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Sex differences and estradiol involvement in hyperalgesia and allodynia in an experimental model of fibromyalgia

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ABSTRACT

Fibromyalgia (FM) is a musculoskeletal chronic pain syndrome. Its prevalence in women is higher than in men possibly by hormonal factors given that symptoms are aggravated during sex hormone-related events, such as the premenstrual period, pregnancy, postpartum or menopause. The aim of the present study was to investigate whether hyperalgesia and allodynia, in reserpine-induced experimental FM, depend on sex, estrous cycle, ovariectomy and replacement with 17β-estradiol. To fulfill this objective, we compared males, intact females with known estrous cycle phases and ovariectomized (OVX) rats treated with 17β-estradiol. Data demonstrated that reserpine administration disrupted the normal estrous cycle and produced that all females entered metestrus/diestrus. In addition, this treatment leads to muscle hyperalgesia and tactile allodynia in a similar manner in male and intact female rats. However, the absence of ovarian hormones (in OVX rats) increased muscle nociception. 17β-estradiol (2.5–10 μg/rat) produced antihyperalgesic and antiallodynic effects 24 h, but not 8 h, after its administration, suggesting a genomic mechanism. The present results support the validity of the reserpine-induced FM model for searching alternatives of treatment, particularly during endocrine phases when pain is exacerbated such as menopause, and that 17β-estradiol replacement might be useful.

1. Introduction

Fibromyalgia (FM) is a musculoskeletal chronic pain syndrome defined by widespread pain. In 2010, the American College of Rheumatology endorsed a new set of criteria for diagnosing fibromyalgia according to which patients must fulfill three conditions: 1) a high widespread pain index (WPI), a moderate symptomatology scale (SS) or lower WPI accompanied by an elevated SS; 2) a similar level of symptoms for at least 3 months, and 3) the absence of another disorder that could explain the pain (Wolfe et al., 2010). The WPI denotes the number of areas with pain and the SS considers the most frequent concomitant symptoms, including disturbed sleep, pronounced fatigue and affective disorders, such as depression (Crofford, 2009; Wolfe et al., 1995a; Wolfe et al., 1995b). FM is a common disorder estimated to affect about 2% of the general population. Its prevalence remains relatively stable in men across their lifetime, while in women increases peaking between ages 55–64 years old (McNally et al., 2006). Criteria for FM, published in 1990, revealed a 9:1 ratio of women to men; such ratio decreased after the criteria modification in 2010, notwithstanding there is a higher prevalence in women (Clauw, 2014; Wolfe et al., 1995a). Gender-specific differences suggest a less effective pain inhibitory mechanism in women as compared to men that may account for the development and maintenance of FM (Staud et al., 2003). Actually, the FM symptoms are aggravated during sex hormone-related events such as the premenstrual period, pregnancy or postpartum (Pamuk and Cakir, 2005). Moreover, the FM patients have higher levels of premenstrual discomfort and dysmenorrhea, as well as less positive mood during the luteal phase than healthy controls, suggesting that cyclic fluctuations in ovarian hormones could be linked to pain perception (Alonso et al., 2004). Indeed, the menopause age-of-onset [worldwide most women enter menopause between 49 and 52 years, (Morabia and Costanza, 1998)] might be a factor that enhances painful and non-painful sensitivity (Martinez-Jauand et al., 2013). These results support the notion that an abrupt decline or a reduced time exposure to ovarian hormones may contribute to hypersensitivity in musculoskeletal chronic pain syndromes such as FM.

A vast literature shows that 17β-estradiol possesses neuroprotective (Garcia-Segura et al., 2001), anti-inflammatory (Vegeto et al., 2008)

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and antiallodynic (Vacca et al., 2016) activities, and strongly influences neuroimmune communication pathways (Brown et al., 2010). All these mechanisms have been proposed to participate in the etiology of FM (Ablin et al., 2008; Arnold, 2006; Legangneux et al., 2001; Russell et al., 1994; Russell et al., 1992; Staud, 2011). Pharmacological treatment for FM includes pregabalin, pramipexole, fluoxetine and duloxetine (Chinn et al., 2016). However, adverse effects and relatively poor response to treatment promote discontinuation in many patients. Therefore, it is necessary to develop novel therapies that have to be tested in fully validated animal models. Thus, several models have been proposed: 1) stress loading models, such as forced swim, chronic restrain, repeated cold, sound stress and neonatal limited bedding stress; 2) chemical injections models using reserpine, acidic saline or carrageenan; and 3) nerve injury models (DeSantana et al., 2013). In the myalgia induced by reserpine, nociception is produced by a dysfunction of the descending pain inhibitory system resulting from monoamine depletion by reserpine (Nagakura et al., 2009).

In the present study, we used the reserpine-induced experimental FM model in male and female [intact and ovariectomized (OVX)] rats to investigate changes in muscle hyperalgesia and tactile allodynia over a time course of three weeks. The group of OVX rats simulates an endocrine condition of absence of estrogens characteristic of menopause. Finally, we investigated the effect of 17β-estradiol replacement.

2. Methods

2.1. Animals

Male and female Wistar rats (200–250 g body weight) obtained from the vivarium of Cinvestav (Unidad Coapa) were used in this study. Rats were housed (five per cage) in acrylic cages (44 cm width × 33 cm length × 20 cm height) with water and food freely available that were placed in a controlled temperature (22 ± 1 °C) and a controlled light inverted cycle (12-h light/12-h dark) (lights off at 10:00 h) room. All experimental procedures followed the national (NOM-062-ZOO-1999) and international guidelines on ethical standards for investigation in animals and were carried out according to a protocol approved by the local Animal Ethics Committee.

The number of experimental animals was kept to the minimum needed to observe significant effects.

2.2. Ovariectomy

Bilateral ovariectomy was conducted under anesthesia with 2% 2,2,2-tribromoethanol (10 ml/kg, i.p.). The ovaries were removed from the lower central abdominal cavity; the abdominal wall and skin were sutured and locally treated with 50% benzalkonium chloride. Animals were allowed a recuperation of a minimum of 15 days after surgery. On this day, the presence of diestrus was confirmed by vaginal smear.

2.3. Drugs

2,2,2-Tribromoethanol was used as anesthetic; it was dissolved in 10% ethanol (96% ethanol diluted in 0.9% saline solution) and administrated intraperitoneally in a volume of 10 ml/kg. 17β-estradiol was dissolved in corn oil. Reserpine was dissolved in acetic acid diluted to a final concentration of 0.5% with distilled water. All drugs were purchased from Sigma (Sigma-Aldrich Co., St Louis, MO) and freshly prepared on the day of experiments. Treatments were subcutaneously administered in a volume of 1 ml/kg.

2.4. Experimental FM model

The reserpine-induced myalgia was induced as previously reported (Nagakura et al., 2009). Reserpine (1 mg/kg) was subcutaneously administered into the loose skin over the neck every 24 h during three consecutive days. From the first day of injection onwards, the animals were placed individually in acrylic Plexiglass boxes (17 cm width × 27 cm length × 20 cm height) with access to water and food ad libitum.

2.5. Muscle pressure threshold

Mechanical hyperalgesia (tenderness to palpation) is a main characteristic of FM; thus, the muscle pressure threshold was measured using the Randall-Selitto apparatus (Ugo-Basile, Italy). For this, rats were immobilized and their midgastrocnemius muscle was submitted to a linear incremental pressure until the hind limb was withdrawn or vocalizations were elicited. The maximum force applied was 250 g (Schafers et al., 2003). The average of three consecutive tests with an inter-stimulus interval of 1 min was considered as the muscle pressure threshold (Nagakura et al., 2009).

2.6. Tactile response threshold

Tactile allodynia is other common characteristic of FM. Thus, the tactile response threshold was determined using the von Frey filaments (North Coast medical, USA) following the up and down method (Chaplan et al., 1994). Rats were individually placed in transparent compartments on an elevated metal grid and allowed to habituate for at least 20 min before testing. The filaments (0.6, 1, 1.4, 2,4, 6, 8, 10 and 15 g) were applied from underneath the grid to the plantar surface of the right hind paw. Brisk paw withdrawal from the pressure of a filament was defined as a positive response and lack of paw withdrawal within 6 s was defined as a negative response. The first filament applied was 2 g, if a positive response occurred, it was assessed the next smaller filament. However, if a negative response occurred, then the next higher filament was applied. The test continued until four responses were collected after the first positive change. The response pattern obtained was converted to the tactile response threshold according to the following formula: $10[Xf - k\delta] / 10000$, where Xf is the log value of the von Frey hair used, k is a tabular value for the pattern of responses (Chaplan et al., 1994) and δ equals the mean difference (log units) between stimuli.

2.7. Experimental design

In order to investigate generalized nociception, the muscle hyperalgesia and tactile allodynia were measured in the right and left hind limbs of rats treated with reserpine or its vehicle. To investigate sex differences, muscle hyperalgesia and tactile allodynia were compared between male and female rats. Baseline thresholds were measured in 12 male, 12 intact females (without registering their estrous cycle), and 14 OVX rats in both right and left hind limb. Rats of the same sex or endocrine condition were divided in two groups (n = 6 or 7), the first group received only the vehicle of reserpine, while the second one was injected with reserpine (1 mg/kg, s.c.) for three consecutive days. Muscle hyperalgesia and tactile allodynia were again measured on days 1, 3, 5, 7, 10, 14 and 21 after the last vehicle or reserpine injection.

To analyze the putative influence of the estrous cycle variations on the changes in nociceptive thresholds induced by reserpine, 14 intact female rats were selected. Their estrous cycle was monitored by daily vaginal smears 14 days before reserpine/vehicle administration and five days after.

In order to determine the influence of estrogen on these responses, 17β-estradiol (1.25, 2.5, 5.0 and 10 μg per rat) was injected to OVX rats. Baseline thresholds were measured in 56 OVX rats that were divided in seven groups (n = 8): five of them received reserpine and five days later were injected with oil or 17β-estradiol at 1.25, 2.5, 5 and 10 μg/rat (Ford et al., 2004; Kramer and Bellinger, 2009; Leuner et al., 2004; Uban et al., 2012). The sixth group received reserpine but no other treatment (FM control group), and the seventh group did not