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Spinal Inhibition of P2XR or p38 Signaling Disrupts Hyperalgesic Priming in Male, but not Female, Mice

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Abstract—Recent studies have demonstrated sexual dimorphisms in the mechanisms contributing to the development of chronic pain. Here we tested the hypothesis that microglia might preferentially regulate hyperalgesic priming in male mice. We based this hypothesis on evidence that microglia preferentially contribute to neuropathic pain in male mice via ionotropic purinergic receptor (P2XR) or p38 mitogen-activated protein kinase (p38) signaling. Mice given a single-priming injection of the soluble human interleukin-6 receptor (IL-6r) and then a second injection of prostaglandin E2 (PGE_2), which unmasks hyperalgesic priming, shows a significant increase in levels of activated microglia at 3 h following the PGE_2 injection in both male and female mice. There was no change in microglia following PGE_2. Intrathecal injection of the P2X3/4 inhibitor TNP-ATP blocked the initial response to IL-6r in both males and females, but only blocked hyperalgesic priming in male mice. Intrathecally applied p38 inhibitor, skepinone, had no effect on the initial response to IL-6r but attenuated hyperalgesic priming in males only. Neither TNP-ATP nor skepinone could reverse priming once it had already been established in male mice suggesting that these pathways must be inhibited early in the development of hyperalgesic priming to have an effect. Our work is consistent with previous findings that P2XR and p38 inhibition can lead to male-specific effects on pain behaviors in mice. However, given that we did not observe microglial activation at time points where these drugs were effective, our work also questions whether these effects can be completely attributed to microglia.

Key words: sex-differences, hyperalgesic priming, chronic pain, ionotropic purinergic receptor (P2XR), p38 mitogen-activated protein kinase (p38), microglia.

INTRODUCTION

It is becoming increasingly clear that the mechanisms underlying the way men and women develop chronic pain and respond to treatments are different. Several studies have demonstrated that the cell types responsible for mediating the development of chronic pain in male and female animals are different (Sorge et al., 2015; Taves et al., 2016). Microglia are the resident immune cells of the central nervous system (CNS) and upon injury or insult they can become activated and begin releasing inflammatory and pro-nociceptive factors, such as cytokines and chemokines (Ekdahl et al., 2003; Block et al., 2007; Scholz and Woolf, 2007; Inoue and Tsuda, 2009; Costigan et al., 2009; Grace et al., 2014). At least two studies have now shown that microglia contribute more prominently to chronic neuropathic pain in male than in female mice (Sorge et al., 2011, 2015; Mogil, 2012; Taves et al., 2016; Lopes et al., 2017; Coraggio et al., 2018). In the spared-nerve injury (SNI) model of neuropathic pain, when microglial activity was blocked, or these cells were depleted with selective toxins, the persistence of mechanical hypersensitivity, which is a key feature of neuropathic pain, was strongly reduced in male CD-1 mice but no effect was observed for these treatments in the same strain of female mice (Sorge et al., 2015). Additional studies have been conducted to demonstrate that these effects are found across laboratories and with different strains of mice creating a strong rationale to continue to explore mechanistic differences in the development of chronic pain in males and females (Mapplebeck et al., 2016).

Our study used the hyperalgesic priming model to examine the transition to a state of persistent pain plasticity where animals are susceptible to a normally sub-threshold stimulus promoting a long-lasting pain state (Reichling and Levine, 2009; Kandasamy and Price, 2015; Price and Inyang, 2015). We hypothesized that in the mouse model of hyperalgesic priming, microglial activation would regulate this plasticity in male but not female mice. In this model we first inject a pro-nociceptive stimulus into the hindpaw to create a transient mechanical hypersensitivity around the site of injection

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(Dina et al., 2008). After this initial hypersensitivity had resolved, we then give a second stimulus, prostaglandin E_2 (PGE_2) into the same hindpaw at a dose that does not promote pain hypersensitivity in naïve mice. In animals that have been primed with an initial pronociceptive stimulus, there is a robust and extended response to the injection of PGE_2 which reveals the presence of hyperalgesic priming (Dina et al., 2008; Reichling and Levine, 2009). Because robust hyperalgesic priming can be induced in male and female mice with a broad variety of stimuli, this paradigm allowed us to test two primary hypotheses. First, we assessed whether microglial activation occurs in the spinal dorsal horn during the initiation of plasticity that causes hyperalgesic priming or at the time that priming is revealed by PGE_2 injection. To our knowledge this has never been tested. Second, we sought to assess whether P2X3/4 antagonists or p38 inhibitors have sexually dimorphic effects in hyperalgesic priming in mice.

Our work demonstrates that modest microglial activation is noted in the spinal dorsal horn in both male and female mice in the hyperalgesic priming model around the time of PGE_2 injection but not when priming is initiated. In contrast, pharmacological inhibition of P2X2/3 or p38 during the initial insult blocks the development of hyperalgesic priming in male mice, but has no impact in female mice. Once hyperalgesic priming has already been established, blocking P2X3/4 or p28 had no effect in either male or female mice. Our results are consistent with a male-specific role for P2X3/4 and p38 in the promotion of chronic pain plasticity but question whether these effects are dependent on microglia.

EXPERIMENTAL PROCEDURES

Animals

In all experiments naïve, 6- to 10-week-old Swiss Webster mice were purchased from Taconic (Hudson, NY). Both male and female mice were used. Mice were housed in same-sex groups of 2–4 animals on a 12: 12-h light/dark cycle. Food and water were available *ad libitum*. Animals were assigned to their experimental groups using a random number generator, with a minimum of one animal per drug treatment in each housing group. The Institutional Animal Care and Use Committee at the University of Texas at Dallas approved all animal procedures.

Drugs

The soluble human interleukin 6 receptor (IL-6r) was obtained from R&D Systems (Minneapolis, MN). Prostaglandin E_2 (PGE_2) from was from Cayman Chemical Company (Ann Arbor, MI). 2′,3′-O-(2,4,5-Trini trophenyl) adenosine-5′-triphosphate tetra (triethylammonium) salt (TNP-ATP) and skepinone-L (skepinone) were obtained from Sigma–Aldrich (St. Louis, MO). IL-6r stock solution was made in sterile phosphate-buffered saline (PBS). PGE_2 stock solution was made in 100% ethanol. TNP-ATP and skepinone stocks were made in dimethyl sulfoxide. All drugs were diluted to final working concentration in sterile 0.9% saline prior to injection.

von Frey testing and hyperalgesic priming

Animals were placed in acrylic boxes with mesh flooring and allowed to acclimate for at least 1 h prior to testing. Calibrated von Frey Filaments were then used to determine the mechanical withdrawal threshold using the up-down method of Dixon with modification (Dixon, 1965; Chapman et al., 1985). Baseline paw withdrawal threshold was determined before all injections.

In order to establish hyperalgesic priming, 0.1 ng of IL-6r was injected into the plantar surface of the left hindpaw. Paw withdrawal threshold was then measured at 3, 24, and 72 h post IL-6r injection. Mice were then allowed to return to their baseline mechanical withdrawal threshold and were then injected with 100 ng PGE_2 in a volume of 10 μL of 0.9% sterile saline at least 7 days after the initial injection of IL-6r. Mechanical withdrawal threshold was then determined at 3 and 24 h after PGE_2 injection.

Rotarod testing

Rotarod testing was used to determine the impact of hindpaw IL-6r and PGE_2 injections on locomotion in animals treated with I.T. Skepinone or vehicle. Prior to experimental testing animals were allowed to acclimate to the stationary rotarod device (rotarod treadmill, 1.25in diameter, IITC Life Science, Woodland Hills, CA) for 120 s prior to baseline measurements. Animals were placed on rotarod rotating at a speed of 10 rotations per minute. Baseline measurements were taken 24 h prior to intrathecal Skepinone injections over 3 trials with a maximal cutoff time of 90 s per trial. Rotarod testing was then performed 3 and 24 h post IL-6r I.Pl. injections and 3 and 24 h post PGE2 intraplantar injections. In this set of experiments locomotion was only assessed in female mice.

Intrathecal injections

Immediately preceding hindpaw IL-6r injections male and female animals were anesthetized using isoflurane gas (4% induction, 1.5% maintenance). The animal was then given an intrathecal injection of either TNP-ATP (5 μg) (Sorge et al., 2015) or skepinone (30 μg) (Taves et al., 2016) in a total volume of 5 μL using a 50-μL Hamilton syringe with a 30-gauge needle (Hylden and Wilcox, 1980). In the second set of experiments males were given hindpaw injections of IL-6r and a minimum of 5 days later given an intrathecal injection of either TNP-ATP (5 μg) or skepinone (30 μg) in a volume of 5 μL immediately before receiving a hindpaw injection of PGE_2.

Immunohistochemistry

In the first set of experiments, animals were injected with IL-6r and 3 h later were deeply anesthetized with isoflurane and sacrificed. In the second set of experiments animals were injected with IL-6r and then 7 days later injected with 100 ng PGE_2. Either 3 or 24 h