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SEX DIFFERENCES IN HYPOTHALAMIC-MEDIATED TONIC NOREPINEPHRINE RELEASE FOR THERMAL HYPERALGESIA IN RATS

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Abstract—Neuropathic pain is treated using serotonin norepinephrine reuptake inhibitors with mixed results. Pain facilitation mediated by α_1-adrenoceptors may be involved, but whether norepinephrine (NE) is tonically released is unclear. The aim of this study was to determine whether NE is tonically released from A7 cells following chronic constriction injury (CCI), and if the lateral hypothalamus (LH) plays a role in this release in male and female rats with nociceptive and neuropathic pain types. Neuropathic groups received left CCI while nociceptive groups remained naïve to injury. Fourteen days later, rats were given intrathecal infusion of either the α_1-adrenoceptor antagonist WB4101, the α_2-adrenoceptor antagonist yohimbine (74 μg), or normal saline for control. Paw withdrawal latency (PWL) from a thermal stimulus was measured. The generalized estimated equation method was used for statistical analysis. Nociceptive rats given WB4101 had a PWL significantly longer than saline control (7.89 ± 0.63 vs. 5.87 ± 0.52 s), while the PWL of neuropathic rats given WB4101 was 13.20 ± 0.52 s compared to 6.78 ± 0.52 s for the saline control rats. Yohimbine had no significant effect. Microinjection of cobalt chloride (CoCl) in the A7 catecholamine cell group to prevent synaptic transmission blocked the effect of WB4101 in all groups, supporting the notion that spinally descending A7 cells tonically release NE that contributes to α_1-mediated nociceptive facilitation. Microinjection of CoCl into the left LH blocked the effect of WB4101 in nociceptive and neuropathic male rats, but had no effect in female rats of either pain type, suggesting differential innervation. These findings indicate that tonic release of NE acts at pronociceptive α_1-adrenoceptors, that this effect is greater in rats with nerve damage, and that, while NE comes primarily from the A7 cell group, LH innervation of the A7 cell group is different between the sexes.

Key words: α_1-adrenoceptors, antinociception, A7 cell group, lateral hypothalamus, neuropathic, nociception.

INTRODUCTION

Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system (Treede et al., 2008) and is refractory to most existing treatments. The serotonin norepinephrine reuptake inhibitors (SNRIs) are a recommended first-line treatment for neuropathic pain, but the effectiveness of these drugs is mixed (Dworkin et al., 2007, 2010). This lack of effect may be due to the functional role of the α-adrenoceptors in the spinal cord dorsal horn.

The SNRIs work in part by presynaptic inhibition of the reuptake of norepinephrine (NE; Lambert and Bourin, 2002), which directly modulates the activity of spinothalamic tract neurons (Westlund et al., 1990; Hagihira et al., 1990; Doyle and Maxwell, 1991a,b). The pontine A7 catecholamine cell group in rats innervates the spinal cord dorsal horn laminae I–IV, the major terminal field for primary nociceptive afferents (Clark and Proudfit, 1991; Light et al., 1992). There are two NE receptor subtypes in the spinal cord dorsal horn that exert a bidirectional effect on nociception. Nociception is increased through the action of α_1-adrenoceptors and is inhibited through the action of α_2-adrenoceptors (Holden et al., 1999; Nuseir and Proudfit, 2000; Holden and Naleway, 2001; Jeong et al., 2012). The net effect is antinociception, but the opposing α_1 activity likely attenuates this antinociception.

Stimulating the lateral hypothalamus (LH) produces antinociception in both male and female rats in both acute nociceptive and neuropathic pain states (Holden et al., 2014). This antinociception is produced in part by substance P immunoreactive (ir) neurons in the LH sending projections to the A7 cell group (Holden and Naleway, 2001; Holden et al., 2002). NE from the A7 cell group acts at α-adrenoceptors in the spinal cord dorsal horn to modulate nociception (Yeomans and Proudfit, 1992; Holden and Naleway, 2001). Evidence of tonic release of NE is seen in nociceptive male (Proudfit and Hammond, 1981; Sagen and Proudfit, 1984) and nociceptive female (Holden and Naleway, 2001) rats. Following tibial nerve transection, male rats also exhibit tonic release, but only with α_2-adrenoceptor activity (Hughes et al., 2013, 2015). However, it is unclear as to the presence of tonic NE release in male and female rats after chronic constriction injury (CCI).

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Abbreviations: CCI, chronic constriction injury; CoCl, cobalt chloride; DβH, dopamine β-hydroxylase; GEE, generalized estimating equation; ir, immunoreactive; LH, lateral hypothalamus; NE, norepinephrine; PWL, paw withdrawal latency; SNRI, serotonin norepinephrine reuptake inhibitor; TH, tyrosine-hydroxylase.

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The aim of this study was to determine if NE is tonically released in male and female rats that received one of two pain conditions: neuropathic pain from CCI or nociceptive pain only from application of a thermal stimulus given to all groups (Loeser and Treede, 2008; Xu et al., 2012). The CCI model is a valid and reliable method of producing thermal hyperalgesia, one of the symptoms of neuropathic pain (Bennett and Xie, 1988; Attal et al., 1990; Bennett, 1993; Kim et al., 1997; Jeong and Holden, 2009; Jeong et al., 2012). The paw withdrawal latency (PWL) was used to test thermal hyperalgesia and has proven reliability and validity (Yeomans and Proudfit, 1994).

We first examined the effect of α-antagonists in the absence of any forebrain stimulation. We then determined the role of the A7 catecholamine cell group in α-adrenergic tonic activity. Finally, we explored the role of the LH in tonic activity. Preliminary accounts of these results have been published as abstracts (Wagner et al., 2013, 2014; Wagner and Holden, 2015).

EXPERIMENTAL PROCEDURES

The Institutional Animal Care Committee at the University of Michigan approved the experimental procedures used in this study. The experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to minimize animal suffering, reduce the numbers of animals used, and to use alternatives to *in vivo* experiments.

Animals

Both male and female Sprague–Dawley rats were used in this study (250–400 g; Charles River, Portage, MI). All rats were maintained on a 12-h day/night schedule with free access to food and water. To reduce the possibility of estrous cycle influence, rats were randomly assigned to either experimental or control groups and no two female rats were taken from the same cage on the same day. To reduce the risk of mirror image effects on the non-ligated paw, we used separate control animals rather than each animal serving as its own control (Kim et al., 1997). A total of 182 rats were used for the study as reported, and each rat was only used once.

CCI procedure

This procedure has been outlined in detail elsewhere (Holden et al., 2014). Briefly, under isoflurane anesthesia, the left thigh of the rat was infused with bupivacaine (0.5%; 0.10 ml). The common sciatic nerve was exposed at the level of the mid-thigh. Approximately 7 mm of nerve was freed of adhering tissue and four ligatures were tied loosely around the nerve approximately 1 mm apart. The muscle was sutured and the incision closed with wound clips. Each rat received a subcutaneous injection of buprenorphine (0.3 mg/ml) at a dose of 0.05 mg/kg, recovered, and returned to its cage.

Analgesiometric testing procedure

To determine the effect of α-adrenoceptor antagonism on thermal nociception, the PWL test was used. The left paw was exposed to a focused beam of radiant heat using an analgesiometer (37360, Ugo Basile, Italy). The time interval between the onset of skin heating and the withdrawal response was measured electronically. In the absence of a response, skin heating was terminated after 15 s to prevent burning. Temperature was measured with a rectal probe pre-injection, then at 10 min and 40 min post injection. Heart rate, blood pressure, and mean arterial pressure were measured pre-microinjection and following the final latency measurement using a tail cuff and Coda monitor (Kent Scientific; Torrington, CT, USA). In all experiments, rats were randomly assigned to pain type (nociceptive or neuropathic). All drugs were made fresh daily and filtered through a 0.2-μm filter immediately before injection. Intrathecal drugs were injected in a volume of 30 μl using an electric syringe pump at a rate of 30 μl/min, and all intracerebral drugs were injected in a volume of 10 μl over 1 min. The injection cannula was left in place for one minute before removal to reduce the flow of drug up the guide cannula.

Experiments

Experiment 1: microinjection of IT α-adrenergic antagonists. Each rat was lightly anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg), which removes conscious perception of pain but leaves withdrawal reflexes intact, and then fitted into a stereotactic apparatus for IT infusion. In each rat, an IT catheter constructed from PE-10 polyethylene tubing was inserted through an incision in the cisterna magna and the tip positioned near the lumbar enlargement. One of the following drugs was then injected into the IT space: the α_2-adrenoceptor antagonist yohimbine, the α_1-adrenoceptor antagonist WB4101 (74 μg each in 30 μl normal saline, Sigma Chemical Co., St. Louis, MO, USA), or normal saline for control. PWLs were measured at 1 min post injection and then every 5 min for 45 min.

Experiment 2: IT microinjection of WB4101 or normal saline followed by microinjection of cobalt chloride (CoCl) in the A7 area. In the second experiment, rats were prepared for IT microinjection as previously described. In addition, a guide cannula was lowered into the A7 area defined by the following stereotactic coordinates: AP +0.2 mm from the interaural line, lateral 2.1 mm, vertical +2.2 mm, incisor bar set at −2.5 mm. A 30-gauge stainless steel injection cannula was connected to a 10-μl syringe by a length of PE-10 polyethylene tubing filled with CoCl (100 mM/0.5 μl). After a baseline response latency, either WB4101 (74 μg) or saline was injected into the IT space and PWLs were measured at 1, 3, and 5 min. Five min after IT injection, the second injection cannula was lowered into the A7 area and extended approximately 3 mm beyond the end of the guide