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Research report

Diffuse noxious inhibitory controls and brain networks are modulated in a testosterone-dependent manner in Sprague Dawley rats

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ABSTRACT

Diffuse noxious inhibitory control (DNIC), which involves endogenous pain modulation, has been investigated as a potential mechanism for the differences in pain modulation observed between men and women, though the literature shows contradictory findings. We used a capsaicin-induced DNIC behavioral assay and resting state functional magnetic resonance imaging (rsfMRI) to assess the effect of testosterone on pain modulation and related brain circuitry in rats. We hypothesized that testosterone is required for DNIC that leads to efficient pain inhibition by increasing descending pain modulation. Male, female, and orchidectomized (GDX) male rats had a capsaicin injection into the forepaw to induce DNIC and mechanical thresholds were observed on the hindpaw. rsfMRI scans were acquired before and after capsaicin injection to analyze the effects of DNIC on periaqueductal gray (PAG), anterior cingulate cortex (ACC) and nucleus accumbens (NAc) connectivity to the whole brain. The strength of DNIC was higher in males compared to females and GDX males. PAG connectivity with prelimbic cortex (PrL), ACC and insula was stronger in males compared to females and GDX males, whereas females and GDX males had increased connectivity between the right ACC, hippocampus and thalamus. GDX males also showed a stronger connectivity between right ACC and NAc, and right NAc with PrL, ACC, insula and thalamus. Our findings suggest that testosterone plays a key role in reinforcing the endogenous pain inhibitory system, while circuitries related to reward and emotion are more strongly recruited in the absence of testosterone.

1. Introduction

Several recent human and animal studies have reported the importance of sex differences in pain perception and modulation in both acute and chronic pain states [1–7]. Multiple physiological systems, including the endocrine system through estrogens, progestins and androgens, may affect the experience of pain directly or indirectly [1,8]. Recently, we have demonstrated that testosterone regulates μ-opioid receptor and cannabinoid 1 receptor (CB1) expression via transcriptional activities of androgen receptor in a trigeminal pain model [9,10]. These findings add novel perspectives on how male gonadal hormones modulate pain responses and have important clinical implications [6,10] since chronic pain conditions are more prevalent in women [11].

Testosterone has been shown to have analgesic effects in several preclinical as well as in clinical pain models [3,6,8,12–15]. Depletion of testosterone by gonadectomy increases formalin-induced nociceptive responses [16] in male rats, while replacement of testosterone to physiological levels decreases nociception in the formalin test [17]. Furthermore, therapy based on testosterone deprivation is associated with increased levels of pro-inflammatory factors and decreased levels of anti-inflammatory cytokines [18,19], while testosterone supplementation reduces inflammatory markers in both young and old hypogonadal men [18]. These analgesic events require activation of several brain circuits [20,21], but the role of testosterone in affecting brain networks in pain have been scarcely investigated.

One of the mechanisms that contribute to generally greater prevalence of pain in females than males is the sexually dimorphic activity in endogenous pain inhibitory systems. Diffuse noxious inhibitory controls (DNIC) require a noxious conditioning stimulus to one part of the body, which inhibits pain in other body regions [22–25]. While previous human studies indicated that DNIC effects were greater in males than females [26,27] sex differences in DNIC in animal models of pain have not been demonstrated. DNIC is mediated by a supraspinal mechanism, partly opioid-dependent [28], that modulates pain processing by the periaqueductal gray (PAG), locus coeruleus, and rostral ventromedial medulla (RVM) projections to the spinal cord [28,29]. DNIC increases activity in the orbitofrontal cortex (OFC) and amygdala, and reduces activations in primary and secondary somatosensory cortices (SI and SII), supplementary motor area (SMA), posterior insula and ACC [30]. However, the brain areas that are engaged in DNIC response

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by sex differences are still unknown.

Human brain imaging studies have shown sex differences in the brain areas involved in pain perception and modulation [31,32]. In irritable bowel syndrome patients, regional brain activity was greater in limbic areas, including the ventromedial prefrontal cortex, right ACC and left amygdala after visceral stimulus in female patients [33]. In contrast, male patients showed greater activation in brain regions involved in cognition and descending pain modulation, including the right dorsolateral prefrontal cortex, insula, and dorsal pons/PAG [33]. These findings suggest that females and males potentially differ in pain-related brain activation through different pathways associated with the multidimensional nature of pain [20,21]. However, very few studies have shown how testosterone in specific affects pain and brain activity [34,35]. Men with low levels of testosterone have greater activation in the pregenual ACC and OFC during thermal noxious stimulus, regions associated with pain-related unpleasantness [34]. Furthermore, while the activation of primary somatosensory cortex, a region associated with pain intensity, did not differ between groups, pain intensity ratings were higher in males with lower testosterone levels, compared to those with higher levels [34]. Additionally, testosterone may be a key factor in modulating pain sensitivity via descending pathways, even in women. For example, on study reported increased RVM activity after thermal noxious stimulus was associated with higher testosterone levels in women using combined oral contraceptive pill [35].

In the current study, we assessed the effects of testosterone on endogenous pain modulation by examining DNIC responses and resting state fMRI in three groups of rats with clearly different circulating testosterone levels: young males, young females, and orchidectomized (GDX) male rats. We hypothesized that testosterone plays a key role in activating the endogenous pain modulatory systems by increasing connectivity of brain regions implicated in descending pain inhibition during DNIC.

2. Methods

2.1. Animals

Age-matched adult male, female, and GDX male Sprague-Dawley rats (8 weeks old; 250–300 g for males and 225–260 g for females; Harlan Laboratories Inc, Indianapolis, IN, USA) were used in the present study. GDX rats received the orchidectomy surgery at the time of order. They were used 3 weeks after the surgery. All other animals were also used 3 weeks following arrival to match the age and weight at the time of experiment. In order to confirm the efficacy of GDX surgery we assessed testosterone serum level from the GDX rats and compared to those obtained from intact male and female rats (The University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core). Total free testosterone level in GDX rats (0.08 ± 0.03 ng/mL) was significantly lower than that of intact male rats (2.4 ± 0.4 ng/mL) and lower than that of female rats (0.29 ± 0.4 ng/mL) (Fig. 1), suggesting that the surgery completely depleted testosterone from GDX rats. The estrous cycle phase in female rats was not determined in this study. Animals were housed in a temperature-controlled room under a 12:12 light-dark cycle with access to food and water ad libitum. Rats in the same experimental groups were housed together as a pair. Male and female rats were housed in the same colony room and each experimental group was tested at a different time. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and under a University of Maryland-approved Institutional Animal Care and Use Committee protocol. For DNIC behavioral testing, 6 rats were used for the intact male group, 7 rats for the GDX male group and 7 rats for the intact female group. The behavioral experiments using phosphate-buffered saline (PBS) as control had 5 rats per group. fMRI study investigated DNIC effects after capsaicin injection in males, GDX males and females from a separate group of rats consisting of 4 rats per group.

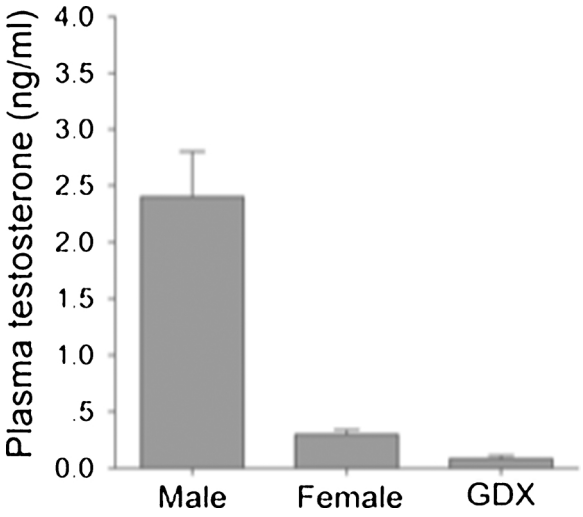

Fig. 1. Blood samples were obtained from the tail vein of normal intact male, female and GDX male rats. Total free serum testosterone levels (ng/mL) from the blood samples were assessed by ELISA assay kits by IBL.

2.2. Behavioral assay

Our model of DNIC in healthy animals was adapted from an earlier study [36]. Hindpaw withdrawal thresholds to noxious mechanical stimulation were measured before and 15, 30, 45, 60 and 90 min following the administration of a small volume of capsaicin (1% in 30 µl) into the left forepaw. The same volume of PBS was injected in the same manner in the control groups. Mechanical sensitivity of the left hindpaw was assessed with the Randall–Selitto test, an established rodent model for testing mechanical hypersensitivity of the paw. Animals were first allowed to habituate to the experimental room for 30 min for three consecutive days. The withdraw response to noxious paw pressure was assessed using a digital paw pressure Randall–Selitto applicator for rodents (IITC Life Science, Woodland Hills, CA). Each rat was placed in a cloth holder suspended in a sling, and the probe of the pressure applicator was placed under the plantar surface of the hindpaw. The probe has a spring load for easy opening and closing of the pressure applicator. The probe closes the pressure applicator and captures the pressure upon reaction. A gradually increasing pressure is applied until the rat withdraws its hindpaw. The lowest pressure necessary to elicit the withdraw response prior to capsaicin treatment was considered as the baseline mechanical threshold. Results were analyzed using the statistical analysis software package SigmaPlot. Two-Way Repeated Measures ANOVA with Holm-Sidak method for correction of multiple comparisons were performed to determine significant group and time effects. Differences were considered statistically significant at $p < .05$ and the data were presented as mean ± standard error of the mean (S.E.M.). The investigators conducting the behavioral study were blinded to the experimental group and drugs.

2.3. rsfMRI data acquisition

Proton density-weighted images were obtained using a 2D RARE (342 × 294 matrix, 24 coronal 1 mm slice thickness, in plane resolution 100 µm, TR 2000 ms, TE 28 ms) for anatomic reference. rsfMRI scans were acquired using an echo planner imaging (EPI) sequence (TR 1500 ms, TE 37 ms, 75 × 63 matrix, in plane resolution 0.45 × 0.45 x 1 mm, 24 coronal slices, 620 volumes per scan). The anatomical and the first rsfMRI scans were performed for each rat as baseline (prior to capsaicin injection, 15.5 min). Rats subsequently received an injection of capsaicin (1% in 30 µl) into the left hindpaw and a second rsfMRI scan was acquired for 15.5 min. Isoflurane concentrations were kept below 1.5% and maintained throughout the scan

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