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Research report

Brain-derived neurotrophic factor (BDNF) in the rostral anterior cingulate cortex (rACC) contributes to neuropathic spontaneous pain-related aversion via NR2B receptors

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ABSTRACT

The rostral anterior cingulate cortex (rACC) plays an important role in pain affect. Previous investigations have reported that the rACC mediates the negative affective component of inflammatory pain and contributed to the aversive state of nerve injury-induced neuropathic pain. Brain-derived neurotrophic factor (BDNF), an activity-dependent neuromodulator in the adult brain, is believed to play a role in the development and maintenance of inflammatory and neuropathic pain in the spinal cord. However, whether and how BDNF in the rACC regulates pain-related aversion due to peripheral nerve injury is largely unknown. Behaviorally, using conditioned place preference (CPP) training in rats, which is thought to reveal spontaneous pain-related aversion, we found that CPP was acquired following spinal clonidine in rats with partial sciatic nerve transection. Importantly, BDNF was upregulated within the rACC in of rats with nerve injury and enhanced the CPP acquisition, while a local injection of a BDNF-tropomyosin receptor kinase B (TrkB) antagonist into the rACC completely blocked this process. Finally, we demonstrated that the BDNF/TrkB pathway exerted its function by activating the NR2B receptor, which is widely accepted to be a crucial factor contributing to pain affect. In conclusion, our results demonstrate that the BDNF/TrkB-mediated signaling pathway in the rACC is involved in the development of neuropathic spontaneous pain-related aversion and that this process is dependent upon activation of NR2B receptors. These findings suggest that suppression of the BDNF-related signaling pathway in the rACC may provide a novel strategy to overcome pain-related aversion.

1. Introduction

Neuropathic pain, as one of the most common types of chronic pain, always behaves in a spontaneous manner (a continuous or paroxysmal pain that is not related to an external stimulus). This non-evoked pain is difficult to measure in animals and its pathogenesis is still poorly understood. A recent study using the principle of negative reinforcement effectively produced conditioned place preference (CPP) in nerve injured rats, which intuitionally mirrored pain-related negative affection and aversive learning produced by neuropathic spontaneous pain (King et al., 2009). In clinical practice, there has been considerable evidence suggesting that patients with chronic pain suffer from much more affective disturbances than pain itself (Crombez et al., 1999; Vlaeyen and Linton, 2000). Thus, increased attention should be paid to the treatment of pain affection.

Brain-derived neurotrophic factor (BDNF) is commonly regarded to be an activity-dependent neuronal modulator in the adult brain that enhances neuronal excitability. Several lines of evidence have demonstrated that BDNF is overexpressed after nerve injury or inflammation in related regions of pain transmission, such as the spinal cord, rostral ventromedial medulla and other cortical areas (Lin et al., 2011; Geng et al., 2010; Guo et al., 2006; Thibault et al., 2014). The increased release of endogenous BDNF is necessary for plasticity changes and central sensitization and thus contributes to the development of chronic pain (Garraway

Abbreviations: BDNF, brain-derived neurotrophic factor; CPP, conditioned place preference; CTX-B, cyclotraxin-B; F-CPA, formalin-induced conditioned place avoidance; LTP, long-term potentiation; NMDAR, Nmethyl-D-aspartate receptors; PFC, prefrontal cortex; rACC, rostral anterior cingulate cortex; SNI, spare nerve injury; TrkB, tropomyosin receptor kinase B.

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et al., 2003; Obata and Noguchi, 2006; Marcol et al., 2007). It is well established that chronic pain produced by BDNF signaling mainly occurs through activation of tropomyosin receptor kinase B (TrkB) receptors (Narita et al., 2000). The hyperalgesia and tactile allodynia caused by sciatic nerve injury are completely blocked by intrathecal application of BDNF inhibitor TrkB-Fc chimera protein; moreover, hyperalgesia and tactile allodynia produced by administration of exogenous BDNF in normal mice are also completely prevented by application of a TrkB receptor antagonist, K-252a (Yajima et al., 2005). Although a great deal of research has focused on the generation of persistent pain mediated by BDNF/TrkB signaling, whether and how this signaling function in neuropathic spontaneous pain-related aversion is not well known.

Nmethyl-D-aspartate (NMDA) receptors are heteromeric complexes including the essential NR1 subunit and one or more of the NR2A-D subunits (Meguro et al., 1992). Activation of NMDA receptors in the ACC were required for the synaptic plasticity and long-term potentiation (LTP), and ultimately contributed to the development of chronic pain as well as pain-associated unpleasantness (Bliss et al., 2016; Zhuo, 2008; Zhuo, 2016). Importantly, the NMDA receptor NR2B subunits participated in nociceptive transmission and pain regulatory in the CNS and played a critical role in chronic pain formation (Yang et al., 2015; Zhuo, 2009). Recently, Geng et al. found that the BDNF/TrkB-mediated signaling pathway in the spinal cord promoted the development of neuropathic pain induced by nerve injury and that this process was dependent upon the activation of dorsal horn NR2B receptors (Geng et al., 2010). Thus, we wondered whether NR2B receptors in the rACC contribute to neuropathic spontaneous pain-related negative emotions induced by BDNF signaling.

Increasing evidence indicates that the rostral anterior cingulate cortex (rACC) plays an important role during the processing of chronic pain (Gao et al., 2004; Johansen et al., 2001; Price, 2000). Animal behavioral studies have shown that the destruction of neurons originating from the rACC prevented the aversive state and negative emotional learning induced by acute noxious stimulation (Johansen and Fields, 2004), and clinical imaging examination studies showed that the rACC area could be activated by both noxious stimuli and pain-induced unpleasantness (Bornhovd et al., 2002; Tolle et al., 1999). However, the underlying mechanism of how neuropathic spontaneous pain-related aversive states arise in the rACC is still unclear. In this study, we hypothesized that the pain-related aversion in the rACC produced by peripheral nerve injuries as well as persistent pain-induced central sensitization in the spinal cord might share a common signaling pathway. Therefore, in this study, we investigate whether BDNF/TrkB signaling in the rACC drives the formation of pain-related negative emotions by activating NR2B receptors in rats following spare nerve injury (SNI) surgery.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200–220 g used in the present study were obtained from the Experimental Animal Center of Shandong University. Rats were housed in separated cages under a 12-h light/dark cycle (lights on at 7:00 am) with free access to food and water. The room temperature was maintained at 24 ± 1 °C, and the humidity was controlled at 40–50%. Animals were given a period of seven days to adjust to their new surroundings before experimental manipulations. In accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983), all experiments were approved by the Animal Care and Use Committee of Shandong University.

2.2. Surgical procedures

2.2.1. Spare nerve injury (SNI) surgery

Spare nerve injury models were established as previously described (Decosterd and Woolf, 2000). All rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (300 mg/kg). The sciatic nerve and its three terminal branches were exposed by directly incising the skin on the lateral surface of the thigh and the deeper biceps femoris muscle. SNI surgery involves an axotomy and ligation of the common peroneal and tibial nerves without touching the sural nerve. The tibial and common peroneal nerves were ligated tightly with 5-0 silk and were severed 2–4 mm from its emergence. Any contact with or stretching of the intact sural nerve was avoided. Skin and muscles were sutured in two layers, separately, and the nerves were kept entirely flattened and transparent after this surgical procedure. Sham-operated rats receiving the same surgical procedure but without sectioning of any nerves were used as a control group.

2.2.2. Intra-rACC catheter implantation and drug injection

The implantation of a catheter was performed as previously described (Xiao et al., 2012). Under intraperitoneal chloral hydrate anesthesia, rats were firmly fastened into a brain stereotactic apparatus with the lambda and bregma at horizontal level. A 30-gauge stainless steel cannula with a 33-gauge stainless steel stylet plug was bilaterally implanted 0.5 mm above the rACC injection site [2.6 mm anterior to bregma, 0.6 mm lateral from the midline, 2.5 mm beneath the surface of the skull] or the prefrontal cortex (PFC) [2.6 mm anterior to bregma, 0.6 mm lateral from the midline, 3.7 mm beneath the surface of the skull] in-line with the atlas of Paxinos and Watson (1998). The cannula was fixed with denture cement, and all surgical procedures were performed under sterile conditions. Animals were allowed to recover for one week before the next experimental procedure. Rats showing any neurological defects after the surgical procedure were removed from the experiment.

For drug injection, rats were transiently anesthetized with isoflurane, and local microinjection was performed through a 33-gauge stainless-steel injection cannula. A 1 μL Hamilton syringe with PE-10 tubing was linked to the cannula that extended 0.5 mm over the tip of the guide cannula. A volume of 1 μL per hemisphere of either drug or vehicle was injected over a 5 min period. The injection cannula was kept in place for another 5 min to minimize diffusion of the drug along the injection syringes.

2.2.3. Intrathecal catheter implantation and drug injection

Implantation of the intrathecal cannula was performed as described by Storkson et al. (1996). Briefly, a PE-10 polyethylene catheter was inserted into the epidural space between the L5 and L6 vertebrae. Correct implantation was determined by observing the behavior of dragging or paralysis of bilateral hind limbs after injection of 2% lidocaine (0.2 mL) after complete recovery from anesthesia. The internal part of the catheter was fixed with the paravertebral muscles, and the outer part of the catheter was plugged and fixed onto the skin after wound closure and sutured at the head. All surgical procedures were performed under sterile conditions. Rats showing neurological deficits within 3 days after the catheter implantation were excluded.

Spinal drug administration was performed by injection of 25 μL of saline or 10 μg of clonidine in 25 μL of saline to elicit CPP in rats with nerve injury. Each injection lasted for at least 5 min. All injections were performed in a separate room, and rats were exposed to the CPP conditions within 5 min after injection.