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GABA-A RECEPTOR ACTIVITY IN THE NORADRENERGIC LOCUS COERULEUS DRIVES TRIGEMINAL NEUROPATHIC PAIN IN THE RAT; CONTRIBUTION OF NAα1 RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX

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Abstract—Trigeminal neuropathic pain is described as constant excruciating facial pain. The study goal was to investigate the role of nucleus locus coeruleus (LC) in a model of *chronic* orofacial neuropathic pain (CCI-ION). The study examines LC's relationship to both the medullary dorsal horn receiving trigeminal nerve sensory innervation and the medial prefrontal cortex (mPFC). LC is a major source of CNS noradrenaline (NA) and a primary nucleus involved in pain modulation. Although descending inhibition of acute pain by LC is well established, contribution of the LC to facilitation of chronic neuropathic pain is also reported. In the present study, a rat orofacial pain model of trigeminal neuropathy was induced by chronic constrictive injury of the infraorbital nerve (CCI-ION). Orofacial neuropathic pain was indicated by development of whisker pad mechanical hypersensitivity. Hypersensitivity was alleviated by selective elimination of NA neurons, including LC (A6 cell group), with the neurotoxin anti-dopamine-β-hydroxylase saporin (anti-DβH-saporin) microinjected either intracerebroventricularly (i.c.v.) or into trigeminal spinal nucleus caudalis (spVc). The $GABA_A$ receptor antagonist, bicuculline, administered directly into LC (week 8) inhibited hypersensitivity. This indicates a valence shift in which increased $GABA_A$ signaling ongoing in LC after trigeminal nerve injury paradoxically produces excitatory facilitation of the *chronic* pain state. Microinjection of NAα1 receptor antagonist, benoxathian, into mPFC attenuated whisker pad hypersensitivity, while NAα2 receptor antagonist, idazoxan, was ineffective. Thus, $GABA_A$-mediated activation of NA neurons during CCI-ION can facilitate hypersensitivity through NAα1 receptors in the mPFC. These data indicate LC is a chronic pain generator. Published by Elsevier Ltd on behalf of IBRO.

Key words: dopamine-beta-hydroxylase, anti-DβH-saporin, mechanical allodynia, spinal trigeminal caudalis, CCI-ION, chronic orofacial neuropathic pain.

INTRODUCTION

The pontine locus coeruleus (LC) nucleus is a major source of norepinephrine/noradrenaline (NA) in the central nervous system and a well-known mediator of descending inhibition of pain. The highly divergent efferent axonal projections of the LC innervate all levels of the neuraxis with an extensive network of ascending and descending projections to accentuate specific responses (Grzanna and Molliver, 1980; Westlund and Coulter, 1980; Westlund et al., 1981, 1982, 1983; Mantz et al., 1988; Aston-Jones et al., 2004; Gompf et al., 2010; Chandler and Waterhouse, 2012; Eschenko et al., 2012). A major NA efferent pathway from the LC innervates the medial prefrontal cortex (mPFC). This circuit optimizes behaviorally relevant, cognitive functions (Aston-Jones and Cohen, 2005; Marzo et al., 2014). For example, salient internal or external events can alter function or "reset" large-scale neural populations. This can be mediated by the targeted release of NA in the mPFC and can then shift the excitatory/inhibitory balance of the mPFC to a more excitable state. Therefore, we hypothesized that continuous activation within the NA LC-mPFC circuit provided by a chronic nerve injury model could shift pain modulation from inhibition to facilitation. To test this, we evaluated neuropathic pain behavior after either: (1) destruction of NA neurons in the LC; or (2) administration of α-adrenergic antagonists into the mPFC. Elimination of ascending and descending NA input was tested, as was the effect of NAα1 and NAα2 receptor activation.

Modulation of nociceptive transmission and pain perception are influenced by direct NA projections to trigeminal and spinal cord dorsal horn neurons. Several studies have shown that neurons of both the LC and the rostral ventromedial nucleus raphe magnus can either inhibit or facilitate spinal pain transmission in different physiological states (Grzanna and Molliver, 1980; Westlund and Coulter, 1980; Nuseir and Proudfit, 2000;

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Abbreviations: ATF3, activating transcription factor 3; BEN, benoxathian hydrochloride; BIC, bicuculline methiodide; CCI-ION, chronic constriction injury of trigeminal nerve maxillary V2 infraorbital branch; DβH, dopamine-β-hydroxylase; $GABA_A$, gamma amino butyric acid receptor A; GAD65, glutamate decarboxylase; i.c.v., intracerebroventricularly; IDA, idazoxan hydrochloride; LC, nucleus locus coeruleus; mPFC, medial prefrontal cortex; NA, noradrenaline; NAα1 or NAα2, noradrenergic receptor alpha 1 or noradrenergic receptor alpha 2; spVc, spinal trigeminal nucleus caudalis; TG, trigeminal ganglion; VEH, vehicle; Vth, trigeminal nerve.

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Millan, 2002). Although the descending feedback inhibition of pain by LC is well understood during acute pain conditions (Jones and Gebhart, 1987), the circuitry and molecular changes associated with central NA neurons that lead to chronic pain facilitation after peripheral nerve injury are still unclear (Martin et al., 1999; Taylor et al., 2000; Viisanen and Pertovaara, 2007; Brightwell and Taylor, 2009). Unique to the present study in comparison to many previous studies is the duration of the behavioral study. The present studies were initiated to understand pain facilitation by the LC through 8 weeks post injury in a chronic orofacial neuropathic pain (CCI-ION) model in contrast to most previous studies that have used pain models persisting 1–3 weeks. In the present study, a model of trigeminal neuropathic facial pain was induced by chronic constrictive injury of the infraorbital nerve, the second branch of the trigeminal nerve coursing across the maxillary bone (Vos et al., 1994). Trigeminal neuropathic pain is described as excruciating, constant burning pain and its treatment is a significant challenge. The nerve injury was confirmed by persistent mechanical hypersensitivity through an 8-week time course and by examining the expression profile of the injury biomarker activating transcription factor 3 (ATF3) in trigeminal ganglion (TG) neurons at the end of the study. The role gamma amino butyric acid (GABA) receptors play in the interneuronal modulation of LC and the effects of NA receptor activation in the mPFC were also investigated.

An initial study determined whether the selective elimination of NA neurons using anti-dopamine-β-hydroxylase saporin (anti-DβH-saporin) alleviates or facilitates the chronic facial neuropathic pain. The immunotoxic anti-DβH-saporin is taken up specifically by NA nerve endings and destroys NA neurons after retrograde transport to the cell bodies. Since the nerve injury increased expression of biomarkers for both NA and GABA (GAD65), the physiological effect of GABA was determined in a second experiment by administering the $GABA_A$ receptor antagonist bicuculline methiodide directly into the LC to block $GABA_A$ activation. Ongoing nerve injury would be expected to increase GABAergic inhibitory tone in the LC. The present study tests an alternative hypothesis that after long standing nerve injury, LC activation can be potentiated by $GABA_A$ receptor mediated neuronal activation (De Koninck, 2007; Doyon et al., 2013; Wei et al., 2013). Then blocking $GABA_A$ signaling with bicuculline would decrease LC activity causing decreased hypersensitivity.

Finally, to test whether LC is providing anti-nociceptive or pro-nociceptive effects on the mPFC in the ongoing pain state, NAα1 and NAα2 receptor antagonists (benoxathian and idazoxan hydrochloride, respectively) were microinjected directly into the mPFC to block the effects of NA input. The mPFC is a key neural region activated by sustained nociceptive input during the transition from acute nociceptive processing to central generation of pain based on numerous fMRI studies (Baliki et al., 2006, 2012; Apkarian et al., 2013). It was hypothesized that if this major ascending NA input from LC to mPFC inhibits chronic neuropathic pain, then injecting a NAα2 receptor antagonist would increase the tactile hypersensitivity that develops after nerve injury. If the ascending LC NA input to mPFC was facilitating chronic facial neuropathic pain, then injecting the NAα1 receptor antagonist would decrease the tactile hypersensitivity that developed after nerve injury.

EXPERIMENTAL PROCEDURES

All experimental procedures were approved by the Institutional Animal Care and Use Committees at the University of Kentucky and VA Medical Center, Lexington, Kentucky and were carried out following the Guidelines of the National Institutes of Health and the American Pain Association regarding the care and use of animals for experimental procedure. All measures were taken to minimize the number of animals used and their discomfort in these studies. Fig. 1 illustrates the experimental timeline for behavioral testing and drug injections.

Chronic constriction injury of the infraorbital nerve

Male Sprague–Dawley rats (n = 53) weighing 250–300 g (Harlan, Indianapolis, IN, USA) were housed under a 12-h light–dark cycle (7 AM–7 PM) with food and water *ad libitum*. Rats were anesthetized with a mixture of ketamine and xylazine (80 mg/kg, i.p. + 10 mg/kg, i.p.) prior to the surgery as previously described (Vos et al., 1994; Ma et al., 2012). The heads of the rats were shaved and stabilized in a stereotaxic frame. After the skin was cleansed with betadine followed by 70% ethanol, the left intraorbital nerve was exposed using a sterile surgical blunt dissection. Two chromic gut (5-0) ligatures were loosely tied around the left intraorbital nerve (2 mm apart). In order to determine the appropriate desired degree of constrictive force, the nerve was observed microscopically while the ligature was tightened so that the circulation through the superficial vasculature was retarded but not occluded. For the sham operation, the intraorbital nerve was exposed but not ligated. Following the nerve injury or sham surgery, the skin incision was closed with wound auto clips.

Assessment of whisker pad mechanical threshold

Bilateral assessment of the whisker pad mechanical withdrawal threshold was performed weekly throughout the 9-week experimental time course. To minimize observer subjectivity, the person performing the behavioral test was blinded to treatment groups. Prior to von Frey testing, each rat was acclimatized by holding them for 15 min in large cloth gloves to minimize stress-induced effects (Aloisi et al., 1994). Baseline behavioral testing was done for 2 weeks. Eight von Frey fibers (0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, 15.0 g; Stoelting, Wood Dale, IL, USA) were used to determine the mechanical sensitivity of the vibrissal whisker pad. A modified up-and-down method was used with a cut off maximal threshold of 18.72 g (Ma et al., 2012). Initially, an intermediate von Frey monofilament (2.0 g) was applied perpendicularly to the vibrissal whisker pad with a slight bending force.