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BDNF contributes to the development of neuropathic pain by induction of spinal long-term potentiation via SHP2 associated GluN2B-containing NMDA receptors activation in rats with spinal nerve ligation

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ABSTRACT

The pathogenic mechanisms underlying neuropathic pain still remain largely unknown. In this study, we investigated whether spinal BDNF contributes to dorsal horn LTP induction and neuropathic pain development by activation of GluN2B-NMDA receptors via Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) phosphorylation in rats following spinal nerve ligation (SNL). We first demonstrated that spinal BDNF participates in the development of long-lasting hyperexcitability of dorsal horn WDR neurons (i.e. central sensitization) as well as pain allodynia in both intact and SNL rats. Second, we revealed that BDNF induces spinal LTP at C-fiber synapses via functional up-regulation of GluN2B-NMDA receptors in the spinal dorsal horn, and this BDNF-mediated LTP-like state is responsible for the occlusion of spinal LTP elicited by subsequent high-frequency electrical stimulation (HFS) of the sciatic nerve in SNL rats. Finally, we validated that BDNF-evoked SHP2 phosphorylation is required for subsequent GluN2B-NMDA receptors up-regulation and spinal LTP induction, and also for pain allodynia development. Blockade of SHP2 phosphorylation in the spinal dorsal horn using a potent SHP2 protein tyrosine phosphatase inhibitor NSC-87877, or knockdown of spinal SHP2 by intrathecal delivery of SHP2 siRNA, not only prevents BDNF-mediated GluN2B-NMDA receptors activation as well as spinal LTP induction and pain allodynia elicitation in intact rats, but also reduces the SNL-evoked GluN2B-NMDA receptors up-regulation and spinal LTP occlusion, and ultimately alleviates pain allodynia in neuropathic rats. Taken together, these results suggest that the BDNF/SHP2/GluN2B-NMDA signaling cascade plays a vital role in the development of central sensitization and neuropathic pain after peripheral nerve injury.

Introduction

The mechanisms underlying the development of neuropathic pain are still not fully understood. Previously, we and others have reported that sensory information processing in the spinal dorsal horn appears to undergo significant plastic changes e.g. long-term potentiation (LTP) in synaptic efficacy following peripheral nerve injury or inflammation (Park et al., 2011; Sandkuhler and Liu, 1998; Xing et al., 2007; Yang et al., 2014), which is well accepted as the underlying mechanisms of central sensitization and post-injury pain hypersensitivity (Ji et al., 2003; Latremoliere and Woolf, 2009; Sandkuhler, 2007; Woolf, 2011). Moreover, we have previously discovered that activation of spinal GluN2B-containing N-methyl-D-aspartate (GluN2B-NMDA) receptors is required for the induction of dorsal horn LTP at C-fiber synapses after nerve injury, implying GluN2B-dependent LTP in the spinal dorsal horn plays a crucial role in the development of long-lasting spinal neurons hyperexcitability (i.e. central sensitization) and neuropathic pain (Qu et al., 2009). In a parallel study, we have demonstrated that increased spinal brain-derived neurotrophic factor (BDNF) contributes to the

Abbreviations: ACC, the anterior cingulate cortex; AMPAR, a-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA) receptor; ANOVA, analysis of variance; AUC, area under the time-course curve; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; ERK, extracellular signal-regulated kinase; GluN2B-NMDA receptors, GluN2B-containing N-methyl-D-aspartate receptors; HFS, high-frequency electrical stimulation; LTP, long-term potentiation; LV-shSHP2, lentivirus-short hairpin SHP2; MAPK, mitogen-activated protein kinase; NMDA receptors, N-methyl-D-aspartate receptors; NS, normal saline; PSD, postsynaptic density; PWT, paw withdrawal threshold; RTK, receptor tyrosine kinase; SHP2, Src homology-2 (H2) domain-containing protein tyrosine phosphatase-2; SNL, spinal nerve ligation; TrkB, tropomyosin related kinase B; WDR neurons, wide dynamic range neurons.

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pathogenesis of neuropathic pain by activation of GluN2B-NMDA receptors in rats with spinal nerve ligation (SNL) (Geng et al., 2010). However, the cellular and molecular mechanisms associated with such a role for BDNF still remain unclear.

Several lines of evidence have shown that in some brain regions BDNF plays a key role in activity-dependent LTP, a synaptic model of memory storage (Escobar et al., 2003; Leal et al., 2014; Lu et al., 2008; Meis et al., 2012; Sakata et al., 2013; Ying et al., 2002), and the GluN2B-NMDA receptor is considered as one of the targets for BDNF-induced modulation of synaptic plasticity (Caldeira et al., 2007b; Carreno et al., 2011; Kim et al., 2006; Levine and Kolb, 2000; Lin et al., 1998; Otis et al., 2014; Yamada and Nabeshima, 2004). In the rat spinal dorsal horn, it is also found that the BDNF-induced LTP at C-fiber synapses is involved in the pathogenesis of mechanical hypersensitivity following peripheral nerve injury (Zhou et al., 2008, 2011). We here hypothesized that induction of spinal LTP at C-fiber synapses via activation of GluN2B-NMDA receptors would be the underlying mechanism by which the increased BDNF in the spinal dorsal horn plays its roles in the development of neuropathic pain following nerve injury.

BDNF, a member of the neurotrophins family, should bind to and activate tropomyosin related kinase B (TrkB), a receptor tyrosine kinase, to exert its effects (Park and Poo, 2013). SHP2, a Src homology-2 (H2) domain-containing protein tyrosine phosphatase-2, acting as a major regulator of receptor tyrosine kinase (Grossmann et al., 2010), has been implicated in numerous neurotrophin signaling (Case et al., 1994; Matozaki et al., 2009; Ohnishi et al., 1999; Stein-Gerlach et al., 1998), including BDNF/TrkB signaling pathway (Araki et al., 2000; Easton et al., 2006; Neel et al., 2003; Okada et al., 1996). In an in vitro study, Lin and colleagues have reported that SHP2 participates in BDNF-mediated GluN2B-NMDA receptors signaling at the postsynaptic site, and BDNF enhances association of SHP2 with the NMDA receptor subunit GluN2B in the cortical postsynaptic density (PSD) (Lin et al., 1999). Recently, Peng et al. have revealed that phosphorylation of spinal SHP2 via signal regulatory protein alpha 1 (SIRPα1) induces SIRPα1-SHP2 interaction, which subsequently triggers SHP2/PSD-95/GluN2B signaling, and thereby playing a role in neuropathic pain development in a rat model (Peng et al., 2012).

In this study, we investigated whether spinal BDNF contributes to dorsal horn LTP induction and neuropathic pain development by activation of GluN2B-NMDA receptors via SHP2 phosphorylation in rats following SNL surgery. We present valid evidence showing that the BDNF/SHP2/GluN2B-NMDA signaling cascade in the spinal dorsal horn plays a vital role in the induction of spinal LTP as well as in the development of central sensitization and neuropathic pain after peripheral nerve injury.

Materials and methods

Chemicals, recombinant lentivirus, and animals

BDNF (Sigma-Aldrich, St. Louis, MO) was first dissolved as a concentrated stock solution (5.0 μg/ml) in 0.9% sterile saline (e.g. normal saline, NS). TrkB-Fc (R&D systems, Minneapolis, USA) and IgG (Sigma) were dissolved in 0.01 M phosphate buffer saline (PBS) containing 0.1% bovine serum albumin (BSA) as a 100 mg/ml store solution. Ifenprodil (Sigma), NSC-87877 (Tocris Bioscience, Bristol, UK), genistein (Sigma) and its inactive analogue genistin (Calbiochem, USA) were dissolved in dimethyl sulphoxide (DMSO, sigma, St. Louis, MO, USA) to make a stock concentration of 50 mM, aliquoted in small volumes and stored at -20 °C. The stock solution was subsequently diluted with sterile normal saline to make desired final concentrations immediately before administration. The final concentration of DMSO was <0.5%.

Recombinant lentivirus-short hairpin SHP2 (LV-shSHP2) was constructed by Genechem (Shanghai, China). Briefly, the plasmids expressed shSHP2-GFP was used to produce lentivirions. Lentivirus-short hairpin RNA (shRNA) was generated using sense small interfering RNA sequence targeting SHP2 (GeneBank number NM-001177593.1): small interfering SHP2 TTAGGAACGTCGATGTCAC. The scrambled sequence LV-GFP was used as negative control. To minimize off-target effects, a BLAST homology search (based on sense and antisense sequences) was systematically performed to ensure that a single mRNA sequence was targeted (http://www.ncbi.nlm.nih.gov). Lentiviral vector is pGV118 (Genechem, Shanghai, China) with U6 promoter. The short hairpin RNAs were cloned into lentivirus vectors. Construction and production of lentivirions were completed by Genechem. The final titer of recombinant virus was around 1.0×10^9 transducing units (TU)/ml.

Male Sprague–Dawley rats weighing 200–250 g at the beginning of the experiment were provided by the Department of Experimental Animal Sciences, Peking University Health Science Center. The rats were housed in separated cages with free access to food and water. The room temperature was kept at 24 ± 1 °C under natural light–dark cycle. All animal experimental procedures were carried out in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by the Animal Care and Use Committee of Peking University. The behavioral experimenters were kept blind from the groupings of the rats. A total of 443 animals were used in our present study.

Spinal nerve ligation (SNL)

Under general anesthesia with chloral hydrate (0.3 g/kg, intraperitoneally, i.p.), the left lumbar 5 (L5) spinal nerves distal to the dorsal root ganglia were tightly ligated with 4-0 silk sutures as described by Kim and Chung (Kim and Chung, 1992). In control animals, sham surgery with identical procedure except for ligation of the L5 spinal nerves was received. Any rats exhibiting motor deficiency or lack of tactile allodynia were excluded from the study.

Implantation of intrathecal catheter

Under chloral hydrate (0.3 g/kg, i.p.) anesthesia, implantation of intrathecal cannula was performed following the method of Storkson et al. (Storkson et al., 1996). Briefly, a PE-10 polyethylene catheter was implanted between the L5 and L6 vertebrae to reach the lumber enlargement of the spinal cord. The outer part of the catheter was plugged and fixed onto the skin on closure of the wound. All surgical procedures were performed under sterile conditions. Rats showing neurological deficits after the catheter implantation were euthanized. Drugs or vehicle were intrathecally injected via the implanted catheter in a 20-μl volume of solution followed by 10-μl of normal saline (NS) for flushing. Each injection lasted at least 5 min. After an injection, the needle remained in situ for 2 min before being withdrawn.

Intrathecal delivery of lentivirions: expression and functional detection

Lentivirions including lentivirus-short hairpin SHP2 (LV-shSHP2) and its control LV-GFP were intrathecally delivered at the final titer of 1.0×10^9 TU/ml in 10-μl volume, respectively. In experiments of knock down SHP2 in naïve rats (Fig. 9), BDNF (100 ng) was intrathecally administrated twice per day, repeated for 2 days on day 5 following lentivirions application. Seven days after BDNF administration and behavioral test, expression of SHP2, SHP1 and GluN2B were detected using Western blot to determine the knockdown of SHP2 by SHP2 siRNA (see Fig. 9I). In experiments of knock down SHP2 in SNL rats (Fig. 10), LV-shSHP2 or LV-GFP was intrathecally delivered at 1.0×10^9 TU/ml in 10-μl volume on day 5 before SNL operation. Seven days after SLN surgery and behavioral test, expression of SHP2, SHP1 and GluN2B were detected also using Western blot to determine the knockdown of SHP2 by SHP2 siRNA (see Fig. 10G).