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Endothelin-1-induced priming to capsaicin in young animals

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HIGHLIGHTS

- Capsaicin produced sex-dependent secondary mechanical hyperalgesia in neonatal rats.
- Secondary mechanical hyperalgesia was prolonged in ET-1 primed females.
- Priming with ET-1 increased capsaicin-induced c-Fos expression in the spinal cord.
- Early-life pain alters responses to painful stimuli at the behavioral and neuronal level.

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ABSTRACT

Endothelin-1 (ET-1) is a known algogen that causes acute pain and sensitization in humans and spontaneous nociceptive behaviors when injected into the periphery in rats. This study sought to examine the effect of ET-1 exposure in the neonatal period on subsequent contralateral capsaicin-induced secondary mechanical hyperalgesia. ET-1 or saline was injected into the left plantar hindpaw on postnatal day 7 (P7). On postnatal day 11 (P11), capsaicin cream or control lotion was applied to the right dorsum hind paw and mechanical paw withdrawal thresholds were measured in the plantar hind paw. In saline control males, P11 administration of capsaicin produced a secondary mechanical hyperalgesia that was still present at 2 h. Neonatal priming with ET-1 did not alter the magnitude or the duration of secondary mechanical hyperalgesia in males. In contrast, in control females, P11 administration of capsaicin produced less than 40 min of mechanical hyperalgesia. Neonatal priming with ET-1 prolonged the duration of secondary mechanical hyperalgesia in females. Priming with ET-1 on P7 led to a significant increase in capsaicin-induced Fos expression in the dorsal horn of the spinal cord in both males and females compared to controls ($p < 0.001$). These findings further suggest that pain in early life may alter future responses to painful stimuli at both the behavioral and neuronal level.

1. Introduction

Vaso-occlusive crisis (VOC) accompanied by extreme pain is a hallmark feature of sickle cell disease (SCD). The vasoactive peptide endothelin-1 (ET-1) has been found to be elevated in SCD patients during an acute VOC [1]. Endothelin-1 is a 21 amino acid peptide known to be a potent vasoconstrictor which causes acute pain and sensitization to mechanical and other noxious stimulation when injected into the human forearm [2–4]. When ET-1 is injected into the rat hindpaw [5] or directly onto the sciatic nerve [6], it induces spontaneous nociceptive behaviors. Repeated administration of ET-1 onto the sciatic nerve of adult male rats causes desensitization as evident by a reduction in nociceptive behaviors [7]. Previous results from our lab have shown that prior exposure to ET-1 alters behavioral responses to subsequent exposure to ET-1 at the same location in a sex dependent manner with sensitization in males and desensitization in females [8]. However, when the second administration of ET-1 was in the contralateral hindpaw a sensitization was observed in both males and females. To determine whether the contralateral increase in ET-1-induced nociception is a result of central sensitization, this study applied capsaicin to the contralateral hindpaw and examined secondary mechanical hyperalgesia and neuronal activation in the dorsal horn of the spinal cord using c-fos. This study tested the hypothesis that a priming dose of ET-1 produces central sensitization, which will cause an increase in capsaicin-induced secondary mechanical hyperalgesia in the contralateral hindpaw and c-fos expression in the spinal cord.

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2. Methods

2.1. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of South Carolina. Efforts were made to limit the amount of distress and the number of animals used. Male and female Sprague–Dawley (Charles River Laboratories, MA) pups were housed with dams on a 12 h light/dark cycle with food and water available ad libitum. Each litter was culled at 10–12 pups.

2.2. ET-1 and capsaicin administration and behavioral analysis

Sterile saline or ET-1 (Enzo Life Sciences, Farmingdale, NY, USA) dissolved in deionized water was administered (5.25 pmol) in 10 μL (1.31 ng/μL) subcutaneously using an insulin syringe into the left plantar hindpaw on postnatal day 7 (P7). Animals were videotaped, and the number of spontaneous paw flinches was counted. On postnatal day 11 (P11), capsaicin cream (0.1% Capzaisin-HP, Chattem, Inc, Chattanooga, TN, USA) or control lotion (Johnson & Johnson Baby Lotion) was applied topically to the dorsum of the right hindpaw. There were four treatment groups for both sexes ($n = 5$–6 for each group and sex): saline (P7) + control (P11), saline (P7) + capsaicin (P11), ET-1 (P7) + control (P11), and ET-1 (P7) + capsaicin (P11). Before application of capsaicin, the baseline paw withdrawal threshold was measured in the plantar right hindpaw using sequential von Frey filaments ranging from 0.04 g to 1.4 g. Each filament was applied a total of five times and the first filament that elicited a sustained response was considered the paw withdrawal threshold. This method was used at 20, 40, 60, and 120 min time points following capsaicin application.

2.3. Fos immunohistochemistry

Two hours post-capsaicin on P11, all animals were deeply anesthetized with isoflurane then transcardially perfused with cold phosphate buffered saline (PBS) and 4% paraformaldehyde followed by isolation of the vertebral columns. Spinal cords were isolated and equilibrated in a cryoprotecting solution (30% sucrose in PBS). Spinal cords were mounted in Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA, USA) and sliced into serial 40 μm transverse sections. Sections were stored in anti-freeze solution (ethylene glycol and sucrose in PBS) at −20 °C until processed for immunofluorescence or immunoperoxidase. Free-floating sections were blocked in normal horse serum before being incubated with polyclonal rabbit anti-c-Fos (EMD Biosciences, Billerica, MA, USA, 1:5000) overnight at 4 °C. Following a wash, the tissue sections were then incubated with donkey anti-rabbit Alexa Flor 594 (Invitrogen, Carlsbad, CA, USA, 1:200) or biotinylated donkey anti-rabbit (Jackson Immunoresearch, West Grove, PA, USA, 1:1000) for 1.5 h at room temperature then washed, slide mounted, and coverslipped using Vectashield (Vector Laboratories, Burlingame, CA, USA). For immunoperoxidase, sections were incubated in HRP-streptavidin (Jackson Immunoresearch, 1:1600) for 1 h at room temperature followed by exposure to DAB and mounting on gelatin coated slides. The total number of c-fos positive cells was counted in the L3-L5 dorsal horns by an experimenter blinded to treatment. Due to the diffuse expression of c-fos expression across the dorsal horn in spinal cords from relatively immature animals, the expression was counted in both superficial and deeper dorsal horns.

3. Data analysis

One-way ANOVA was used for comparing the number of ET-1 induced paw flinching on postnatal day 7 (treatment and sex). Two-way ANOVA was used for comparing time versus treatment between sexes in the capsaicin-induced mechanical hyperalgesia study. One-way ANOVA was used for comparing the number of c-fos positive neurons (treatment and sex). The conservative Bonferroni post-test was used for all analysis and a p-value <0.05 was considered significant. All statistical analysis was done with GraphPad Prism 5 (GraphPad Software, Inc, San Diego, CA).

4. Results

4.1. Behavioral analysis

Administration of ET-1 on P7 elicited a significantly greater number of paw flinches compared to saline administration in both male and female rats ($p < 0.05$; Fig. 1a). No differences between sexes was observed on P7 (ET-1 female vs. ET-1 male, $p > 0.05$; saline female vs. saline male, $p > 0.05$).

Administration of capsaicin to the contralateral dorsal hind paw on P11, produced secondary mechanical allodynia in the plantar hind paw (Fig. 1b and c). In saline controls animals, the duration of secondary allodynia was sex-dependent (Fig. 1b and c). In control males not previously exposed to ET-1, topical capsaicin produced secondary mechanical allodynia at all time points examined including 120 min post-capsaicin (Fig. 1b). In control females not previously exposed to ET-1, topical capsaicin produced secondary mechanical allodynia of a short duration (Fig. 1c). Secondary mechanical allodynia was only observed at 20 min after capsaicin administration. At 40 min post-capsaicin administration control saline females had significantly higher paw withdrawal thresholds compared to capsaicin-treated control saline males ($p < 0.05$). This suggests sex-dependent capsaicin-induced secondary allodynia in neonatal rats.

Priming with ET-1 on P7, did not alter the magnitude or duration of capsaicin-induced secondary mechanical hyperalgesia in males. There were no significant differences found between the primed and unprimed males at any of the time points (Fig. 1b). In contrast, priming with ET-1 on P7, sensitized females to capsaicin induced secondary mechanical hyperalgesia ($p < 0.01$ versus control across the time course) demonstrated by a significant reduction in paw withdrawal threshold across the entire 120 min (Fig. 1c). Interestingly, females primed with ET-1 on P7 had greater capsaicin induced secondary mechanical hyperalgesia as measured by a lower paw withdrawal threshold when compared to males primed with ET-1 on P7 ($p < 0.05$ across time). Administration of vehicle cream did not alter paw withdrawal thresholds across the time course in primed and unprimed males and females (data not shown).

4.2. Fos expression

ET-1 priming significantly increased capsaicin-induced c-Fos expression in both males and females ($p < 0.001$) compared to the unprimed animals and compared to control (Fig. 1d–f). C-fos expressing neurons were found in both superficial and deeper dorsal horn lamina (see Fig. 1d). Animals that were primed with ET-1 and received capsaicin on P11 had significant c-Fos expression in the right dorsal horn (Fig. 1d–f) compared to the animals who received saline on P7 and capsaicin on P11. Primed males treated with capsaicin had significantly greater c-Fos compared to primed females treated with capsaicin ($p < 0.001$). Interestingly, priming also increased c-Fos expression on the contralateral paw in males but not females (data not shown).