Neuroscience 270 (2014) 226–235

DIFFERENCES IN CARBACHOL DOSE, PAIN CONDITION, AND SEX FOLLOWING LATERAL HYPOTHALAMIC STIMULATION

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Abstract—Lateral hypothalamic (LH) stimulation produces antinociception in female rats in acute, nociceptive pain. Whether this effect occurs in neuropathic pain or whether male–female sex differences exist is unknown. We examined the effect of LH stimulation in male and female rats using conditions of nociceptive and neuropathic pain. Neuropathic groups received chronic constriction injury (CCI) to induce thermal hyperalgesia, a sign of neuropathic pain. Nociceptive rats were naive for CCI, but received the same thermal stimulus following LH stimulation. To demonstrate that CCI ligation produced thermal hyperalgesia, males and females received either ligation or sham surgery for control. Both males and females demonstrated significant thermal hyperalgesia following CCI ligation ($p < 0.05$), but male sham surgery rats also showed a significant left–right difference not present in female sham rats. In the second experiment, rats randomly assigned to CCI or nociceptive groups were given one of three doses of the cholinergic agonist carbachol (125, 250, or 500 nmol) or normal saline for control, microinjected into the left LH. Paw withdrawal from a thermal stimulus (paw withdrawal latency; PWL) was measured every 5 min for 45 min. Linear mixed models analysis showed that males and females in both pain conditions demonstrated significant antinociception, with the 500-nmol dose producing the greatest effect across groups compared with controls for the left paw ($p < 0.05$). Female CCI rats showed equivalent responses to the three doses, while male CCI rats showed more variability for dose. However, nociceptive females responded only to the 500-nmol dose, while nociceptive males responded to all doses ($p < 0.05$). For right PWL, only nociceptive males showed a significant carbachol dose response. These findings are suggestive that LH stimulation produces antinociception in male and female rats in both nociceptive and neuropathic pain, but dose response differences exist based on sex and pain condition.

Key words: lateral hypothalamus, sex differences, nociception, antinociception, analgesia.

INTRODUCTION

The lateral hypothalamus (LH) is part of a descending system that modifies nociception in the spinal cord dorsal horn. Both electrical (Lopez and Cox, 1992; Dafny et al., 1996; Franco and Prado, 1996) and chemical stimulations with *S*-glutamate (Behbehani et al., 1988), morphine (Dafny et al., 1996; Franco and Prado, 1996), carbamylcholine (carbachol; Holden and Naleway, 2001; Holden et al., 2002, 2005, 2009; Holden and Pizzi, 2008; Safari et al., 2009), or nociception/orphanin FQ (Geraschenko et al., 2011) increase response latencies to acute (nociceptive) pain in rats. This nociceptive modulation was found in both male (Behbehani et al., 1988; Dafny et al., 1996; Franco and Prado, 1996; Safari et al., 2009; Geraschenko et al., 2011) and female rats (Lopez and Cox, 1992; Holden and Naleway, 2001; Holden et al., 2002, 2005, 2009; Holden and Pizzi, 2008). The role of the LH in modulating neuropathic pain states is not known for either male or female rats. However, there is evidence that neuropathic pain, including hyperalgesic pain states, creates a shift from opioid-mediated responses to noradrenergic-mediated mechanisms (Schroder et al., 2010; Schiene et al., 2011; Hartrick, 2012; Rojo et al., 2012). This finding is in line with recent reports from our laboratory and others that the LH modifies nociception in the spinal cord dorsal horn in part through descending alpha adrenergic brainstem neurons (Holden and Naleway, 2001; Holden et al., 2002, 2005, 2009; Holden and Pizzi, 2008; Safari et al., 2009) and supports the hypothesis that LH stimulation will modify both nociceptive and neuropathic pain in male and female rats.

The purpose of this study was to determine the effect of three different doses of carbachol stimulation of the LH on paw withdrawal responses in male and female

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Abbreviations: ANOVA, analysis of variance; CCI, chronic constriction injury; LH, lateral hypothalamus; PAG, periaqueductal gray; PWL, paw withdrawal latency; RVM, rostral ventromedial medulla.

http://dx.doi.org/10.1016/j.neuroscience.2014.04.020
0306-4522/

Sprague–Dawley rats that received one of two pain conditions: neuropathic pain from chronic constriction injury (CCI), or naïve rats in which the thermal stimulus was analogous to acute, or nociceptive, pain (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). The paw withdrawal latency (PWL) was used to test responses to a thermal stimulus and has proven reliability and validity (Yeomans and Proudfit, 1994). A preliminary account of these results has been published as an abstract (Wagner et al., 2012).

EXPERIMENTAL PROCEDURES

The Institutional Animal Care Committee at the University of Michigan approved the experimental protocols used in this study. The experiments were conducted in accordance with the National Institutes 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 use alternatives to *in vivo* experiments.

Animals

Male and female Sasco-derived Sprague–Dawley rats (250–350 g; Charles River, Portage, MI, USA) were used. 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, female rats were randomly assigned to either experimental or control groups and no two rats were taken from the same cage on the same day. To reduce the risk that mirror image effects on the non-ligated paw may be occurring, (Kim et al., 1997) we used separate control animals rather than having each animal serve as its own control. Two hundred ninety-one rats were used for the study as reported, and each rat was used only once.

CCI procedure

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 by blunt dissection through the biceps femoris. Proximal to the sciatic trifurcation, about seven mm of nerve was freed of adhering tissue and four ligatures (4.0 chromic gut) were tied loosely around the nerve, about 1 mm apart. The length of the nerve affected was 4–5 mm long. The ligatures were tied to lightly constrict the diameter of the nerves when viewed with 40× magnification, and standardized by observing for the initial twitching of the paw as the ligature was tightened. The muscle was sutured with 4.0 chromic gut 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 procedures

To determine the effect of LH stimulation on thermal nociception, the PWL was used. The paw was exposed to a focused beam of radiant heat using an analgesiometer (37360, Ugo Basile, Italy). Using a heat flux radiometer, the radiant beam of each machine was adjusted to a maximum intensity of 130 mW/cm^2. 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 20 s to prevent burning. Paw response latencies were measured at one place on the hairy surface of each hind paw. Baseline response latencies were approximately 6–8 s. Temperature was measured with a rectal probe pre-injection, then at 5-min post injection and every 10 min thereafter. Heart rate, blood pressure and mean arterial pressure were measured preinjection and following the final latency measurement using a tail cuff and Coda monitor (Kent Scientific; Torrington, CT, USA).

Estrodial/progesterone measurement

After thermal testing, cardiac puncture was used to draw blood for determining the serum estradiol/progesterone ratio in females to use for covariate analysis if indicated. Following completion of analgesic testing, female rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, IP) and the chest opened. Using a 26-g needle attached to a 3-cc syringe, the cardiac ventricle was punctured and 1.5 cc of blood withdrawn. The blood was then injected into a serum separator tube, gently inverted two to three times and left to sit for 30–60 min. The tube was then spun on a microcentrifuge for 15 min at 3100 rpm, and refrigerated at −70 °C until a batch was ready to send for analysis (Antech Diagnostics; Morrisville, NC, USA).

Experiment 1: Nociceptive responses in CCI vs. non-CCI rats. To demonstrate that CCI ligation produced thermal hyperalgesia, male and female rats received CCI ligation of the left leg or sham surgery for control. In sham surgery, the sciatic nerve was exposed to the air but not ligated. Two weeks later each rat was lightly anesthetized with sodium pentobarbital (35 mg/kg, IP). Response latencies were then measured at 1 min and then every 5 min for 45 min.

Experiment 2: Carbachol or saline microinjection in the LH. Male and female rats were randomly assigned to pain condition (nociceptive or neuropathic) and to carbachol dose (125, 250, 500 nmol in 0.5 µl normal saline). Rats in the neuropathic group received CCI ligation, as described above, 2 weeks prior to Experiment 2. Rats in the nociceptive group did not receive ligation. Each rat was lightly anesthetized as described above and the scalp infused with the local anesthetic lidocaine (1%; 0.15 ml). Supplemental doses of pentobarbital were given during the procedure if the rats vocalized or moved without stimulation, but were rarely required. Immediately after anesthesia, the midline scalp was shaved and the rats were immobilized