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A Tropomyosine Receptor Kinase Inhibitor Blocks Spinal Neuroplasticity Essential for the Anti-Hypersensitivity Effects of Gabapentin and Clonidine in Rats With Peripheral Nerve Injury

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Abstract: Spinally released brain-derived nerve growth factor (BDNF) after nerve injury is essential to anatomic and functional changes in spinal noradrenergic and cholinergic systems, which are engaged or targeted by commonly used treatments for neuropathic pain. Since BDNF signals via tropomyosine receptor kinases (trks), we tested whether trk blockade by repeated spinal injection of the trk inhibitor K252a would reduce anatomical (spinal noradrenergic and cholinergic fiber density), functional (α2-adrenoceptor-mediated direct stimulation of spinal cholinergic terminals), and behavioral (anti-hypersensitivity from systemic gabapentin and spinal clonidine) plasticity, which depends on BDNF. Spinal K252a treatment did not alter hypersensitivity from spinal nerve ligation (SNL), but blocked the SNL-associated increase in dopamine-β-hydroxylase (DβH) fiber density in the spinal cord dorsal horn while reducing spinal choline acetyltransferase (ChAT)-immunoreactivity. K252a treatment also abolished the facilitatory effect of dexmedetomidine on KCl-evoked acetylcholine release in spinal cord synaptosomes and reduced the anti-hypersensitivity effects of oral gabapentin and spinal clonidine. These results suggest that spinal trk signaling is essential for the anatomic and functional plasticity in noradrenergic and cholinergic systems after nerve injury and consequently for the analgesia from drugs that rely on these systems.

***Perspective:** Many drugs approved for neuropathic pain engage spinal noradrenergic and cholinergic systems for analgesia. This study demonstrates that spinal trk signaling after nerve injury is important to neuroplasticity of these systems, which is critical for the analgesic action of common treatments for neuropathic pain.*

***Key words:** Neuropathic pain, noradrenergic, cholinergic, spinal cord, tyrosine kinase receptor, K252a.*

Spinal processing and transmission of sensory signals are modulated by local circuits and supraspinal neurons, which project to the spinal cord.[20] Bulbospinal noradrenergic pathways inhibit pain and hypersensitivity after peripheral nerve injury in animals, and many approved treatments for neuropathic pain, including gabapentin, the noradrenaline-mimetic clonidine, and noradrenaline re-uptake inhibitors, engage or mimic this mechanism to relieve neuropathic pain.[9,12,21-23,25,32] Although noradrenergic inhibition can be demonstrated in the normal animal, peripheral nerve injury fundamentally alters the structure and function of this system, and these alterations are essential to efficacy of this pathway after nerve injury. For one, noradrenergic fibers in the spinal cord dorsal horn sprout at dermatomes surrounding the site of input, allowing for great and more anatomically extensive release of norepinephrine when this pathway is activated.[14] For another, spinally released norepinephrine, which normally inhibits spinal cholinergic interneurons, excites them after nerve injury, and this release acetylcholine is critical to analgesic effects of spinal norepinephrine release.[21] Gabapentin, commonly used to treat neuropathic pain, activates neurons in the brainstem to induce spinal noradrenaline release, which stimulates α2-adorenoceptors and subsequent release of acetylcholine for analgesia in rodents with neuropathic hypersensivity.[11,32] A combination of gabapentin with cholinesterase inhibitors produces synergistic analgesia, which is dependent on spinal muscarinic receptors,[11,12,32] and these muscarinic receptors are themselves up-regulated in primary sensory

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afferents after nerve injury.[13] The clinical relevance of these laboratory observations is underscored by demonstration in patients with chronic pain that oral administration of gabapentin, in a dose that produces postoperative analgesia, increases noradrenaline concentrations in cerebrospinal fluid.[9] These clinical and laboratory data validate spinal noradrenergic-cholinergic circuits as important targets for treatment of neuropathic pain.

In both normal and neuropathic pain states, α2-adrenoceptors are coupled to Gi/o-proteins and Gi/o signaling by these metabotropic receptors results in antinociception in part by reducing release of pronociceptive neurotransmitters including substance P and glutamate from primary afferent terminals,[20,24] and in part by hyperpolarizing spinal neurons via activation of potassium channels.[30] We have previously demonstrated that activation of α2-adrenoceptors by clonidine or dexmedetomidine inhibits KCl-evoked acetylcholine release in spinal cord slices and lumbar dorsal horn synaptosomes in normal rats, consistent with this classical Gi/o-mediated effect of α2-adrenoceptors.[10,21] Interestingly, after peripheral nerve injury, activation of α2-adrenoceptors results in Gs-mediated facilitation rather than Gi/o-mediated inhibition of acetylcholine release from synaptosomes,[10] consistent with behavioral data that analgesia from intrathecal clonidine is blocked by intrathecal atropine in nerve-injured rats, but not in normal rats.[23,25]

Brain-derived neurotrophic factor (BDNF) plays an important role in spinal noradrenergic neuroplasticity after nerve injury. BDNF can be released from the terminals of primary afferents and resident glia,[3,7,14,29] and primarily acts on its high-affinity receptor, tropomyosine receptor kinase B (trkB), to regulate survival and differentiation of neurons during development and in adulthood.[19] We recently demonstrated that peripheral nerve injury increases BDNF content and dopamine-β-hydroxylase-immunoreactive (DβH-IR) axon density in the spinal dorsal horn, that spinal infusion of BDNF antibody blocks this increase in noradrenergic axon density in rats after nerve injury, and that intraspinally-injected BDNF in normal rats increases noradrenergic axon density.[14] Additionally, spinal infusion of BDNF antibody also reduces choline acetyltransferase (ChAT)-IR in the dorsal horn and abolishes the shift from inhibition to excitation by dexmedetomidine of KCl-evoked acetylcholine in dorsal horn synaptosomes after nerve injury.[10] Finally, spinal infusion of BDNF antibody reduces anti-hypersensitivity from intrathecal clonidine in nerve-injured rats in parallel with its effect on anatomic and functional changes in the spinal cord from injury.[14] These results suggest that spinal BDNF drives spinal noradrenergic-cholinergic neuroplasticity and hence efficacy for analgesia from drugs, which rely in part on engagement of this pathway, after nerve injury.

The current study extends these observations by determining the role of trk signaling in this plasticity. Efficacy of spinal treatment with the anti-BDNF antibody to prevent this plasticity assumes that this antibody acts specifically and solely to sequester BDNF and experiments using this tool do not elucidate the mechanisms by which BDNF acts. For these reasons, we tested whether spinal infusion of the trk inhibitor, K252a, would block anatomic and functional neuroplasticity of spinal noradrenergic systems after nerve injury and behavioral anti-hypersensitivity from 2 clinically used treatments for neuropathic pain that are presumed to rely on this plasticity.

Methods

Animals

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), weighing 180 to 280 g, were used. Animals were housed under a 12-hour light-dark cycle, with free access to food and water. All experiments were approved by the Animal Care and Use Committee at Wake Forest University (Winston Salem, NC).

Animal Surgery and K252a Treatment

Animals were anesthetized with 2% isoflurane in oxygen and intrathecal catheterization was performed as previously described.[35] A small puncture was made in the atlanto-occipital membrane of the cisterna magnum and a 7.5-cm polyethylene catheter (ReCathCo LLC, Allison Park, PA) was inserted so that the caudal tip reached the lumbar enlargement of the spinal cord. At least 5 days after intrathecal catheterization, L5-L6 SNL was performed as previously described.[17] Briefly, under anesthesia with 2% isoflurane, the right L6 transverse process was removed and the right L5 and L6 spinal nerves were tightly ligated using 5–0 silk suture. For the behavior and synaptosome studies, animals received intrathecal injection of saline or K252a (Sigma Chemical Co., St. Louis, MO; 2 μg in 10 μl of saline) followed by 10 μl of saline 15 minutes prior to the surgery, and then saline and K252a were repeatedly injected at 1, 3, 5, 7, 9, 11, and 13 days after surgery. Other animals for immunohistochemistry were also treated with saline and K252a, but their spinal cord tissues were collected at 10 days after surgery.

Behavioral Testing

Hypersensitivity to light touch following SNL was assessed using calibrated von Frey filaments (Stoelting, Wood Dale, IL) applied to the plantar surface of the paw. Filaments were applied to the bending point for 5 seconds, and a brisk paw withdrawal was considered a positive response. Withdrawal threshold was determined using an up–down statistical method.[2] At 10 days after SNL, gabapentin solution (Neurontin 50 mg/mL, Parke-Davis, New York, NY) was diluted in .5% carboxymethylcellulose solution and orally administered by a feeding tube (100 mg/5 mL/kg) 2 hours prior to measurement of withdrawal threshold in saline- and K252a-treated animals (n = 6 in each group). At 12 days after SNL, these same animals received intrathecal injection of clonidine (Sigma Chemical Co.; 15 μg in 10 μl of saline) followed by 10 μl of saline 1 hour prior to the measurement. At 14 days after SNL, spinal cord tissues from those animals were used for the synaptosome study. The person