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SEX-DEPENDENT ROLES OF PROLACTIN AND PROLACTIN RECEPTOR IN POSTOPERATIVE PAIN AND HYPERALGESIA IN MICE

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Abstract—Although surgical trauma activates the anterior pituitary gland and elicits an increase in prolactin (PRL) serum levels that can modulate nociceptive responses, the role of PRL and the PRL-receptor (PRL-R) in thermal and mechanical hyperalgesia in postoperative pain is unknown. Acute postoperative pain condition was generated with the use of the hindpaw plantar incision model. Results showed endogenous PRL levels were significantly increased in serum, operated hindpaw and spinal cords of male and female rats 24 h after incision. These alterations were especially pronounced in females. We then examined the role of the PRL system in thermal and mechanical hyperalgesia in male and female mice 3–168 h after plantar incision with the use of knock-out (KO) mice with PRL or PRL-R gene ablations and in wild-type (WT) mice. WT mice showed postoperative cold hyperalgesia in a sex-dependent manner (only in females), but with no effect on heat hyperalgesia or mechanical allodynia in either sex. Studies in KO mice showed no effect of PRL and PRL-R gene ablation on heat and cold hyperalgesia in male mice, while heat hyperlgesia were reduced 3–72 h post-surgery in female PRL and PRL-R KO mice. In contrast, PRL and PRL-R ablations significantly attenuated mechanical allodynia 3–72 h post-surgery in both male and female mice. Overall, we found elevated PRL levels in serum, hindpaws and spinal cords after incision, and identify a contributory role for the PRL system in postoperative pain responses to thermal stimuli in females and to mechanical stimuli in both males and females. © 2013 Published by Elsevier Ltd. on behalf of IBRO.

Key words: prolactin, prolactin receptor, post operative model, pain, sex-dependence.

INTRODUCTION

The management of postoperative pain is an important healthcare issue since ineffective treatment of postoperative pain can prolong recovery, and increase both morbidity and mortality after surgery. Even though considerable progress has been made in understanding mechanisms contributing to postoperative pain (Pogatzki et al., 2002; Martin et al., 2005; Pogatzki-Zahn and Zahn, 2007; Wu, 2011; Steyaert, 2012; Deumens et al., 2013), a large percentage of patients still experience inadequate pain relief following surgical procedures (Phillips, 2000; Raghunathan, 2013).

Findings from clinical studies indicate that women are at a substantially greater risk than men for certain aspects of postoperative and procedural pain (Fillingim et al., 2009). However, studies show male and female mice baseline responses to heat and mechanical nociception are not statistically different in a model of acute postoperative pain (Banik et al., 2006). Nevertheless, a number of physiological and pharmacological differences have been revealed regarding sex-dependency in responses to anesthesia and analgesic therapy in humans (Fillingim and Gear, 2004). Postoperative pain is conventionally managed with non-steroid anti-inflammatory drugs, acetaminophen, opioids and peripheral acting anesthetics (Kehlet et al., 2006; Butler et al., 2011; Wu, 2011). In this respect, women are more sensitive than men to opioid receptor agonists and to certain neuroblocking agents (Campesi et al., 2012) and these differences suggest that optimum analgesic regimes may be sex-dependent.

The sex-dependency in postoperative pain remains poorly understood, yet it appears that sexual hormones may play an important role (Fillingim et al., 2009; Campesi et al., 2012). The response of the hypothalamic–pituitary–adrenal axis in humans to surgery is well documented (Noel et al., 1972; Anand, 1986; Reiner and Oreskovic, 1987). Stress, surgical trauma and postoperative conditions activate the anterior pituitary gland in humans and elicit an increase in serum levels of prolactin (PRL), an important sex hormone (Noreng et al., 1987; Yardeni et al., 2007). Plasma PRL concentrations in humans remain increased for up to 1–2 weeks postoperatively with a gradual return to normal levels (Chernow et al., 1987). Certain long-acting local anesthetics can decrease plasma levels of PRL in humans as well (Reiz et al., 1989). Inflammation is present postoperatively in both animals and humans (Moore et al., 1994; Wu, 2011),

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Abbreviations: ANOVA, analysis of variance; KO, knock-out; PRL, prolactin; PRL-R, PRL-receptor; PRL KO, PRL null-mutant; PRL-R KO, PRL-R null-mutant; WT, wild-type.

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and can contribute to elevated systemic as well as local PRL levels (Berczi et al., 1984; Mateo et al., 1998; Scotland et al., 2011). Animal studies demonstrate that PRL can modulate the immune system (Bernton et al., 1988; Tseng and Kessler, 1997) that plays a critical role in activation of nociceptors (Woolf et al., 1997). Importantly, PRL directly sensitizes nociceptors in rats (Diogenes et al., 2006). Moreover, pro-nociceptive actions of PRL could vary in males versus females, since PRL levels during inflammation are sex-dependent in humans and animals (Jimena et al., 1998; Mateo et al., 1998; Giraldo et al., 2008; Scotland et al., 2011). Altogether, despite the wealth of information on PRL elevation in serum of humans and animals during postoperative conditions, certain issues are still not clear. Questions remain on whether acute operative interventions trigger an increase in endogenous PRL locally at the site of surgical intervention or at distant sites such as the spinal cord that are important in processing pain signals, the specific contribution of the PRL system to modulating nociceptive responses during the acute postoperative pain period and if this contribution is sex-dependent? To investigate these questions, we measured endogenous PRL levels in serum, operated and non-operated hindpaws and spinal cords of male and female rats 24 h after incision procedures on hindpaws. We also compared pain behaviors in wild-type (WT) versus PRL or PRL receptor (PRL-R) null-mutant (knockout; KO) male and female mice with the use of the hindpaw plantar incision postoperative pain model.

EXPERIMENTAL PROCEDURES

Animals

All animal experiments conformed to APS's Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training, and to protocols approved by the University Texas Health Science Center at San Antonio (UTHSCSA) Animal Care and Use Committee (IACUC). We also followed guidelines issued by the National Institutes of Health and the Society for Neuroscience to minimize the number of animals used and their suffering.

Adult male and female Sprague–Dawley rats (200–250 g, Charles River Laboratories, Wilmington, MA) were housed three per cage under a 12-h-light/12-h-dark cycle with food and water available *ad libitum*. Adult female and male PRL null-mutant (PRL KO), PRL-R null-mutant (PRL-R KO) and corresponding littermate WT mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). PRL KO and PRL-R KO mice are viable, normal in size and do not display any gross physical or behavioral abnormalities. The homozygous PRL KO females are infertile and have an irregular estrous cycle. Male and female homozygous PRL-R KO mice are completely sterile, and the serum PRL levels are increased 60–100-fold (Ormandy et al., 1997). The serum estradiol and progesterone levels are moderately decreased in estrus PRL-R KO females (estradiol: from 53 pg/ml for WT to 37 pg/ml for PRL-R KO; progesterone: from 17 ng/ml for WT to 7 ng/ml for PRL-R KO; (Clement-Lacroix et al., 1999). The serum total testosterone levels are at similar levels in WT and PRL-R KO male mice (Clement-Lacroix et al., 1999). PRL KO mice were generated via targeted disruption of coding exon 4, which results in a truncated 11 kDa-long PRL protein (normal PRL size is 24 kDa) that lacks any detectable bioactivity (Horseman et al., 1997). PRL-R KO mice were produced via creating an in-frame stop codon in exon 5 (Ormandy et al., 1997). The lack of functional PRL-R in homozygous mutant animals was confirmed using northern, western, and binding assays (Ormandy et al., 1997). PRL and PRL-R KO mice were produced in C57BL/6J line. Adult mice weighing 20–30 grams were used in the study. All animals were housed in a 12-h light–dark cycle.

Measurement of endogenous PRL

We have selected male and female rats for measurement of endogenous PRL, because reliable anti-mouse PRL are not available. Tissues and serum were collected 24 h after sham and incision operative procedures on hind paws of animals (Pogatzki and Raja, 2003). Rat paw samples were collected with 6-mm biopsy punches (Healthlink®, Fray Corp., Buffalo, NY). The L4-L6 lumbar spinal cord was isolated. Serum samples were collected in blood collection tubes with sodium citrate (BD Biosciences, Franklin Lakes, NJ, USA). Protein extracts were generated by adding 200–500 µl T-PER solution (Thermo Scientific, Rockford, IL, USA) to samples, and disrupting them with TissueLyser LT (Millipore, Billerica, MA, USA) at 50 oscillations per min for 5 min. Protein extracts were stored at −20 °C until assayed using a commercially available rat PRL EIA kit (SPIbio, Montigny le Bretonneux, France, distributed by Cayman Chemical). Amount of protein in extracts was measured by Bradford assay (Scotland et al., 2011). Endogenous PRL levels in serum were presented as ng/ml of serum. Endogenous PRL levels in tissues were presented as ng/ml of protein extract. These values were normalized against total amount of protein in extracts.

Acute postoperative pain model

All behavioral experiments were conducted by a blinded observer. Since PRL KO and PRL-R KO female mice have irregular estrous cycles, surgical procedures on female mice were conducted in the morning on mice in estrus phase (Caligioni, 2009). The reproductive stage of cycling females was determined by vaginal lavage using methods previously described (Marcondes et al., 2002). Thermal and mechanical nociception were mainly measured in mice in estrus cycle, which have approximately 40–65 pg/ml of estradiol (Clement-Lacroix et al., 1999). However, 7 days post-incision, nociception in some female mice was measured in metestrus phase that is associated with low levels of E2. The plantar incision in mice was used as a model of acute postoperative pain as previously described (Pogatzki and Raja, 2003). Mice were anesthetized using 2% isoflurane. The right hindpaw was prepared for incision

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