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Research Report

Specificity of female and male sex hormones on excitatory and inhibitory phases of formalin-induced nociceptive responses

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Abstract

Several factors have been proposed to account for the differences observed between men and women in pain perception. One of these is female and male gonadal hormones. In order to verify this assumption, a hormone replacement (pellets inserted subcutaneously) of (1) 17β-estradiol, (2) progesterone, (3) 17β-estradiol + progesterone or (4) testosterone have been performed in gonadectomized female and male Sprague–Dawley rats. Twenty-one days after the hormonal replacement, a formalin test was performed. The nociceptive responses were divided in three distinct phases: acute (phase I), inhibitory (interphase) and tonic (phase II). After analysis, we observed that testosterone has a hypoalgesic effect on phases I and II of the formalin test. At the opposite, female hormones act only on the interphase: the combination of 17β-estradiol and progesterone in gonadectomized rats reestablishes the weaker nociceptive pain reduction during the interphase as it is observed in the intact female. These effects were not gender specific since they had the same action in female and male. Our results permit to believe that testosterone plays a protective role in pain perception. Moreover, the female hormones act mainly on pain inhibition mechanisms (interphase), suggesting that the prevalence of certain chronic pain conditions in women could be related to a deficit of these pain inhibitory mechanisms rather than an increased nociceptive activity.

Theme: Sensory systems
Topic: Pain modulation: anatomy and physiology

Keywords: Formalin test; Sex difference; Testosterone; Interphase; Nociception; Sex hormone

1. Introduction

It is well established that there is sex and gender differences in pain and analgesia. These sex differences have been demonstrated in experimental as well as in clinical pain studies. In experimental pain studies, women present lower pain and tolerance thresholds and evaluate an equivalent stimulus as being more painful than men [9]. In clinical pain, women are more likely to have pain that is severe, prolonged and recurrent [33]. Moreover, women are more susceptible to present chronic pain conditions than men [9]. Experimental pain studies carried out on animals coincide with the results obtained in humans: females have lower nociceptive thresholds than males in the majority of experimental modalities (see the review in [28]).

Several factors have been proposed to account for these differences, from psychosocial to purely physiological factors. However, growing attention is now given to the presence and fluctuation of gonadal hormones. It makes sense because binding sites for the gonadal hormones are widely distributed through the central nervous system (CNS) areas involved in pain transmission and inhibition [4,29,30,34]. Estrogen receptors are present in brain areas such as the periaqueductal gray matter (PAG), the arcuate

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nucleus and the amygdala [27]. Estrogens could also act on many neurotransmitters implicated in pain modulation including serotonin, acetylcholine, dopamine, opioids and γ-amino butyric acid (GABA) [4]. Progesterone receptors are also present in CNS areas involved in autonomic regulation and pain processing including: solitary tract, ventrolateral medulla and parabrachial nucleus [23]. Finally, androgens are likely to play an active role in pain processes because they seem to be protective in chronic inflammatory diseases [18].

Many animal studies have tried to establish the role of gonadal hormones on nociception. Recently, Stoffel et al. [31] published a study where all gonadal hormones were given, alone or in combination, to male and female Sprague–Dawley rats in order to identify their potential role on morphine antinociception. The authors found that gonadal steroid manipulations modulate basal nociception only in females and that these hormones modulate morphine's antinociceptive potency in both males and females in the hot plate test. More recently, Hau and colleagues [19] demonstrated that testosterone reduces nociceptive responses to a thermal stimulus in male house sparrows.

The studies mentioned above are of interest but we think that a sustained stimulus is more representative of clinical pain than a phasic nociceptive test. The formalin test is thus a good tool for studying the role of gonadal hormones on nociception. This test allows the separation of both early (acute) and later tonic (chronic) nociceptive phases and also the isolation of an active inhibitory phase (interphase) [17,20]. Using this test, Aloisi and Ceccarelli [5] showed that injection of estradiol intracerebroventricularly in male rats increases licking and flexing duration and decreases the paw jerk frequency of the injected paw. More recently, they observed the same difference when a male rat is in presence of bisphenol A (a xenoestrogen) perinatally [6]. These results appear to demonstrate that estrogens play a key role in pain modulation. However, the role of other gonadal hormones, such as progesterone, still needs to be elucidated. The role of testosterone in repetitive nociceptive stimulation was also recently studied in male rats by the same group [10]. In this study, it was observed that the male rats were "habituated" to repetitive nociceptive stimulations while the castrated males lost that capacity. In 2004, Aloisi and her colleagues [7] gave testosterone propionate in a supra-physiological dose to female and male Sprague–Dawley rats. They observed that only females displayed a modification in formalin-induced nociceptive responses after administration of testosterone propionate and that the only behavior that had changed was the licking behavior (being now similar to normal males). These results suggest a hypoalgesic role for testosterone.

Further supporting the idea that gonadal hormones play a central part in nociceptive processing, we demonstrated that there is no difference between male and female rat nociceptive responses, following gonadectomy [17]. Moreover, we found that female and male sex hormones act differently on each phase of the formalin test (including the interphase).

The main goal of the present study was to measure the effect of sex hormone replacement on the different tonic, phasic and inhibitory phases of the formalin test to verify if different sex hormones are truly phase dependant as suggested by the results from our previous study [17].

The specific goals were to verify:

- the isolated effect of each sex hormone on each phase of the formalin test;
- if the effects of progesterone, estradiol, testosterone and the combination of progesterone and estradiol are gender specific;
- if, in comparison with normal rats, hormone replacement in gonadectomized rats produces hypoalgesic or hyperalgesic effects.

2. Materials and methods

2.1. Animals

Ninety-four 4-month-old Sprague–Dawley rats (Charles River, St-Constant, Québec, Canada) were used. They were housed two per cage (same sex, same hormonal condition) under a 12-h light/12-h dark cycle (lights on at 0700 h). The temperature and the relative humidity were maintained constant (25 °C, 55%). Food and water were available ad libitum. The rats were manipulated every day over a 1-month adaptation period in order to reduce the stress induced by the manipulations and the new environment [1]. The formalin test was always done at 0900 AM to reduce any variation related to circadian rhythm. The rats were divided into 12 groups: females (n = 8), ovariectomized females (OVX) (n = 8), OVX with 17β-estradiol replacement (OVX + E2) (n = 8), OVX with progesterone replacement (OVX + P) (n = 7), OVX with 17β-estradiol and progesterone replacement (OVX + E2 + P) (n = 8), OVX with testosterone replacement (OVX + T) (n = 8), males (n = 8), castrated males (CAST) (n = 7), CAST with 17β-estradiol replacement (CAST + E2) (n = 8), CAST with progesterone replacement (CAST + P) (n = 8), CAST with 17β-estradiol and progesterone replacement (CAST + E2 + P) (n = 8) and CAST with testosterone replacement (CAST + T) (n = 8).

2.2. Gonadectomy

Female ovariectomy and male castration was performed by the company Charles River 2 months after birth (young adults). A period of 1 month separated the arrival of the animals and the beginning of testing to allow for the recuperation from the surgery and the elimination of any