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Ovariectomy-induced chronic abdominal hypernociception in rats: Relation with brain oxidative stress

[Hipernocicepción abdominal crónica inducida por ovariectomía en ratas y su relación con el estrés oxidativo cerebral]

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Abstract

Context: Ovarian hormone deficiency observed in menopausal women increases the production of reactive oxygen species, which could be implicated in central sensitization subjacent in chronic functional pain syndromes.

Aims: To examine the hyperalgesic state induced by ovariectomy in adult rats and its relation to some oxidative stress outcomes.

Methods: The female Wistar rats were divided into normal, sham ovariectomized (OVX) and OVX groups, which were tested for mechanical and thermal hypernociception during 6 weeks and a single acetic acid-induced test 6 weeks after surgery. Redox biomarkers determinations of superoxide dismutase (SOD) enzyme activity, glutathione (GSH) and nitrates/nitrites as an indicator of nitric oxide (NO) concentrations were determined in the brain and cerebellum of 6 animals of each group.

Results: Exclusivity OVX rats developed a robust state of mechanical hypernociception and allodynia in the abdomen, hindlimbs and proximal tail. Besides, thermal pain thresholds (hot plate) decreased. That was established 3-4 weeks after OVX and lasted for the 6 weeks of the experiment. Increases in visceral sensitivity were also observed in OVX rats. SOD enzyme activity decreased in OVX rats, which showed major deficit for this enzymatic defense under visceral inflammatory injury. However GSH concentrations were increased in brain of OVX animals that allow the balance during acute inflammation. NO concentrations were raised only in OVX rats exposure to chemical inflammatory injury.

Conclusions: OVX in rats provide a useful model, which mimics the functional pain in females that could be related with brain oxidative stress.

Keywords: Functional pain syndromes; hyperalgesia; hypernociception; ovariectomized rats; ovariectomy; pain.

Resumen

Contexto: La deficiencia de las hormonas ováricas en la mujer menopáusica incrementa la producción de especies reactivas de oxígeno que han sido implicadas en la sensibilización central subyacente en los síndromes de dolor funcional crónico.

Objetivos: Examinar el estado hiperalgésico inducido por ovariectomía en ratas adultas y su relación con algunas variables de estrés oxidativo.

Métodos: Ratas Wistar femeninas fueron divididas en grupos: normal, falsas ovariectomizadas (OVX) y OVX, para la evaluación de hipernocicepción mecánica y termal durante 6 semanas, así como la prueba de contorsiones abdominales inducidas por ácido acético a las 6 semanas tras la cirugía. La actividad de la enzima superóxido dismutasa (SOD), concentraciones de glutatión reducido (GSH) y de nitratos/nitritos como indicador de la producción de óxido nítrico (NO) fueron determinadas en cerebros y cerebelos de 6 animales de cada grupo.

Resultados: Las ratas OVX desarrollaron hiperalgesia mecánica y alodinia en abdomen, patas traseras y cola proximal, así como un descenso de sus umbrales al dolor térmico (plato caliente). Estos cambios fueron establecidos 3-4 semanas post-OVX y mantenidos durante las 6 semanas del experimento. La sensibilidad visceral también fue incrementada. La actividad de SOD disminuyó en las ratas OVX, que mostraron mayor deficiencia para la defensa enzimática ante la injuria inflamatoria visceral. El GSH fue incrementado en el cerebro de los animales OVX, lo cual podría facilitar el balance durante la inflamación aguda. El NO solo incrementó en las ratas OVX expuestas al daño químico inflamatorio.

Conclusiones: OVX en ratas provee un modelo beneficioso que mimetiza el dolor funcional en mujeres y que podría estar relacionado con el estrés oxidativo cerebral.

Palabras Clave: Hiperalgesia; hipernocicepción; dolor; ovariectomía; ratas ovariectomizadas; síndromes de dolor funcional.

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INTRODUCTION

Functional pain syndromes (FPS) grouped a certain functional somatic and visceral disorders that present pain or discomfort in the absence of physiological or organic causes (Mayer and Bushnell, 2009). Nowadays, irritable bowel syndrome, chronic pelvic pain, fibromyalgia, temporomandibular joint disorder, vulvodynia and painful bladder syndrome/interstitial cystitis are receiving attention from researchers to find the possible pathophysiological mechanisms (Phillips et al., 2011).

The majority of patients diagnosed with functional abdominal pain are women, and it appears to be associated with its reproductive history, although post-menopausal women are affected (Lathe et al., 2006; Phillips and Clauw, 2011). After that is suggested that the pain correlates with the levels of circulating estrogens rather than with an organic disease of the abdominal or pelvic organ (Sanoja and Cervero, 2005). In general, the experimental and clinical data suggests a close relationship between sex gonadal hormones and pain perception (Greenspan et al., 2007). Both androgen and estrogen receptors, as well as synthetic pathways for sex hormones, have been found in pain circuits in the peripheral and central nervous system (CNS) (Hassan et al., 2014; Reichling et al., 2013). Estrogens may also indirectly affect pain via their modulation of skeletal, cardiovascular and immune systems. Subsequently, the estrogenic modulation of pain is an exceedingly complex, multi-faceted phenomenon, with estrogens producing both pro- and anti-nociceptive effects that depend on the extent to which each of these systems of the body is involved in a particular type of pain (Craft, 2007). Despite controversial results in humans and animals concerning estrogens effects on pain signaling, the data suggest that its acute administration tested in acute noxious procedures results in enhanced nociception. Whereas long-term loss of estrogens produces a hyperalgesic state, that may be prevented and reversed by exogenous estrogens (Cervero and Gebhart, 2009). A plausible explication suggested by these authors implicate the genomic effects of nuclear estrogen receptors in chronic effects and the faster estrogen membrane coupled to intracellular second-messenger pathways (GPR30) in acute actions (Craft 2007; Cervero and Gebhart, 2009).

On the other hand, different studies suggest that reactive oxygen species (ROS) are important for central or peripheral sensitization in pain (Kim et al., 2006; Gao et al., 2007; Salvemini et al., 2011).

Also, antioxidant agents are today accepted as a therapeutic option for chronic pain, and these are incorporated in the novel neuroprotective strategies for the neuropathic pain management (Bordet and Pruss, 2009). Particularly, the ovarian hormones show a recognized neurotrophic and neuroprotective effects (Baker et al., 2004; Suzuki et al., 2009; Arevalo et al., 2010; Cerciat et al., 2010). Estrogen acts as a free radical scavenger and degrades the ROS produced during cellular oxidation processes (Borrás et al., 2005; Evsen et al., 2013). Ovarian hormone deficiency observed in menopausal women also increases the production of ROS, which could result in oxidative stress and cell damage that could facilitate many diseases at this lifetime (Shrivastava et al., 2005). Ovariectomized (OVX) female rats are recognized as an animal model of the clinical features of the postmenopausal period related to its high free radical generation in brain tissues (Evsen et al., 2013). Moreover, pain hypersensitivity was reported in OVX rats, which could be reversed by administration of exogenous estrogen (Gaumond et al., 2002; 2005). For dissecting this complex phenomenon a useful rational model of functional visceral pain induced by ovariectomy in adult mice was developed (Sanoja and Cervero, 2005). The authors documented a chronic abdominal hyperalgesic state of slow onset and long duration, as well as its sensibility to estrogen replacement therapy (Sanoja and Cervero, 2005; 2008).

The present study examines the hyperalgesic state induced by ovariectomy in adult rats to investigate if it could induce a similar state of hyperalgesia that mimics the process of functional pain in females. As well, its relation with some brain oxidative stress biomarkers, which could be implicated in the central hiperexcitabiliy proposed for FPS.

MATERIAL AND METHODS

Experimental animals

Experimental procedures were carried out in accordance with European regulations on animal protection (Directive 86/609), the Declaration of Helsinki, and/or the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institute of Health (NIH Publication № 85-23, revised 1996). All experimental protocols were approved by the Institutional Animal Care and Ethical Committee from the Center for Drugs Research and Development (CIDEM, La Habana, Cuba). Pubertal female Wistar Albino (five weeks) but virgin rats about 120–150 g were obtained from the Center for Experimental Animals Production (CENPALAB, La Habana, Cuba). They were kept under controlled conditions (22 ± 0.5°C, relative humidity 40-60% at 7 a.m. to 7 p.m. alternate light-dark cycle, food, and water *ad libitum*). The experiments took place during the light period, and animals belonging to the various treatment groups were tested in randomized order.

Animal model of functional abdominal pain induced by ovariectomy in rats

Ovariectomy

Ovariectomy was performed by a dorsal approach under anesthesia with xylazine (13 mg/kg, i.m.) and ketamine (87 mg/kg, i.m.). A small incision was made in the abdominal wall on each side of the rat. The ovary, oviduct, and top of the fallopian tube were ligated and removed. Abdominal wall and skin were sutured. In sham-operated controls, an identical operation was performed but without remove of reproductive organs. Postoperative periods were uneventful.

Experimental protocols

Development of referred hypernociception after OVX: In the first experiment were used 44 rats. The aim of this experiment was to reproduce the estrogen-dependent hyperalgesia model describing in OVX adult female C57/BL6 mice (Sanoja and Cervero, 2005). To investigate if OVX rats could show referred hypernociception and its time course according to the experimental protocol utilized by the authors, considering possible differences between species. The rats were divided into normal control (n = 10), sham OVX (n = 17) and OVX (n = 17) groups and were tested for mechanical and thermal hypernociception two or three times per week for six weeks.

Visceral pain and OVX: In the second experiment were used 42 rats divided into six groups normal control, sham and OVX groups exposed to chemical irritant or control saline (n = 7 animals each). A single test of visceral pain was carried out during six weeks after surgery. It was performed the acetic acid-induced writhing, a tonic model of visceral pain (Deyama et al., 2007; Tanimoto et al., 2003).

Vaginal smears

Vaginal smears were taken from control, sham-operated and OVX rats and processed (Nelson et al., 1982). In first experiment, vaginal smears were taken from all rats at the end of each test session to record the phase of the cycle at the time of the test. After 6 - 7 weeks of post-surgery all animals were sacrificed by an overdose of ether and necropsies were performed to confirm the OVX or the sham procedures and to remove the internal reproductive organs for weighing.

Behavior tests

Behavioral tests began a week after surgery and continued, between one and three times per week, until week sixth after surgery.

Mechanical stimulation

The frequency of withdrawal responses to the application of von Frey filaments to the abdomen, forepaws, hindpaws, and the first two cm of the proximal tail was examined as tests of referred mechanical hypernociception (Sanoja and Cervero, 2005). It was used a plastic box divided into six small chambers (21 x 16 x 27 cm each) with a wire mesh floor. A rat was placed in each of the boards so that six rats were tested sequentially. The animals were allowed to adapt to the chamber for 20 min before

testing began. Five von Frey filaments with increasing exponential forces of 1, 4, 8, 16 and 32 mN were applied five times each in ascending order of force and the number and intensity of withdrawal responses noted. The filaments were applied for 1–2 s with an interstimulus interval of 5 s. Care was taken not to stimulate the same point twice in succession to avoid learning or sensitization. Only an sharp withdrawal from the filament was considered to be a positive response.

Thermal stimulation

The hot-plate test for rats was used to measure the latency of hindpaw withdrawal response to thermal stimulation (Eddy and Leimbach, 1953). The plate was held at 52 ± 0.2°C, and the test ended when the rat licked one of the hindpaws or jumped with both hindpaws. Cut off time was set at 20 s. This test was applied 20 min after the end of mechanical stimulation.

Visceral pain test

The writhing test was carried out in rats (Deyama et al., 2007; Tanimoto et al., 2003) with minor modifications. Acetic acid solution (2%) in saline was injected i.p. (10 mL/kg), and the animals were placed in an acrylic observation chamber (34 x 30 x 17 cm). The number of writhing responses (abdominal cramps) was counted in 30 min after the injection of acetic acid.

Determination of redox biomarkers

Following the single test of visceral pain at six weeks after OVX surgery, the brain and cerebellum of six animals of each group were selected at random. Homogenates were prepared by homogenizing tissue samples in cold phosphate- buffered saline (0.01 M, pH 7.4) in ice, in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 4 000 ×*g* for 20 min at 4°C, and the resultant supernatant was used. Redox biomarkers were determined by spectrophotometric methods using a Pharmacia 1000 Spectrophotometer (Pharmacia, Uppsala, Sweden). Total protein content was measured with bovine serum albumin as standard (Bradford, 1976).

Estimation of reduced glutathione concentration

After precipitation of thiol proteins, the reduced glutathione (GSH) levels were measured with Ellman`s reagent (5,5`dithiobis-2-nitrobenzoic acid) (Sigma, USA) at 412 nm. Purified GSH (Sigma) was used to generate standard curves (Sedlak and Lindsay, 1968).

Estimation of superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined by using RANSOD kit (catalogue No. SD 125, Randox Labs, Crumlin, UK), where xanthine and xanthine oxidase were used to generate superoxide anion radicals ($O_2^{\bullet-}$). This free radical reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the inhibition degree of this reaction (Boehringer, 1987).

Estimation of nitrates/nitrites concentrations

Nitrates/nitrites (NO_3^-/NO_2^-) level, as a surrogate marker of nitric oxide ($NO^{\bullet}$), was determined by converting nitrates to nitrites using nitrate reductase (Boehringer Mannheim, Milan, Italy). Then, Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl)-ethylenediaminedihydrochloride in 0.25% phosphoric acid) was added (Granger et al., 1996). Samples were incubated at room temperature for 10 min and absorbance was measured at 540 nm.

Statistical analysis

Data were analyzed using the statistical program Graph Pad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Inter-group statistically significant differences were tested using one-way analysis of variance (ANOVA) followed by Bonferroni's or Newman-Keuls post-hoc tests for multiple comparisons. The results are presented as mean ± standard error of mean (SEM). $P < 0.05$ was considered statistically significant.

RESULTS

Long-term referred mechanical hypernociception after ovariectomy in rats

OVX rats showed a strong mechanical hypernociception restricted to the abdomen, proximal tail, and hindpaws but spared the forepaws (Figs. 1 and 2), which was evident from weeks fourth after surgery and continued significant for the week sixth of the observation ($p < 0.05$). Particularly for the abdominal region and lower extremities, augmented responses to mechanical stimulus were observed from the third week. Sham OVX and naïve control animals did not show signs correlated with allodynia or hyperalgesia. In general, mechanical hypernociception was detected in the application of 4 - 30 mN von Frey filaments used in this experiment (ranging from innocuous to noxious forces).

Long-term referred thermal hypernociception after ovariectomy in rats

As seen in Fig. 3A, OVX rats developed a progressive reduction of paw withdrawal latency to the hot plate that became significant from the third week after surgery and remained significant for the experimental period during six weeks ($p < 0.01$ and $p < 0.001$).

Visceral hypernociception after ovariectomy in rats

Injection of intraperitoneal acetic acid (2%) evoked a robust visceral pain behavior in OVX rats that was significantly different compared with sham and control animals ($p < 0.001$). The number of writhing responses increased in all animals respect to its controls treated with saline. Nevertheless, the OVX rats exhibited a significantly nociceptive behavior after saline injection (mechanical stimulus) compared with naïve control and sham animals ($p < 0.001$) (Fig. 3B).

Effects of the estrous cycle

The direct observation of vaginal smears in optical microscopy at the end of each test session showed that OVX rats remained in a diestrus-like phase during the experiment. The naïve and sham animals were found to be in different stages of the estrous cycle (Fig. 4). These rats exhibited responses significantly increased to mechanical stimulus when they were in the estrus phase ($p < 0.05$) (Fig. 4A). No changes in thermal sensitivity (hot plate) were detected in normal rats.

Weight of reproductive organs and changes in body weight after ovariectomy

OVX induced a substantial involution of the internal reproductive organs (uterus and vagina) indicated by a marked reduction in organ weight compared with control and sham animals ($p < 0.001$) (Fig. 4B).This fact in addition to diestrus-like phase in a vaginal smear of OVX rats corroborated the efficacy of OVX surgery. A significant increase in body weight in OVX animals seven weeks after surgery compared with its controls was observed ($p < 0.001$) (Fig. 4C).

Redox biomarkers in hyperalgesic OVX rats under chemical peritoneal inflammatory injury

Redox biomarkers (SOD enzyme activity, GSH, and NO concentrations) in the brain of OVX and sham animals exposure to viscero-somatic inflammatory injury and its controls injected with saline (mechanical injury) are given in Table 1. Sham-acetic acid animals showed SOD activity significantly reduced with respect sham-saline ($p < 0.01$). Likewise, OVX-acetic acid compared with OVX-saline exhibited a similar pattern ($p < 0.01$). However, enzyme activity in both groups of OVX rats was significantly reduced compared with its sham controls ($p < 0.05$ and $p < 0.01$, respectively). There was no significant difference between GSH concentrations in sham animals. OVX-saline rats showed an increase of GSH concentrations compared with sham-saline rats ($p < 0.001$). Nevertheless, GSH concentration in OVX-acetic acid animals was similar to sham rats and significantly different from OVX-saline group ($p < 0.001$). The concentrations of nitrites as an indicator of NO were similar in the different groups, except for the OVX-acetic acid rats. NO was increased in these animals compared with sham-acetic acid ($p < 0.01$) and with OVX-saline group ($p < 0.05$).

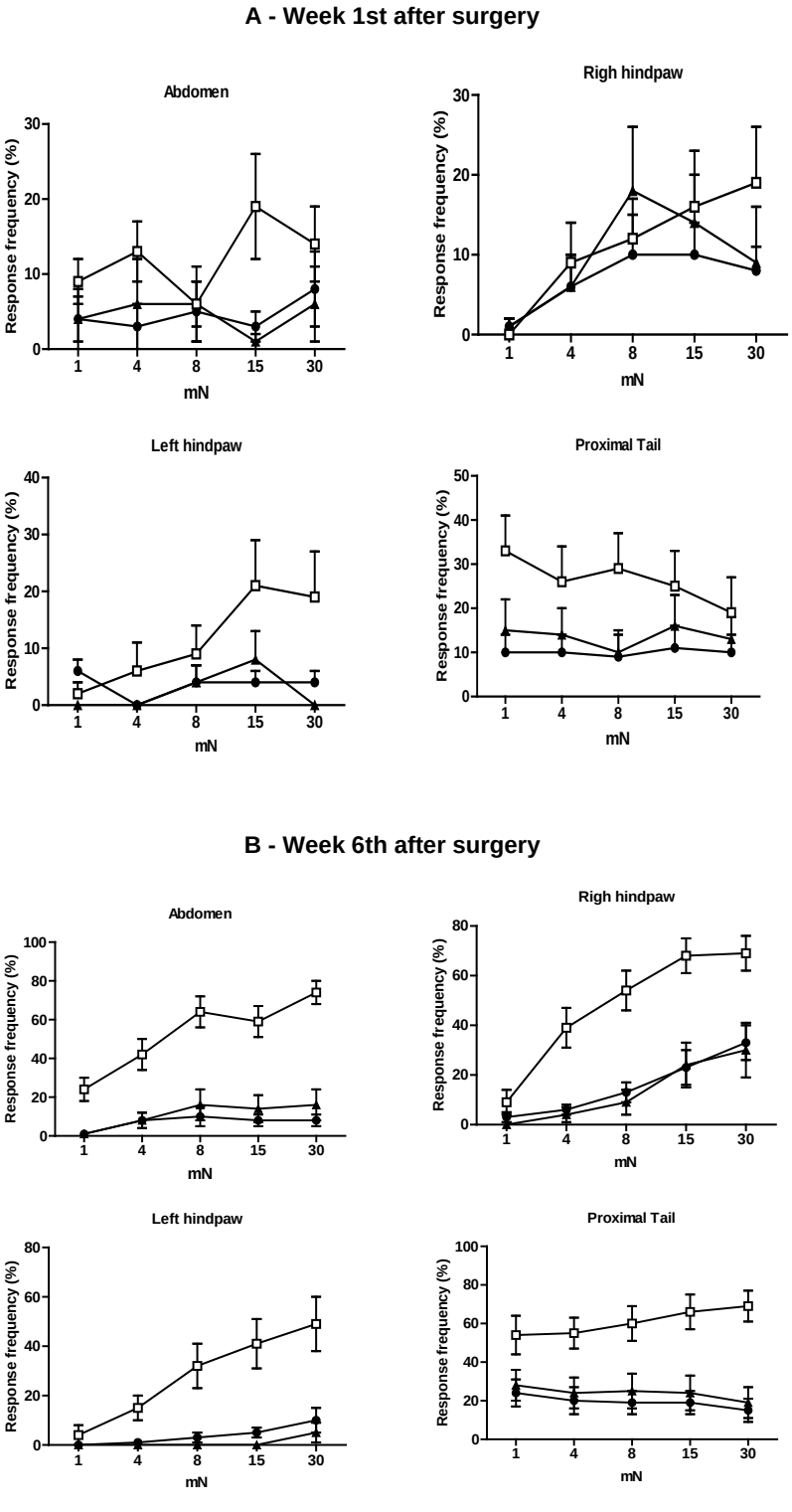

Figure 1. Responses to mechanical stimulation (von Frey filaments) of the abdomen, hindpaws and the proximal tail in ovariectomized (OVX), sham-operated (Sham) and control rats. The same animals were tested a week **(A)** and six weeks **(B)** after surgery. Data are shown as mean percent response frequency (± SEM) to five applications of each von Frey filaments. Significant differences ($P < 0.05$) were detected on week 6 after surgery between OVX animals and the other two groups (n = 10 - 17 rats per group).

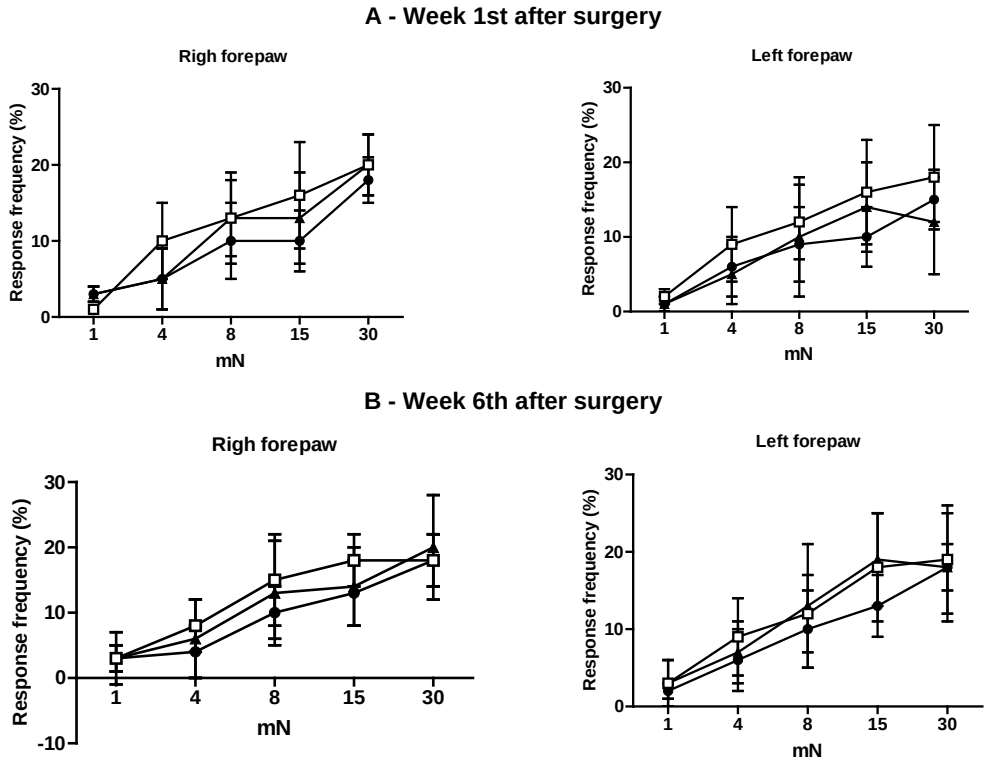

Figure 2. Responses to mechanical stimulation (von Frey filaments) of the forepaws in ovariectomized (OVX), sham-operated (Sham) and control rats. The same animals were tested a week **(A)** and six weeks **(B)** after surgery. Data are shown as mean percent response frequency (± SEM) to five applications of each von Frey filaments. No significant differences were detected in week sixth after surgery between OVX animals and the other two groups (n = 10 - 17 rats per group).

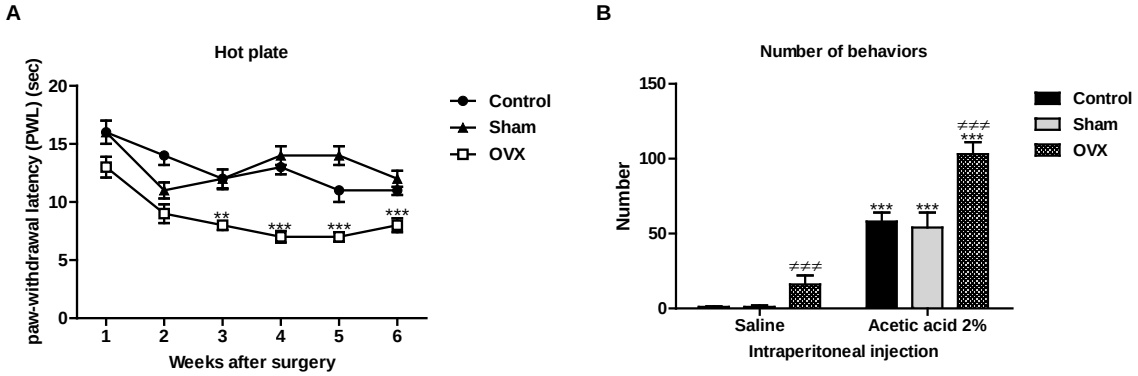

Figure 3. Responses to thermal and visceral chemical stimulation of ovariectomized (OVX), sham-operated (Sham) and control rats (n = 7 - 17 per group). **A.** Time course of paw withdrawal latency in the hot plate test. Animals were tested once a week for six weeks. Data are shown as mean latency response (± SEM). Significant differences; $^{**}p < 0.01$, $^{***}p < 0.001$ were detected in the OVX group from week third until the end of the experiment at week sixth. **B.** A single test of acetic acid-induced writhing, a tonic model of visceral pain was carried out six weeks after surgery. Data are shown as mean of a number of writhing responses (± SEM.), $^{***}p < 0.001$ represents the groups that were significantly differents from their respective saline-treated controls, $^{\#\#\#}p < 0.001$ represents significant differences between OVX rats and sham or control animals.

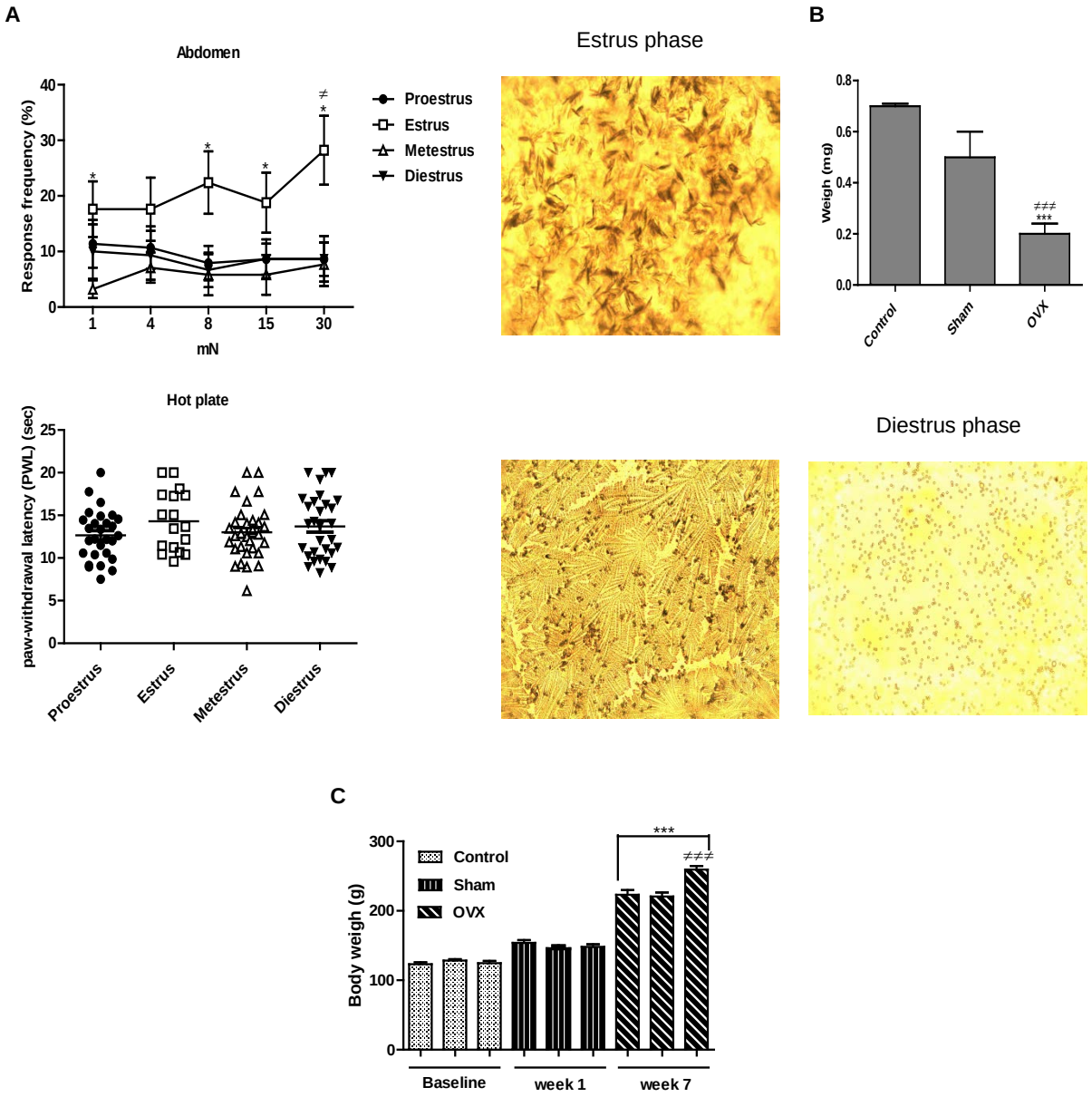

Figure 4.A. Responses to mechanical stimulation (von Frey filaments) of the abdomen in sham-operated (Sham) and control rats in relation with estrous cycle state. Each animal was tested at least twice in each of the four phases of the estrous cycle (evaluated by direct observation of vaginal smears taken at the end of each behavioral test). Each point of each the curves is the mean of between 17 and 31 observations. $^{*}P < 0.05$ indicates the significant differences in responses of sham rats in estrus with respect the other phases of the cycle. $^{\#}P < 0.05$ indicates the significant differences in responses of control rats in estrus with respect the other stages of the cycle. Down graphic: Hot plate test data from the same animals. The image at the right shows vaginal smears and at downs the crystallized cervical mucus in the presence of saline of these animals in estrous phase. **B.** The weight of reproductive organs in control, sham and OVX rats. Data are shown as mean weight ± SEM. Significant differences ($^{***}p < 0.001$ and $^{\#\#\#}p < 0.001$) were detected in the OVX group with respect Sham and control rats respectively. **C.** Body weights in control, sham and OVX rats. Data are shown as mean weight ± SEM. Body weights increased throughout the course of the experiment (seven weeks) in all animals, but at seven weeks OVX rats were significantly different compared with sham and control rats, $^{***}p < 0.001$ represents statistical differences with respect baseline data, $^{\#\#\#}p < 0.001$ represents significant differences between groups.

Table 1. Brain redox biomarkers in the exposure of hyperalgesic OVX rats to acetic acid-induced writhing test.

Treatment	Reduced glutathione (µM/mg protein)	Superoxide dismutase (U/mg protein)	Nitrates (µM/mg prot)
Sham-SS	6.11 ± 0.13	120.10 ± 13.62	109.0 ± 3.70
Sham-AcA	6.80 ± 0.67	73.86 ± 5.30$^{\#\#}$	140.2 ± 6.16
OVX-SS	12.26 ± 1.54***	87.18 ± 12.81*	164.8 ± 8.89
OVX-AcA	6.32 ± 0.80$^{\#\#\#}$	40.86 ± 4.36**,$^{\#\#}$	252.0 ± 28.51**,$^{\#}$

Sham-AcA and OVX-AcA rats were injected with acetic acid 2% (10 mL/kg, i.p.). Its controls sham-SS and OVX-SS received only saline (10 mL/kg, i.p.) and observed during 30 minutes. Values are given as mean ± SEM, n = 6 per group. $^{*}P < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ represent the statistical differences between each OVX group and its respective sham control. $^{\#}P < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ represent statistical differences between groups in the presence or absence of saline or acetic acid (one-way ANOVA following of Newman-Keuls)

DISCUSSION

Studies on changes in pain sensitivity in OVX rats shows controversial results, probably since the variability of experimental designs make it difficult unified the results. Several of these experiments using fast nociceptive tests reported the decrease of tail flick and hot plate thresholds of these animals (Forman et al., 1989; Kepler et al., 1989). Other studies report no effects on acute pain as the evoked by electric shocks (Beatty and Fessler, 1977). Otherwise, on thermal pain thresholds and formalin-induced pain in long term OVX rats (six months) (Ceccarelli et al., 2003) described very small or no changes in the responses to these noxious stimuli. On the other hand, there are also reports of increases in pain sensitivity in OVX rats that could be reduced by exogenous administration of estrogen (Gaumond et al., 2002; 2005; Mannino et al., 2007).

The model of chronic functional visceral pain induced by ovariectomy in adult mice (Sanoja and Cervero, 2005) provides a rational protocol to mimic the long-term hormonal decreased (as menopauses process). It is a similar paradigm to the clinical problem and could unify the ideas about the changes in pain sensitivity in females and the controversial role of estrogens. A significant observation of the present study was that OVX rats, similar to OVX mice, developed a powerful state of referred mechanical hyperalgesia and allodynia restricted to the abdomen and lower limbs of slow onset (3 - 4 weeks) and long duration (seven weeks). The main interpretation of the mechanisms of referred hyperalgesia is based in the theory of central sensitization, whereby CNS neurons become sensitized by the enhanced afferent drive from the primary focus (Woolf, 1983). In consequence, it would be enhanced pain sensitivity from the originating injury and the appearance of areas of hyperalgesia in the somatic areas whose afferent innervations converges with that of the damaged site in the spinal cord (Cervero and Gebhart, 2009). Particularly, extracellular signal-regulated kinases activation plays an important and specific role in the transcriptional events underlying the maintenance of referred visceral hyperalgesia (Galan et al., 2003).

Ovariectomy induces changes as atrophy in the internal reproductive organs that could generate a peripheral focus of nociceptive activity, however, in OVX mice hormone replacement pellets were unable to reverse the involution of the reproductive organs even though they reversed the hyperalgesic state (Sanoja and Cervero, 2005). This fact suggests that the referred hyperalgesia correlates with the levels of circulating estrogens rather than with an organic injury of the pelvic organ (Sanoja and Cervero, 2010). Estrogen receptors are localized in neurons of the superficial dorsal horn and lamina X (Amandusson et al., 1999) as well as in dorsal root ganglion (DRG) neurons of the lumbosacral segments (Bennett et al., 2003), which constitutes a neurochemical substrate for the action of circulating estrogens.

Several studies have reported that estradiol administration to OVX rats increases the concentration of µ opioid receptor protein in the hypothalamus (Quiñones-Jenab et al., 1997) as well as enkephalin mRNA expression in the spinal cord (Amandusson et al., 1999). These elements support the

notion the estrogens can increase endogenous antinociception. Subsequently, the hyperalgesic state under long-term loss of estrogen after OVX could be backed by a deficit of endogenous antinociceptive mechanisms may render a condition of disinhibition in the spinal cord. Also, estrogens could have influences in the modulation of noradrenergic and serotonergic systems also involved in pain transmission (Craft 2007; Hassan et al., 2014). Nowadays is accepted that via multiple mechanisms estrogens modify monoamine levels within the brain areas to play roles in cognitive and affective functions (Lubbers et al., 2010). Some which, as frontal cortex and hippocampus also are implicated in the central processing of pain (Tu et al., 2010; Phillips and Clauw, 2011).

Maladaptive neuroplasticity and aberrant processing at the level of the forebrain are now implicated in the etiology of FPS (Mayer and Bushnell, 2009). Also, it has been accepted that brain regions involved in cognitive pain modulation or more general bodily awareness identified by functional imaging are most susceptible to menstrual cycle effects (Tu et al., 2010). The estrogens receptors subtypes (ER-α and-β) are also located in areas of the brain that mediate stress, anxiety, and pain, such as the hypothalamus, amygdala, periaqueductal gray (PAG) and dorsal raphe nucleus (Shugrue et al., 1997; Veldhuijzen et al., 2013). The presence of ER in PAG suggests that estrogens influence the function of descending nociceptive modulatory pathways (Bernal et al., 2007; Craft, 2007).

In the present experiment, thermal pain thresholds (hot plate) were also decreased in OVX rats from three week after surgery and lasted for the six weeks. This result is in agreement with the reports of OVX mice in the second and most extensive analysis of thermal hypersensitivity (Sanoja and Cervero, 2008) and also in OVX rats under another experimental paradigm (Kepler et al., 1989). Some evidence has proved an increased expression of tumor necrosis factor alpha in estrogen deficiency animals. Its expression in L5 DRG has been directly related to thermal and mechanical hyperalgesia in OVX rats after eight weeks of surgery (Chen et al., 2012).

A limitation of the present study was that our protocol did not include the hormone replacement pellets reversion, element that was previously demonstrated (Sanoja and Cervero, 2005). However, it was controlled a long-term loss of estrogen state in OVX rats by vaginal smears (diestrus-like phase) and confirmed the efficacy of surgical procedures by necropsies. OVX also induced a substantial involution of reproductive organs after lacking in of ER-α estrogenic stimulus.

On the other hand, other observations in women suffering from irritable bowel syndrome (IBS) reported that IBS symptoms, including pain, are exacerbated at the time of menses with the lowest levels of circulating estrogen (Houghton et al., 2002). In women affected by temporomandibular disorders, pain levels rose toward the end of the cycle and peaked during menstruation at times of lowest estrogen, but rapid estrogen change, as around the date of ovulation, may also be associated with increased pain (LeResche et al., 2003). It was evaluated specifically possible cyclic changes in pain sensitivity in normal rats and detected significant enhanced in mechanical hypernociception in the estrous phase, at this phase estrogen levels are high in the morning, but they drop precipitously as the day progresses (Walmer et al., 1992). This result is congruent with early reports also in rats (Martinez-Gomez et al., 1994; Kayser et al., 1996). However, the cyclic changes in pain sensitivity (mechanical or thermal) was not detected in normal mice, perhaps because of the very short time course of the cycle that may be insufficient to allow the expression of its changes (Mogil et al., 1993; Sanoja and Cervero, 2005). It was not detected evidence for variations in thermal pain sensitivity (hot plate) in normal rats, but the increase in referred abdominal mechanical sensitivity in estrous phase was evident.

Also, increases in visceral sensitivity were observed in OVX rats in basis to an augmented number of pain behaviors in acetic-acid induced writing (chemical stimuli). It is a chemogenic tonic model of visceral pain with an acute inflammatory component that affect both visceral and parietal layers of the peritoneum (Tanimoto et al., 2003; Deyama et al., 2007). OVX rats showed evidence of increased visceral sensitivity to mechanical stimuli (saline) like OVX mice in a visceral model of colonic instillations (capsaicin or saline) (Sanoja and Cervero, 2005).

Particularly, mechanosensitivity has been the focus of major attention in the study of visceral nociceptive mechanisms (Cervero and Gebhart, 2009). Many of low- and high-threshold mechanosensitive visceral afferents can encode the noxious range and become sensitized (Sanoja and Cervero, 2010). Studies have been directed to transient receptor potential vanilloid 1 and 4, acid-sensing ion channels 3, purinergic P2X and sodium channels. Also, chemicals including bradykinin, adenosine triphosphate, serotonin, and capsaicin are reported to activate serosal and mesenteric receptive endings in the splanchnic innervations of abdominal organs (Cervero and Gebhart, 2009). Then the present result suggests that the sensitization of serosal endings could be facilitated in OVX rats exposure to chemical and mechanical stimulus.

This study shows that OVX rats increased in body weight seven weeks after surgery. Estrogens exert an inhibitory effect on food intake in a variety of species, its deficiency also contributes to the onset of changes in body composition in both humans and animals. A greater magnitude in body mass and especially the accumulation of adipose tissue in sedentary OVX rats were reported (Geary and Asarian, 1999; Braga et al., 2015).

Today is accepted the role of ROS in peripheral and central sensitization (Salvemini et al., 2011). It has been shown that nociceptive stimulation generates an increase in glucose uptake in the spinal cord, which is evidenced by a rise in metabolic activity in this tissue during pain transmission (Guedes et al., 2009; Reichling et al., 2013). On the other hand, oxidative stress increases in women after menopause, this may contribute to the pathogenesis of menopause-related neurological condition (Suzuki et al., 2009; Braga et al., 2015). In line with this idea increased total oxidant status, oxidative stress index and decreased total antioxidant status have been reported in brain tissue of OVX female rats. These effects were reversed by estrogen, estrogen/progesterone and genistein treatments (Evsen et al., 2013).

Recently has been accepted that ROS contribute to changes in pain processing in the central nucleus of the amygdala (CeA), an important contributor to emotional–affective aspects of pain (Ji et al., 2015). Particularly these species serve as a mechanism of visceral pain, which increases the excitatory drive of amygdala output neurons in CeA via metabotropic glutamate receptors (Ji and Neugebauer, 2010). It is resulting in increased excitability and neuronal activity to generate spontaneously and evoked pain behaviors (Ji et al., 2015).

The loss of protective up-regulation of the antioxidant, longevity-related genes by estrogens (Borrás et al., 2005) could have an influence on the central processing of visceral pain in a menopausal woman. Then, brain oxidative stress was also observed in the present study particularly related to the increases in visceral sensitivity in OVX rats. SOD enzyme activity decreased in OVX rats, which showed a major deficit for this enzymatic defense under visceral inflammatory injury. Other authors reported the low activity of enzymatic antioxidants system SOD, catalase, and glutathione peroxidase in OVX rats susceptible to the estrogen treatment, its effect was enhanced by the addition of vitamin E (Ulas and Cay, 2011). The finding of the present study is relevant in OVX hyperalgesic state considering the pivotal role of $O_2^{\bullet-}$ in glutamate-mediated hyperalgesia (Muscolia et al., 2004; Lee et al., 2007). Since the activation of N-methyl-D-aspartate (NMDA) receptor by glutamate induces an increase in the production of $O_2^{\bullet-}$ and NO, leading to the formation of peroxinitrite, which in turn promotes SOD inactivation and enhances hyperalgesia (Muscolia et al., 2004; Salvemini et al., 2011). About this evidence, some authors hypothesized that nitroxidative species activity within the RVM contributes to central sensitization (Salvemini et al., 2011). However, GSH concentrations were compensatory increased in the brain of OVX animals that allow the balance during acute inflammation induced by acetic acid observed in this experiment. ROS production and compensatory enhancement of antioxidant mechanisms also were seen in the spinal cord after peripheral nerve injury (Guedes et al., 2009). NO concentrations were raised exclusivity in the exposure of OVX rats to inflammatory chemical injury. Previous studies did not describe changes in the brain NO concentration in OVX rats or these were lower in serum and hippocampal tissues related to learning and memory impairments in these animals (Sadeghian et al., 2012). It is recognized the role of NO

as a critical mediator of synaptic plasticity, long-term potentiation, and consolidation of long-term memory (Yamada and Nabeshima, 1998). Also, it has been suggested that NO mediates the effects of estradiol in many organs (Cury et al., 2011). Estradiol affects the release of NO and the activity of nitric oxide synthase isoforms in the brain (Lopez-Jaramillo and Teran, 1999; Gotti et al., 2010). However, OVX rats with increased visceral pain sensitivity under tonic inflammatory condition may show increased NO in the brain. The role of the glutamate-NMDA-NO pathway has been recognized in central hyperexcitability subjacent in hyperalgesic states (Latremoliere and Woolf, 2009). The normal level of NO in OVX-SS rats could explicate the GSH preservation observed in this experiment since peroxinitrite-mediated nitration of excitatory amino acid channel 1 in neurons reducing the uptake capacity of cysteine has been shown. In neurons, cysteine is the rate-limiting substrate for GSH synthesis; subsequently this inactivation may lead to a depletion of intracellular GSH (Salvemini et al., 2011).

CONCLUSIONS

OVX in rats also provides a useful model, which mimics the process of functional pain in females that could be related to brain oxidative stress, an important emergent field in the understanding of pain signaling and development of therapeutic strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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