Published in final edited form as:

Neurosci Lett. 2014 May 1; 567: 15–18. doi:10.1016/j.neulet.2014.03.017.

Endothelin-1-induced priming to capsaicin in young animals

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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.

Keywords

Endothelin-1 (ET-1); Capsaicin; Neonatal rat; Nociception

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

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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.

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 μ L (1.31 ng/ μ L) 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 cyroprotecting 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 μ m 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 Fluor 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).

5. Discussion

5.1. Effect of nociception in neonatal period

The nociceptive response to capsaicin was sex dependent with males developing a longer lasting capsaicin induced secondary mechanical hyperalgesia compared to females. Priming with ET-1 on P7 sensitized females to capsaicin induced secondary mechanical hyperalgesia. These results also demonstrate that capsaicin causes a greater increase in Fos expression in the dorsal horns of ET-1 primed animals compared to ET-1 naïve animals, with a larger induction in males as compared to females. The results of this study provide more evidence for the hypothesis that pain early in life can alter the response to subsequent noxious stimuli and this alteration may be sex dependent. The neonatal period has been found to be a critical period for injury-induced changes to nociceptive processing, and these changes affect subsequent responses to noxious stimuli [9]. In children with sickle cell disease, the risk of experiencing a painful crisis occurs in the first year of life and increases thereafter [10].

5.2. Sex-specific changes in pain response

In female rats, capsaicin induced a short duration of secondary mechanical hyperalgesia on postnatal day 11. Priming with ET-1 on postnatal day 7 increased the duration of capsaicin-induced secondary mechanical hyperalgesia. In addition, priming with ET-1 led to greater capsaicin induced secondary mechanical hyperalgesia in females as compared to males. These results correspond with research that has reported sexually dimorphic differences in nociceptive thresholds in adulthood after neonatal injury [11]. They also relate to clinical research that has shown that female sickle cell anemia patients have a higher incidence of painful crisis compared to male sickle cell anemia patients [10].

5.3. Effect of priming on expression of Fos in dorsal horn of spinal cord

The significantly higher Fos expression seen in the ET-1 pre-treated animals suggests that the priming effect of ET-1 may be centrally mediated at the level of the spinal cord. However, it cannot be ruled out that ET-1 priming may also have supraspinal effects on descending modulation, such as enhancing facilitation of nociceptive inputs or reducing descending inhibition [12,13]. It is possible that similar to inflammatory pain, ET-1-induced nociception may cause a shift in the balance from inhibition to facilitation in the capsaicin treated animals [14]. In contrast to prior reports showing no Fos expression in the contralateral dorsal horns of adult rats after treating with capsaicin in the neonatal period, we show Fos expression in the contralateral dorsal horns [13]. However, this difference may be explained by the fact that in the prior report, Fos expression was measured in adulthood as opposed to the neonatal period. At this time it is not exactly clear what the functional significance is of the activation of contralateral neurons. One possible explanation is that the activation of these contralateral neurons could be due to the activation of polysynaptic pathways involving neuronal connections to the brainstem [15]. The observation of Fos expression in the deeper dorsal horns may be explained by an increase in the response of wide dynamic range and low-threshold neurons, which has been shown to happen after neonatal chronic inflammation [16,17].

The repetitive nociceptive pain experienced by sickle cell patients early in life may contribute to the development of chronic pain experienced by some patients later in life [18]. Because SCD patients may experience repeated painful crises throughout the infant and childhood periods, it is important to find better ways to effectively relieve pain during these critical periods.

Together, the results of this study suggest that pain in early life may alter future responses to painful stimuli at both the behavioral and neuronal level and the response at the behavioral level is sexually dimorphic.

Acknowledgements

This work was funded by National Institutes of Health grants R01 DA023593 and NS26363, a South Carolina Post-Baccalaureate Research Education Programs for Minorities funded by grants R25 GM066526 and R25 GM076277 from the National Institutes of Health, and the Alfred P Sloan Foundation.

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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.

Fig. 1.

Sex-dependent ET-1 priming on capsaicin induced secondary allodynia and spinal cord c-fos expression. (A) Intraplantar ET-1 administration increased the total number of spontaneous paw flinches on postnatal day 7 ($*p < 0.05$ ET-1 vs saline same sex). (B) In males, priming had no effect on capsaicin induced secondary allodynia in the contralateral hind paw ($*p < 0.05$ vs time 0 baseline, $\#p < 0.05$ vs primed animals). (C) In females, priming prolongs capsaicin-induced secondary allodynia in the contralateral hind paw ($*p < 0.05$ vs baseline, $\#p < 0.05$ vs primed animals, $^{\alpha}p < 0.05$ vs. males). (D) Representative micrographs of c-Fos staining in the right dorsal horn of the spinal cord. (D1) Ipsilateral and contralateral dorsal horn from male ET-1 + Capsaicin treatment. Large arrows point to deep dorsal horn c-fos positive neurons. Small arrows point to superficial dorsal horn c-fos positive neurons. Box indicates area that is shown in higher magnification in (D2). (D3) Ipsilateral dorsal horn from female ET-1 + Capsaicin treatment. Scale bar represents 500 μ m in D1 and 50 μ m in D2 and 3. (E) In females, priming increases capsaicin-induced c-fos in the dorsal horn ($***p < 0.001$ vs. control). (F) In males, priming increases capsaicin-induced c-fos in the dorsal horn ($***p < 0.001$ vs. control; $^{\alpha\alpha\alpha}p < 0.001$ vs. females).