Physiology & Behavior 165 (2016) 278–285

Contents lists available at ScienceDirect

Physiology & Behavior

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

Hormonal and molecular effects of restraint stress on formalin-induced pain-like behavior in male and female mice

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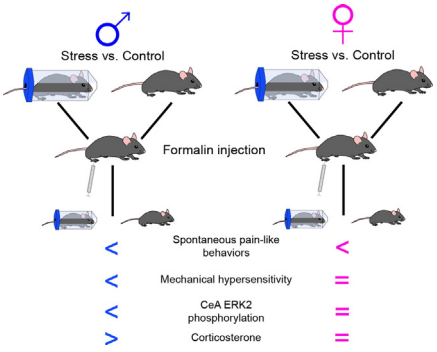
HIGHLIGHTS

- Restraint stress modulates spontaneous formalin behavior in both male and female mice.
- Restraint stress decreases formalin-induced mechanical hypersensitivity only in male mice.
- Restraint in males prevents formalin-induced ERK2 phosphorylation in the CeA.
- Corticosterone levels differ in male and female mice 180 min post-formalin injection.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 April 2016
Received in revised form 15 July 2016
Accepted 8 August 2016
Available online 09 August 2016

Keywords:
Sex differences
Stress-induced analgesia
Acute restraint
Pain
Central nucleus of the amygdala

ABSTRACT

The evolutionary advantages to the suppression of pain during a stressful event (stress-induced analgesia (SIA)) are obvious, yet the reasoning behind sex-differences in the expression of this pain reduction are not. The different ways in which males and females integrate physiological stress responses and descending pain inhibition are unclear. A potential supraspinal modulator of stress-induced analgesia is the central nucleus of the amygdala (CeA). This limbic brain region is involved in both the processing of stress and pain; the CeA is anatomically and molecularly linked to regions of the hypothalamic pituitary adrenal (HPA) axis and descending pain network. The CeA exhibits sex-based differences in response to stress and pain that may differentially induce SIA in males and females. Here, sex-based differences in behavioral and molecular indices of SIA were examined following noxious stimulation. Acute restraint stress in male and female mice was performed prior to intraplantar injections of formalin, a noxious inflammatory agent. Spontaneous pain-like behaviors were measured for 60 min following formalin injection and mechanical hypersensitivity was evaluated 120 and 180 min post-injection. Restraint stress altered formalin-induced spontaneous behaviors in male and female mice and formalin-induced mechanical hypersensitivity in male mice. To assess molecular indices of SIA, tissue samples from the CeA and blood samples were collected at the 180 min time point. Restraint stress prevented formalin-induced increases in extracellular signal regulated kinase 2 (ERK2) phosphorylation in the male CeA, but no changes associated with pERK2 were seen with formalin or restraint in females. Sex differences were also seen in plasma corticosterone concentrations 180 min post injection. These results demonstrate sex-based

Abbreviations: CeA, central nucleus of the amygdala; CORT, corticosterone; CRF, corticotropin releasing factor; CRF1 receptor, corticotropin releasing factor receptor 1; CRF2 receptor, corticotropin releasing factor receptor 2; ERK, extracellular signal-regulated kinase; GR, glucocorticoid receptor; HPA axis, hypothalamic pituitary adrenal axis; PAG, periaqueductal gray; SIA, stress-induced analgesia; RVM, rostral ventromedial medulla.

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http://dx.doi.org/10.1016/j.physbeh.2016.08.009

differences in behavioral, molecular, and hormonal indices of acute stress in mice that extend for 180 min after stress and noxious stimulation.

1. Introduction

Although pain has evolved as a defensive response to noxious stimuli, the suppression of pain during stressful events is evolutionarily advantageous and known as stress-induced analgesia (SIA). SIA is generated through supraspinal integration of the physiological stress response and descending pain inhibition. The amygdala is a limbic brain region involved in both of these processes, and thus a potential modulator of SIA. Signaling molecules localized in the central nucleus of the amygdala (CeA) provide direct evidence for this specific region's link to the underlying mechanisms of SIA; stimulus-induced expression of corticotropin-releasing factor (CRF) [1] and phosphorylated extracellular signal regulated kinase 2 (pERK2), [2] couple the CeA to the stress response and pain modulation, respectively. Additional anatomical evidence comes from CeA projections to CRF-rich regions of the hypothalamus [3,4], linking it to the HPA axis, and heterogeneous CeA projections through the periaqueductal gray (PAG) [5] to the rostral ventromedial medulla (RVM) [6] linking it to descending pain transmission.

Interestingly, the CeA exhibits sex-based differences in response to stress and pain. For instance, basal levels of CRF in the CeA vary between the sexes [7] and psychological stress and foot shock differentially regulate expression of this hormone in male and female rats [8]. In the context of pain, men exhibit increased functional connectivity between the amygdala and PAG as compared to women [9]. Additionally, localized injections of female sex hormones in the amygdala alter pain-like responses to visceral stimulation in rats [10]. Taken together, these data suggest that sex-dependent variability in amygdaloid processing of pain and stress may differentially induce SIA in males and females.

In this paper, we evaluated sex-based differences in behavioral and molecular indices of SIA. Specifically, we performed acute restraint stress in male and female mice prior to intraplantar injections of formalin, a noxious inflammatory agent. At various time points following injection, we observed pain-like behaviors, quantified circulating stress hormones, and analyzed ERK activation in the CeA. We hypothesized that females would exhibit more robust SIA since they have higher basal levels of the stress hormone corticosterone [11], greater variability in hormone responses to stress [12], and increased pain sensitivity [13].

2. Materials and methods

2.1. Animal care

All protocols were done in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Duquesne University, Pittsburgh, PA (Protocol Number: 1412-16). Male and female C57BL/6J mice aged 9–12 weeks were used for all experiments. Animals were housed on a 12 h light/dark cycle (7 am–7 pm) with ad libitum access to rodent chow and water.

2.2. Behavioral testing

Only one sex was tested at a time to avoid odorant cues influencing testing. All cohorts were tested in the same room and time of day over the course of one week. Mice were placed in 25 × 25 × 35 cm ventilated Plexiglas enclosures on a wire mesh rack and habituated for at least 2 h with background white noise. Male and female experimenters who were blinded to restraint stress condition performed all additional behavioral testing. All experimenters spent at least 30 min in the testing room prior to any behavioral assay to account for experimenter effects on pain-like behavior [14].

2.2.1. Acute restraint stress

Mice were restrained to induce stress before receiving a formalin paw injection (see below). Mice were restrained for 30 min in a 50 mL plastic conical tube fitted with air holes and a stopper so animals were not able to fully turn around in the tube; non-restrained control mice remained in Plexiglas enclosures. Restraint stress has been used for decades to investigate the neurobiological, behavioral, and clinical aspects of stress on the development and expression of numerous disorders [15]; it produces a significant stress response without causing physical injury to the animal. All mice were then allowed a 15-min grooming period before further testing.

2.2.2. Spontaneous formalin behavior

As previously described [16], spontaneous behaviors following intraplantar formalin injection were measured. Animals were injected subcutaneously in the plantar surface of the right hind paw with 10 μL of 2% formalin in saline (Sigma, St. Louis, MO). Restrained and control mice were videotaped (Logitech Pro 9000) following intradermal formalin injection and analyzed for nociceptive behaviors (defined as licking, lifting, and flinching of the injected paw) in 5 min bins for 60 min following formalin injection. The first phase of spontaneous behavior was defined as 0–10 min after injection and the second phase of testing was defined as 10–60 min after injection. The entire period was also analyzed from 0 to 60 min using an area under the curve analysis to determine the presence or absence of sex differences in this assay.

2.2.3. von Frey mechanosensory assessment

All behavioral testing occurred between 8 am and 3 pm. von Frey filaments (North Coast Medical, San Jose, CA; [17]) were used to evaluate hind paw mechanical sensitivity. As previously described [16], mechanical testing consisted of applying von Frey filaments to the left and right hind paws until bent at approximately 30° for no longer than 2 s. If the animal removed its paw before this time, it was recorded as a withdrawal. Each filament, beginning with the smallest force filament and increasing in force thereafter, was applied five times. The mechanical threshold was determined as the smallest filament that evoked a withdrawal response in at least three of the five trials. Three to five baseline withdrawal thresholds were averaged for each hind paw. One day following baseline testing, mice were again habituated in Plexiglas enclosures. After 2 h, mice were subjected to restraint stress (or control, as described above), allowed to groom for 15 min, and then injected with formalin (as described above). Mechanical sensitivity was measured 120 and 180 min following formalin in both the formalin-injected paw and the contralateral (uninjected) paw.

2.3. Blood and tissue collection

Mice were habituated as described in Plexiglas enclosures for at least 2 h with background white noise; tissue and blood collection occurred between 12 pm and 3 pm. Restraint and formalin (or saline) injections were performed as described and then mice were returned to their Plexiglas enclosures and remained undisturbed until 180 min post-injection. At the 180 min time point, animals were transferred one at a time to another room for sacrificing. The 180 min time point was chosen for analysis because this is the time at which the CeA modulates