Physiology & Behavior 135 (2014) 44–48

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Gender-dependent effect on nociceptive response induced by chronic variable stress

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HIGHLIGHTS

- Chronic stress can induce a decrease in the nociceptive threshold in male rats.
- The nociceptive response in females was independent of the cycle phase.
- Chronic stress can induce an increase in the nociceptive threshold in female rats.

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 20 May 2014
Accepted 28 May 2014
Available online 5 June 2014

Keywords:
Stress
Chronic variable stress
Nociception
Rats
Gender
Estrous cycle

ABSTRACT

It has previously been reported that exposure to repeated restraint stress induces hyperalgesia in male rats, an effect that was not observed in females. The aim of the present study was to investigate the effects of chronic variable stress over 40 days on nociception threshold indexed by tail-flick latency in male and female adult rats. The results showed different behavior in chronically stressed animals when compared to the control group: male rats showed a decrease in tail-flick latency while females presented an increase in this parameter. For female rats this effect was independent of the phase of the estrous cycle. Several sources of data indicate that behavioral and physiological responses to stress are sexually dimorphic, including in nociception, and the estrous cycle appears to be a factor that influences opioid analgesia in female. These effects are modulated by the strain and conditions of nociception assay. Additional studies concerning the mechanisms involved in the hyperalgesic response in males and the differences on nociceptive response in females chronically exposed to stress are needed.

1. Introduction

Stress is an important phenomenon that may interfere with the perception of and response to pain and modulate behavioral responses. Pain syndromes are associated with chronic stress, since chronic exposure to pain produces suffering, which activates the hypothalamic–pituitary–adrenal (HPA) axis [1]. Unlike acute stress, which has been associated with a reduction in pain sensitivity – probably mediated by brain stem pain modulation [1] – chronic stress has been associated with increased sensitivity to pain, producing hyperalgesia (decreased pain threshold) [2–4] and allodynia (pain induced by no noxious stimuli) [4]. Previous studies have suggested that this could be related to changes in central or peripheral opioid activity [3,5,6]. The absence of novelty-induced antinociception in these animals supports this theory [3], since the exposure of rats to a novel environment induces naloxone-reversible mild antinociception [7]. The opioid system can be highly plastic, as reflected by its susceptibility to modifications through various pharmacological and behavioral manipulations [8]. In accordance with this, a previous study showed decreases in binding of opioid receptors in the hippocampus and cerebral cortex [6]. Additionally, Torres and colleagues (2003) demonstrated that animals subjected to chronic restraint stress for 6 weeks needed high doses of morphine to exhibit an analgesic response, suggesting that prolonged stress could lead to longer-lasting changes in the neural systems involved in nociceptive modulation [5]. On the other hand, in acute stress, the opiate system seems to be modulated in the opposite direction. In fact, a study has demonstrated that animals subjected to acute stress show

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http://dx.doi.org/10.1016/j.physbeh.2014.05.033
0031-9384/

an increase in the magnitude and duration of the analgesic effect of some opiate agonists [9].

Moreover, sex-related differences in humans and animals with regard to nociception as well as the efficacy of analgesic drugs have been the subject of previous study, particularly in the case of opioids [10]. Different numbers and distribution of central opioid receptors in male and female rodents have been described as associated with differences in the expression of opioid analgesia [11]. In addition, pain thresholds may vary in females in different phases of the estrous cycle [12]. This raises the possibility of gender differences in a variety of components associated with the regulation of nociception. For example, gonadal hormones influence pain sensitivity, with higher pain threshold and tolerance levels during the follicular phase, when the levels of estrogen are higher. Evidence indicates that estrogen is involved in the regulation of analgesia and nociception [13,14] and appears to stimulate the release of brain endorphins [15]. The interaction between female gonadal hormones and the opioid system might also contribute to men and women's differential sensitivity to the analgesic effects of opioids [16].

Previous study showed that repeated restraint stress induced hyperalgesia in males, while no effect was observed in females [2]. Besides the different gender of experimental animals used, the type, duration, or severity of the stressors used may modify the responses to stress [17,18]. Since studies using repeated restraint present a certain degree of predictability when compared to models using different stressors [19–22], it is possible that the effects of chronic variable stress may present different degrees of adaptation.

In this study, we investigated possible sex and estrous cycle differences in the effects of chronic variable stress on nociception, examining the hypothesis that sex differences may be dependent upon natural hormonal variations associated with the estrous cycle. Therefore, we measured pain threshold in two different phases of the estrous cycle, with adrenal weight as stress control.

2. Material and methods

2.1. Animals

Experimentally naïve male and female adult Wistar rats (60 days, 160–170 g weight — female and 223–230 g — male) from our breeding stock were used. The animals were housed in groups of 4 per cage, separated according to sex. They were maintained on a 24-hour dark–light cycle (lights on 7:00 AM) at 22 ± 2C before and throughout the experimental period. Rats had free access to food (standard laboratory-rat chow) and water, except when either food or water restriction was used as a stressor. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Ethical Committee of Universidade Federal do Rio Grande do Sul.

2.2. Chronic variable stress procedures

The animals were divided into 2 groups: the control group (C; females, n = 11; males, n = 8) that were kept in home cages during the treatment, and the stressed group (S; females, n = 11; males, n = 8). The animals were stressed using a model by Gamaro et al. (2003) [23], which is a version of other models of variable stress [21,24–27]. The animals were submitted to 7 different stressors: restraint for varying periods (30 minutes to 3 hours), food deprivation for 24 hours, water deprivation for 24 hours, forced swimming for 10 or 15 minutes, restraint on cold (4 °C, from 30 minutes to 3 hours), isolation (2–4 days), and flashing light (40 W), during different periods (Table 1). The stress treatment was maintained over 40 days. Control animals were kept in their home cages.

Table 1
Schedule of stressor agents used during the chronic stress treatment. These stressors were applied to all animals in the stressed group. Intact controls were kept undisturbed in their home cages during the 40 days of treatment.

Day of treatment	Stressor used	Duration
1	water deprivation	24 hours
2	food deprivation	24 hours
3	isolation	24 hours
4	isolation	24 hours
5	isolation	24 hours
6	flashing light	3 hours
7	food deprivation	24 hours
8	forced swimming	10 minutes
9	restraint	1 hour
10	water deprivation	24 hours
11	—————	—————
12	—————	—————
13	restraint + cold	2 hours
14	flashing light	2.5 hours
15	food deprivation	24 hours
16	forced swimming	15 minutes
17	isolation	24 hours
18	isolation	24 hours
19	isolation	24 hours
20	water deprivation	24 hours
21	food deprivation	24 hours
22	flashing light	3 hours
23	restraint	2 hours
24	isolation	24 hours
25	isolation	24 hours
26	restraint + cold	1.5 hours
27	forced swimming	10 minutes
28	flashing light	3.5 hours
29	————	————
30	food deprivation	24 hours
31	restraint	3 hours
32	flashing light	2 hours
33	water deprivation	24 hours
34	restraint + cold	2 hours
35	forced swimming	15 minutes
36	isolation	24 hours
37	isolation	24 hours
38	—————	————
39	flashing light	3 hours
40	forced swimming	10 minutes

2.3. Cycle determination

Cycle determination was started three weeks prior to testing. The cycle of each female rat was determined by observation of vaginal smears, which were taken using a plastic tip. The procedure was performed on all animals between 8:00 and 9:00 AM. Distilled water was placed on the vaginal opening, aspirated, and then placed on a microscopic slide. After the sample had dried, it was stained with hematoxylin-eosin. When the dye was removed, the slide was washed in de-ionized water and examined under a binocular microscope. The slide specimens were compared and matched. Briefly, estrus was identified by a majority of cornified epithelial cells and diestrus by small amounts of leukocytes, round and cornified epithelial cells.

2.4. Tail-flick measurement

Nociception was assessed with a tail-flick apparatus [28]. Rats were wrapped in a towel and placed on the apparatus; the light source positioned below the tail was focused on a point 2–3 cm rostral to the tip of the tail. Deflection of the tail activated a photocell and automatically terminated the trial. Light intensity was adjusted so as to obtain a baseline tail-flick latency (TFL) of 3 to 4 s. A cut-off time of 10 s was used to prevent tissue damage. On day 1, the animal was familiarized with the apparatus. This first measure was done in order to familiarize the animals with the procedure, since it has been observed that novelty itself can