; SNI, spared nerve injury; SD, Sprague-Dawley

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1. Introduction

Neuropathic pain, generally defined as a chronic pain state resulting from peripheral or central nerve injury, is characterised by symptoms such as allodynia, hyperalgesia and spontaneous pain (Woolf and Mannion, 1999). A growing body of evidence from diverse animal models of neuropathic pain showed that injury to the peripheral nervous system could lead to activation of microglia in the spinal cord and the consequent neuropathic pain, in which the activated microglia has an important role to play (Cao and Zhang, 2008; Milligan and Watkins, 2009; Tsuda et al., 2005; Watkins et al., 2001). Subsequent to peripheral nerve injury, the resting microglia in the spinal dorsal horn is converted to an activated state through a series of cellular and molecular modifications.

Numerous studies have demonstrated that hypersensitivity to neuropathic pain is involved in P2 purinergic receptors (Burnstock, 2006a; Di Virgilio, 2006), especially P2X4R, which is a subtype of ionotropic purinoceptor. Spinal nerve injury can upregulate the expression of the P2X4Rs in the microglia in the spinal dorsal horn ipsilateral to the nerve injury, instead of the neurons or astrocytes wherein the upregulation of the P2X4Rs parallels the increase in pain hypersensitivity, while blockade of P2X4Rs can significantly attenuate allodynic effects (Tsuda et al., 2003, 2008 Ulmann et al., 2008). The activated P2X4Rs cause the phosphorylation of p38-MAPK, resulting in the release of brain-derived neurotrophic factor (BDNF), all of which are essential to the persistence of pain hypersensitivity after nerve injury (Trang et al., 2009). It seems plausible that blockade of P2X4Rs-p-p38-MAPK-BDNF pathway in spinal cord may provide a novel therapeutic strategy for neuropathic pain.

It is well established that α_2-adrenoceptor agonists, like clonidine, have anti-nociceptive properties, and the pain modulatory action of the α_2-adrenoceptor in the spinal cord has been most extensively studied (Duflo et al., 2002; Paqueron et al., 2003). Moreover, it has been found that the activation of α_1-adrenergic receptor agonist interferes with α_2-mediated analgesia (Gil et al., 2009). Therefore, it has been proposed that dexmedetomidine (DEX), a highly selective agonist of α_2-adrenergic receptors clinically applied to sedation. Accumulating evidence has exhibited that DEX has a significant analgesic effect in many models of chronic pain (Guneli et al., 2007; Kimura et al., 2012; Liu et al., 2012). Despite the proposed mechanisms concerning DEX-induced analgesia, the possible interaction between DEX and P2X4Rs at a molecular level has not been elucidated. Our experiment was designed to investigate whether consecutive IP injections of DEX can attenuate neuropathic pain in rats with SNI by regulating the expressions of P2X4Rs, p-p38-MAPK and BDNF in spinal cord.

2. Results

2.1. *Decrease in the mechanical withdrawal threshold in SNI rats*

There were no significant differences in MWT between Normal and Sham groups ($p>0.05$). In the SNI group, the MWT in the hind limb ipsilateral to injury was significantly reduced as from postoperative day 1 until day 14 versus the Normal and Sham animals ($p<0.05$) (Fig. 1).

2.2. *Upregulation of P2X4R expression in hyperactive microglia in spinal cord in SNI rats*

Western blot showed apparently upregulated expression of P2X4Rs in the spinal dorsal horn in SNI rats versus the Normal and Sham groups. Immunofluorescence assay indicated that the average fluorescence intensities of P2X4Rs in the spinal dorsal horn ipsilateral to injury were significantly increased at days 7 and 14 after SNI versus the control groups ($p<0.05$). Immunofluorescence assay results showed that the intensity of ionised calcium binding adapter molecule 1 (Iba-1, a microglial marker) in the spinal dorsal horn ipsilateral to injury in SNI rats was increased, and the activated microglia exhibited enlargement of cell bodies with shrunk or thickened processes. One-way analysis of variance (ANOVA) showed that the average fluorescence intensities of Iba-1 in the spinal dorsal horn ipsilateral to injury began to increase on day 1 after SNI. Besides, on days 7 and 14, the average fluorescence intensities of Iba-1 were significantly greater in SNI rats versus the Normal and Sham groups ($p<0.05$). On day 14, the average fluorescence intensities of P2X4Rs and Iba-1 were still greater, and exhibited yellow in colour, indicating that they were double-labelled. The results showed that P2X4Rs were mainly expressed in the microglia in the spinal dorsal horn ipsilateral to injury 14 days after sciatic nerve injury (Fig. 2A–D).

2.3. *Time course of protein expression of p-p38 and BDNF in the spinal cord*

Western blotting showed that the protein expression levels of p-p38 and BDNF in the spinal dorsal horn in SNI rats were apparently upregulated versus the Normal and Sham groups

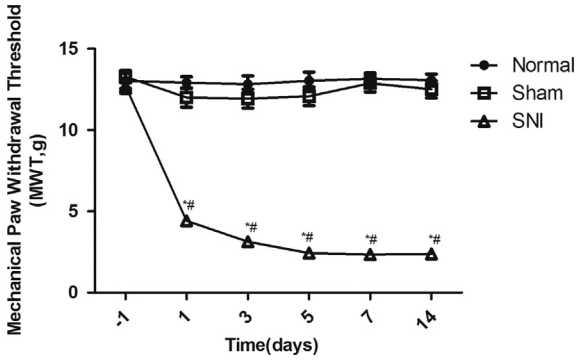

Fig. 1 – von Frey test for the assessment of mechanical paw withdrawal threshold (MWT) in the Normal, Sham and SNI groups. SNI-induced rats show a significant drop in the MWT from day 1 after operation, and maintain a lower level throughout the course of the experiment compared to Normal and Sham groups ($n=8$, $^{*}p<0.05$ versus Normal, $^{\#}p<0.05$ versus Sham).