Experimental Neurology 288 (2017) 38–50

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Complete spinal cord injury (SCI) transforms how brain derived neurotrophic factor (BDNF) affects nociceptive sensitization

Yung-Jen Huang [a,*,1], Kuan H. Lee [b,1], James W. Grau [a]

[a] Behavioral and Cellular Neuroscience, Department of Psychology, Texas A&M University, College Station, TX 77843, USA
[b] Center for Pain Research, Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form 25 October 2016
Accepted 1 November 2016
Available online 3 November 2016

Keyword:
BDNF
GABA
$GABA_A$ receptor
KCC2
Central sensitization
Allodynia
Pain
SCI

ABSTRACT

Noxious stimulation can induce a lasting increase in neural excitability within the spinal cord (central sensitization) that can promote pain and disrupt adaptive function (maladaptive plasticity). Brain-derived neurotrophic factor (BDNF) is known to regulate the development of plasticity and has been shown to impact the development of spinally-mediated central sensitization. The latter effect has been linked to an alteration in GABA-dependent inhibition. Prior studies have shown that, in spinally transected rats, exposure to regular (fixed spaced) stimulation can counter the development of maladaptive plasticity and have linked this effect to an up-regulation of BDNF. Here it is shown that application of the irritant capsaicin to one hind paw induces enhanced mechanical reactivity (EMR) after spinal cord injury (SCI) and that the induction of this effect is blocked by pretreatment with fixed spaced shock. This protective effect was eliminated if rats were pretreated with the BDNF sequestering antibody TrkB-IgG. Intrathecal (i.t.) application of BDNF prevented, but did not reverse, capsaicin-induced EMR. BDNF also attenuated cellular indices (ERK and pERK expression) of central sensitization after SCI. In uninjured rats, i.t. BDNF enhanced, rather than attenuated, capsaicin-induced EMR and ERK/pERK expression. These opposing effects were related to a transformation in GABA function. In uninjured rats, BDNF reduced membrane-bound KCC2 and the inhibitory effect of the $GABA_A$ agonist muscimol. After SCI, BDNF increased KCC2 expression, which would help restore GABAergic inhibition. The results suggest that SCI transforms how BDNF affects GABA function and imply that the clinical usefulness of BDNF will depend upon the extent of fiber sparing.

1. Introduction

Over the last two decades, there has been a plethora of studies examining how brain-derived neurotrophic factor (BDNF) affects neural function (for reviews, see Boyce and Mendell, 2014; Cunha et al., 2010; Hollis and Tuszynski, 2011; Merighi et al., 2008; Smith, 2014; Waterhouse and Xu, 2009). Interest in this protein has been fueled by evidence that it promotes neuronal growth, synaptogenesis, cell survival and neurogenesis (Smith, 2014; Weishaupt et al., 2012). At a functional level, BDNF has been implicated in learning and memory, with research demonstrating that it is released in an activity dependent manner, promotes the development of long-term potentiation (LTP), and fosters memory formation (Cunha et al., 2010; Waterhouse and Xu, 2009). The action of BDNF has been linked to the TrkB receptor and its downstream effectors, including phospholipase C (PLC), extracellular signal-regulated kinase (ERK), and Akt, which have been tied to synaptic plasticity, axonal growth, and cell survival, respectively (Minichiello, 2009). Interestingly, how BDNF affects neural function appears to be regulated by the cellular environment, leading some to suggest that it acts to maintain a form of homeostasis that promotes adaptive plasticity (Swanwick et al., 2006; Wenner, 2014).

These findings have led researchers to posit that BDNF could foster cell survival and adaptive plasticity after spinal cord injury (SCI; Boyce and Mendell, 2014; Hollis and Tuszynski, 2011; Weishaupt et al., 2012). BDNF has been shown to enhance axonal growth, enable locomotor function in spinally transected animals, and promote respiratory function after cervical injury (Baker-Herman et al., 2004; Boyce et al., 2012; Boyce et al., 2007; Cote et al., 2011; Tashiro et al., 2015; Vaynman and Gomez-Pinilla, 2005). In addition, both locomotor training and spinally-mediated learning have been shown to up-regulate BDNF expression after SCI (Gomez-Pinilla et al., 2007; Gomez-Pinilla et al., 2001; Huie et al., 2012). Exogenously applied BDNF, microinjected by

Abbreviations: aCSF, artificial cerebrospinal fluid; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; Cap, capsaicin; CNS, central nervous system; EMR, enhanced mechanical reactivity; ERK, extracellular signal-regulated kinases; GABA, Gamma-aminobutyric acid; i.t., intrathecal; KCC2, K^+-Cl^- cotransporter 2; LTP, long-term potentiation; MAPK, mitogen-activated protein kinases; Mus, muscimol; N-CAD, N-cadherin; NKCC1, Na^+-K^+-Cl^- cotransporter 1; NMDAR, *N*-methyl-D-aspartate receptor; pERK, phosphorylated ERK; PLC-γ, phospholipase C-γ; SCI, spinal cord injury; Shk, shock; 5HT, serotonin; T2, second thoracic; TrkB, tropomyosin receptor kinase B; Unshk, unshock; Veh, vehicle.

* Corresponding author at: Department of Neuroscience, 301 Old Main Dr ILSB 3149, Texas A&M University, College Station, TX 77843, USA.
E-mail address: redkie@gmail.com (Y.-J. Huang).

[1] Both authors contributed equally to the study.

http://dx.doi.org/10.1016/j.expneurol.2016.11.001
0014-4886/

means of an intrathecal (i.t.) catheter, has been shown to foster spinal learning and block the learning impairment induced by variable intermittent stimulation (shock) applied at an intensity that engages pain (C) fibers (Gomez-Pinilla et al., 2007; Huie et al., 2012). Interestingly, spinal learning is also disrupted by treatments that induce nociceptive sensitization within the spinal cord (e.g., the peripheral application of the irritant capsaicin) that manifests behaviorally as enhanced mechanical reactivity (EMR; Ferguson et al., 2006; Ferguson et al., 2012a; Ferguson et al., 2012b; Hook and Grau, 2007). Prior work has shown that the induction of nociceptive sensitization, and the associated spinal learning impairment, are inhibited by training with either response-contingent or temporally predictable stimulation (Baumbauer and Grau, 2011; Baumbauer et al., 2012; Hook and Grau, 2007). We have linked the restoration of spinal learning to the release of BDNF, demonstrating that the protective effect of training is blocked by pretreatment with the BDNF sequestering antibody TrkB-IgG (Baumbauer et al., 2009; Huie et al., 2012). The present paper extends these findings by testing whether TrkB-IgG also blocks the protective effect of training on nociceptive sensitization. Conversely, we explore whether pretreatment with BDNF inhibits the development of capsaicin-induced EMR in spinally transected rats.

The idea that BDNF may counter the development of spinally-mediated nociceptive sensitization after SCI runs counter to a large literature implicating this ligand in the development of central sensitization (Merighi et al., 2008; Pezet et al., 2002; Smith, 2014). Prior work has shown that treatments that increase spinal BDNF in uninjured animals induce EMR (Beggs and Salter, 2013; Coull et al., 2005; Garraway et al., 2003; Kerr et al., 1999; Latremoliere and Woolf, 2009; Lu et al., 2009; Merighi et al., 2008; Miletic and Miletic, 2008; Thompson et al., 1999). Conversely, the EMR induced by the application of peripheral irritants (e.g., formalin) is inhibited by TrkB-IgG (Kerr et al., 1999). Further, electrophysiological studies indicate that the application of BDNF can sensitize nociceptive activity in lamina II of the dorsal horn (Garraway et al., 2003). These pronociceptive effects have been tied to a BDNF-induced reduction in GABAergic inhibition linked to the down-regulation of membrane-bound K^+-Cl^- cotransporter 2 (KCC2; Beggs and Salter, 2010; Coull et al., 2005; Smith, 2014). How BDNF affects KCC2 and neuronal excitability within the dorsal horn appears to be regulated by descending fibers (Boulenguez et al., 2010; Garraway et al., 2005; Garraway and Mendell, 2007; Shulga et al., 2008). By interrupting these fibers, SCI could potentially transform how BDNF affects nociceptive sensitization. We show that SCI not only attenuates the pronociceptive effect of BDNF, SCI reverses its action, unveiling an antinociceptive effect that inhibits the development of capsaicin-induced EMR. We relate this transformation to a BDNF-induced increase in KCC2 and GABAergic inhibition.

2. Experimental procedures

2.1. Subjects

Subjects were male Sprague-Dawley rats obtained from Envigo (Houston, TX) that were 80–100 days old, and between 325 and 400 g. All subjects were pair housed and maintained on a 12-hour light/dark cycle, with all behavioral testing performed during the light cycle. Food and water was available ad libitum. All experiments were carried out in accordance with NIH standards for the care and use of laboratory animals (NIH publications No. 80-23), and were approved by the University Laboratory Animal Care Committee at Texas A&M University. Every effort was made to minimize suffering and limit the number of animals used.

2.2. Surgery

Prior to surgery, the fur over the thoracic portion of the vertebral column was shaved and disinfected with betadine solution. Subjects were anesthetized with isoflurane gas. Anesthesia was induced at 5% isoflurane and maintained at 2–3% isoflurane. Each subject's head was rendered immobile in a stereotaxic apparatus, and a small (5 × 4 × 2.5 cm) gauze pillow was placed under the subject's chest to provide support for respiration. An anterior to posterior incision over the second thoracic vertebrae (T2) was made and the tissue just rostral to T2 was cleared using rongeurs, and the cord was exposed. A cautery device was then used to transect the cord and a 25-cm polyethylene cannula (PE-10, VWR International, Bristol, CT, USA) was subsequently threaded 9 cm down the vertebral column, into the subarachnoid space between the dura and the white matter so that it lay on the dorsal surface of the spinal cord. After surgery, the incision was closed using Michel clips (Fine Science Tools, Foster, CA, USA), and the exposed end of cannula tubing was fixed to the skin with cyanoacrylate.

Following surgery, rats were placed in a temperature-controlled environment (25.5 °C) and monitored until awake. All rats were checked every six to eight hours during the 18–24 h post-surgical period. During this time, hydration was maintained with supplemental injections of saline, and the rats' bladders and colons were expressed as needed. As in prior studies (Garraway et al., 2014; Huie et al., 2012; Lee et al., 2016; Lee et al., 2015), testing began 24 h after surgery.

Spinal transections were confirmed by inspecting the cord at the time of surgery and observing the behavior of the subjects after they recovered to ensure that they exhibited paralysis below the level of the forepaws and did not exhibit any brain-dependent responses to stimulation caudal to injury.

2.3. Drug administration

Drugs were administered intrathecally (i.t.) using a 10 μL Hamilton syringe attached to the exposed end of each subject's i.t. cannula. BDNF (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 μL of artificial cerebrospinal fluid (aCSF) + 0.1% bovine serum albumin (BSA) vehicle. The BDNF sequestering agent TrkB-IgG (R&D Systems, Minneapolis, MN) was also injected intrathecally, as described above. Muscimol (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline. Each compound was administered at a constant rate over a period of several minutes. Following the injection, the cannula was flushed with 20 μL of 0.9% saline.

2.4. Fixed spaced leg shock

All fixed spaced (ISI: 2 s) shock was administered to one hind leg. An electrode was inserted through the skin over the distal portion of the tibialis anterior muscle (1.5 cm from the plantar surface of the foot). Next, the proximal portion of the tibialis anterior (approximately 1.7 cm proximal to the wire electrode) was probed with a 2.5-cm stainless steel pin attached to a shock lead to locate a position that elicited a robust flexion response. The pin was then inserted 0.4 cm into the muscle. Shock intensity was set to 0.4 mA shock,

2.5. Mechanical testing

Mechanical reactivity was assessed using von Frey filaments (Stoelting, Wood Dale, IL) that were applied while rats were loosely restrained in Plexiglas tubes. Sensitivity was determined by applying calibrated filaments to the mid-plantar surface of each hindpaw in an ascending order until a flexion response was elicited. Stimuli were presented twice to each paw in an ABBA counterbalanced fashion (A = left, B = right), with testing on the same leg separated by a 2 min interval. Filament thickness/force was related to behavior using the transformation provided by the manufacturer: Intensity = log10 (10,000 g). This transformation yields a scale that is approximately linear and amenable to parametric analyses. Data were converted to change from baseline scores for purposes of analysis. The experimenter performing the behavioral tests was unaware of the subject's treatment condition.