Published in final edited form as:
Neurosci Lett. 2012 August 30; 524(2): 111–115. doi:10.1016/j.neulet.2012.07.018.

Effects of peripheral κ opioid receptor activation on inflammatory mechanical hyperalgesia in male and female rats

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Abstract

Activation of peripheral κ opioid receptors (KOR) effectively relieves pain and hyperalgesia in preclinical and clinical models of pain. Although centrally located KOR activation results in sexually dimorphic effects, it is unclear whether peripheral KOR also produces sex dependent effects in persistent inflammatory pain conditions. In this study, we investigated whether local administration of a specific KOR agonist, U50, 488 relieve mechanical hyperalgesia induced by the injection of complete Freund's adjuvant (CFA) in the rat hindpaw, and whether there are sex differences. The effects of U50, 488 were assessed three days after the induction of CFA-induced inflammation, a time point at which mechanical hyperalgesia was most prominent. There were no sex differences in baseline and CFA-induced changes in mechanical thresholds between male and female rats. Local treatment of U50, 488 produced moderate, but significant, anti-hyperalgesia in both male and female rats. However, U50, 488 was significantly more effective in male rats at the highest dose of U50, 488. We confirmed that the highest dose of U50, 488 used in this study did not produce systemic effects, and that the drug effect is receptor specific. On the basis of these results, we suggest that local KOR agonists are effective in mitigating mechanical hyperalgesia under a persistent inflammatory pain condition and that sex differences in anti-hyperalgesic effects become more evident at high doses.

Keywords

Sex; peripheral; kappa opioid receptor; inflammation; mechanical hyperalgesia

Targeting peripheral opioid receptors that are expressed on primary afferent terminals as effective means of treating a wide variety of pain conditions has been a topic of substantial amount of research in the past two decades [31,37]. Recently, considerable interest has been generated in developing kappa opioid receptor (KOR) ligands that are restricted to the

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There are no conflicts of interest associated with the present study.

periphery since activation of peripheral KOR does not produce many of the side effects mediated by mu or delta opioid receptors (MOR and DOR, respectively) [14,29,35,39,40].

Peripheral KORs are particularly effective in a variety of preclinical visceral pain models [6,19,32]. Consistent with the animal data, human clinical and experimental studies indicate that peripherally restricted KOR agonists produce significant analgesic effects on visceral pain [2,22]. However, peripheral KOR effects have been also demonstrated in somatic neuropathic and inflammatory pain models [4,20,24]. More recent studies showed that local KOR activation also decreases temporomandibular joint (TMJ) pain as well as inflammation and prevents the loss of alveolar bone and periodontal tissue [3,7,26,27].

It is well established that humans and animals show sex differences in KOR-mediated analgesia. While most studies that examined sex differences in KOR analgesia characterized centrally-mediated effects [10,15], few available studies that report sex differences in peripheral KORs [5,8,21] utilized persistent pain models as opposed to acute pain models that have been predominantly used to assess systemic effects of KOR treatments. Of those peripheral studies, two report a greater KOR effect in females [5,8] and one in males [21]. Thus, as with sex differences mediated by KOR in the CNS, multiple factors such as the type of stimulus modality and the intensity of a stimulus might contribute to sex differences in peripheral KOR [10,16]. However, additional studies utilizing different pain conditions are required to increase our overall understanding on neurobiological mechanisms underlying sex different responses to opioid treatments at the site of injury. In this study, we examined whether a locally applied KOR agonist attenuates complete Freund's adjuvant (CFA)-induced mechanical hyperalgesia in a sexually dimorphic manner using a rat hindpaw model.

One hundred and two age-matched adult male and female Sprague Dawley rats (8 weeks old; 250–300 g for males and 225–260 g for females, Harlan, Indianapolis) were used in all experiments. The estrus cycle phase in female rats was not determined in this study. All animals were housed in a temperature-controlled room under a 12:12 light-dark cycle with access to food and water *ad libitum*. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and under a University of Maryland approved Institutional Animal Care and Use Committee protocol.

Mechanical sensitivity of the hindpaw was assessed with the Randall-Selitto test, an established rodent model for testing mechanical hypersensitivity of the paw. Animals were first allowed to habituate to the experimental room for 30 min for three consecutive days. The withdraw response to noxious paw pressure was assessed using a digital paw pressure Randall-Selitto applicator for rodents (IITC Life Science, Woodland Hills, CA). Each rat was placed in a cloth holder suspended in a sling, and the probe of the pressure applicator was placed under the plantar surface of the hindpaw. The probe has a spring load for easy opening and closing of the pressure applicator. The probe, which is placed under the paw, close the pressure applicator, captures and stores the pressure upon reaction. A gradually increasing pressure was applied until the rat withdraws its hindpaw. The lowest pressure necessary to elicit the withdraw response prior to inflammation was considered as the baseline mechanical threshold.

Inflammation was induced by the injection of complete Freund's adjuvant (CFA, 50 μl; 1:1 isotonic saline) into the plantar surface of the right hindpaw with a 27-gauge needle over 5–10 s. Control animals were injected with the same volume of isotonic saline (ISO) in the same manner. Anti-hyperalgesic effects of U50, 488 (Tocris), a specific KOR agonist, was measured on day 3 after intraplantar injection of CFA or saline, during which mechanical hyperalgesia was most profound. On day 3, three different doses of U50, 488 (1, 30, or 100

μg/20 μl) were dissolved in phosphate buffer solution (PBS) or the same volume of vehicle control were administered into the plantar surface of the inflamed hindpaw of both male and female rats. A pre-injection mechanical threshold for evoking a hindpaw withdrawal response was determined 15 min prior to drug injection. Changes in mechanical sensitivity of the hindpaw were assessed 30, 60, 120 and 180 min after the administration of the drug. To rule out the possibility that local administration of U50, 488 produced systemic effects by activating KOR in the CNS, additional groups of male and female rats received the highest doses of U50, 488 (100 μg) injections into the hindpaw contralateral to the mechanical stimulation. To test the receptor specificity of the agonist, a selective antagonist for KOR, nor-BNI (Tocris; 200 μg/20 μl) was administered 10 min before U50, 488 (100 μg) treatment in male and female rats. The doses of the KOR agonist and the antagonist were adapted from previous studies [33]. All experimental and control groups consisted of 6 animals per group.

One-Way ANOVA was used to compare the differences in baseline mechanical thresholds between male and female rats. The inflammation-induced or drug-induced changes in mechanical thresholds were analyzed with a Two-Way ANOVA with repeated measures. In order to compare sex differences in the effect of individual doses of U50, 488, the mechanical thresholds were also normalized to the pre-injection threshold and the percent changes following treatment were assessed. All comparisons between multiple groups were followed by a *post hoc* test (Holm-Sidak method). All data are presented as mean ± standard error of the mean (SE), and differences were considered significant at $p < 0.05$.

The average mechanical thresholds needed to evoke a nocifensive hindpaw withdraw response before any treatments ranged between 158 – 202 g. There was no significant difference in the baseline mechanical thresholds between male and female rats that received either CFA or ISO (Fig 1A; F=2.19, $p > 0.05$). Following the CFA treatment, there was a significant group effect (F=53.2, $p < 0.001$) and time effect (F=21.3, $p < 0.001$). The ISO treatment did not significantly alter the mechanical thresholds at any time point during the 28 days of observation in either male or female rats (Fig 1B). In contrast, the CFA treatment caused a significant and prolonged mechanical hyperalgesia in both male and female rats. The mean mechanical thresholds were significantly reduced from day 1, reaching the peak around day 3 (41.3 g for males and 44.7 g for females), and then showed a tendency of gradually returning to baseline over 28 days. However, a significant mechanical hyperalgesia was still present on day 28. There were no significant differences in the magnitude and the extent of CFA-induced mechanical hyperalgesia between male and female rats. A significant interaction between treatment and time was observed (F=5.89, $p < 0.005$).

On day 3 when the CFA-induced mechanical hyperalgesia was most prominent, local injection of U50, 488 dose-dependently reversed the hyperalgesia in male rats (Fig 2A). There was a significant group effect (F=5.23, $p < 0.05$) without a significant time effect (F=2.4, $p > 0.05$). A significant interaction between dose and time was observed (F=8.1, $p < 0.001$). PBS injection further increased the hyperalgesia possibly due to an injection procedure in the inflamed paw (Fig 2C). U50, 488 at 1 μg did not attenuate either the injection-induced or the CFA-induced component of mechanical hyperalgesia, while U50, 488 at 30 μg slightly attenuated the CFA-induced hyperalgesia (Fig 2A). U50, 488 at 100 μg not only blocked the injection-induced exacerbation of mechanical hyperalgesia, but also significantly attenuated the CFA-induced hyperalgesia. Similarly, U50, 488 dose-dependently reversed mechanical hyperalgesia in female rats (Fig 2B; F= 9.19, $p < 0.01$). There was also a significant time effect at 30 and 60 min (F=5.9, $p < 0.05$). A significant dose and time interaction was also observed in the female group (F=4.62, $p < 0.05$)

In order to compare sex differences in the effectiveness of U50, 488, we normalized the data to the pre-treatment value and plotted and analyzed the responses to vehicle and each dose of U50, 488 separately (Fig 2C). PBS injection in the inflamed paw exacerbated mechanical hyperalgesia to a similar extent between male and female rats. There was a significant time effect (F=6.4, $p < 0.001$), but no significant sex effect (F=0.28, $p > 0.05$). Following PBS injection, the mechanical threshold was further reduced by approximately 30 % at 30 min and remained significantly depressed for 180 min. There was neither a significant sex effect nor time effect following the treatment with 1 μg of U50, 488 (F=0.47, $p > 0.05$; F=1.73, $p > 0.05$, respectively). The 30 μg dose of U50, 488 significantly attenuated the hyperalgesia for 120 minutes (F= 5.05, $p < 0.05$), but no sex differences were observed at this dose (F= 0.24, $p > 0.05$). More profound and prolonged anti-hyperalgesic effect was observed with U50, 488 at 100 μg. The significant anti-hyperalgesic effect was maintained for 180 min (F=25.3, $p < 0.01$). Interestingly, a significant sex effect was observed at this high dose with males exhibiting a greater anti-hyperalgesic effect than females (F=6.0, $p < 0.05$). There was no significant interaction between sex and time in any of the treatment conditions.

The highest dose of U50, 488 (100 μg) injected into the paw contralateral to the inflamed paw neither caused a further reduction of mechanical hyperalgesia nor attenuated the CFA-induced hyperalgesia in both male and female rats (Fig 3A,B). There was a significant drug effect (Male: F=8.06, Female: F=13, $p < 0.05$) and time effect (Male F=4.27, Female: F=5.3, $p < 0.05$) for both male and female groups. These data suggested that the anti-hyperalgesic effect produced by U50, 488, even at the highest dose utilized in this study, is mediated by local KOR. Finally, we confirmed that the anti-hyperalgesic effects produced by U50, 488 are receptor mediated by pre-treating the hindpaw with nor-BNI prior to U50, 488 administration. The nor-BNI pre-treatment completely prevented the anti-hyperalgesic effects of U50, 488 in both male and female rats (Fig 3C).

The obvious clinical advantages of avoiding the centrally mediated side effects of opioids by targeting peripheral opioid receptors continues to generate a substantive amount of data under various pain conditions. [2,12,13,14,36]. Peripheral KORs are demonstrated as a particularly important target due to their lower abuse potential and generation of fewer side effects [38]. Peripherally restricted or systemically low doses of KOR agonists reliably attenuate visceral pain in animals [6,19,32]. In humans, a peripherally restricted KOR agonist, CR665, significantly attenuates visceral pain while paradoxically enhancing somatic pain [2]. Asimadoline, another peripherally restricted KOR agonist, provides a significant relief of pain and discomfort in patients with irritable bowel syndrome [22], and ADL-10-0101 in patients with chronic pancreatitis [14]. Although preclinical studies indicate that peripheral KORs also play a role in somatic and joint pain conditions [8,11,25,27], additional studies are warranted to take full advantage of peripheral KOR agonists that are currently undergoing clinical trials [37].

To that end, we provide additional evidence that a local dose of U50, 488 can partially, but significantly, attenuate CFA-induced inflammatory mechanical hyperalgesia in somatic tissue. Previous studies that utilized inflammatory agents such as carrageenan, capsaicin, or formalin examined the anti-hyperalgesic effects of KOR agonists under pain conditions that are resolved in a relatively shorter duration and during the development phase of inflammatory pain [1,8,20,21]. Our data is particularly interesting in that U50, 488 was anti-hyperalgesic 3 days after the induction of inflammation, a time point at which the hyperalgesia was fully developed. The data is also consistent with the anti-hyperalgesic effects of asimadoline on mechanical hyperalgesia in an adjuvant-induced arthritis model [5].

In the CNS, animal studies generally report greater KOR mediated analgesia among males [9], whereas human studies with mixed action κ-agonist-antagonists have shown better analgesia in women than men in a dental pain model [16,17]. At present, there is no clear picture as to how peripheral KOR agonists produce different responses between the sexes. Peripheral KOR agonists produce a greater reduction of formalin-induced nociceptive responses in female rats [8], which is consistent with human clinical data [16,17]. In contrast, the peripheral action of KOR agonists is more potent in males whereas sex differences are not observed with mixed action opioids in capsaicin-induced hyperalgesia in rats [21]. Finally, sex differences in the effect of asimadoline are evident in attenuating only thermal, but not mechanical, hyperalgesia in a rat model of persistent arthritis [5]. Our data showed that U50, 488 dose-dependently attenuate CFA- and injection-induced mechanical hyperalgesia in both male and female rats. However, albeit small but significant sex differences can be observed at a higher dose. A small sex related difference we observed could be due to the fact that we tested the drug effect when the hyperalgesia is most prominent. It is also possible that a higher dose of U50, 488 could exaggerate sex differences. We did not test higher doses since U50, 488 may penetrate the blood brain barrier. Our data still provides important clues to studying the mechanistic basis regarding sex differences in peripheral KOR analgesia.

Although cellular mechanisms of sex differences in peripheral KOR-mediated analgesia have not been systematically investigated, it is likely that the nature of sex differences in peripheral KOR effects involve multiple mechanisms. There may exist sex differences at the level of KOR expression, KOR trafficking, and KOR signaling pathways in sensory neurons. For example, systemic activation of KORs is thought to produce sex differences via NMDA dependent mechanisms in acute pain models [18], while peripheral KOR mediated sex differences do not involve NMDA receptors [21]. Sex differences in peripheral DOR effect is in part mediated by sex differences in KATP expression, one of the downstream effectors of ORs in trigeminal sensory neurons [23,30].

Puehler et al. [28] report significant upregulation of KOR mRNA in dorsal root ganglia following the induction of paw inflammation by CFA, an effect measured only in male rats. We have shown that CFA-induced inflammation in the masseter muscle significantly upregulates MORs and KORs in trigeminal ganglia of male, but not in female, rats [41]. Male specific upregulation of KORs under inflammatory conditions could explain a lack of asimadoline effect in female patients with irritable bowel syndrome [34]. In our study, it is possible that a higher level of KOR in male rats rendered greater anti-hyperalgesic effects that became apparent with higher doses of U50, 488.

Taken together, our data bear clinical significance since the management of many types of chronic pain conditions is sexually dimorphic, and since there is increasing clinical as well as pre-clinical evidence that indicates peripheral KORs as potential therapeutic targets. Understanding the mechanical basis regarding the sex differences in KOR function will help develop sex-specific management strategies that can be directed at the periphery to ameliorate persistent types of pain.

Acknowledgments

This study was supported by NIH grant RO1 DE19448 (J.Y.R.).

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Highlights

- CFA induced long lasting mechanical hyperalgesia in the rat hindpaw.
- No sex differences in baseline or changes in mechanical sensitivity were observed.
- Activation of peripheral KOR attenuated inflammatory mechanical hyperalgesia.
- High dose of KOR agonist produced moderate but significant sex differences.

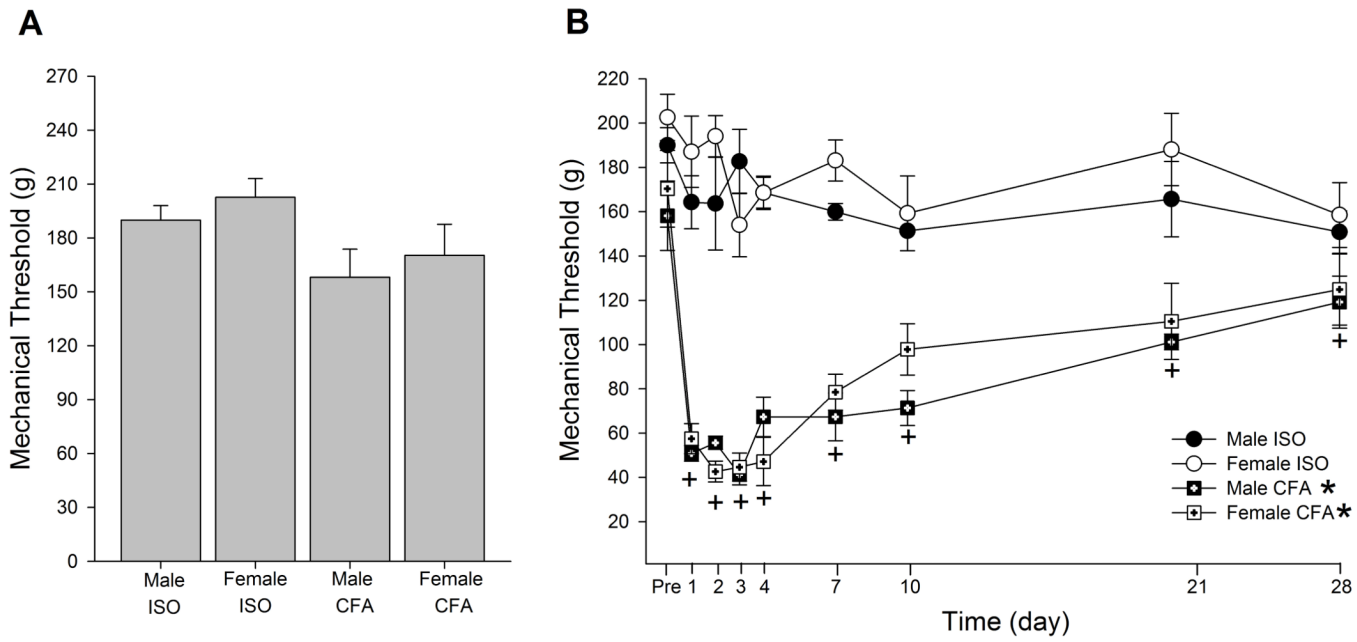

Figure 1.

Effects of i.pl injection of CFA on hindpaw mechanical sensitivity. (A) Baseline thresholds of CFA and ISO treated male and female rats. (B) Time course of changes in mechanical thresholds of CFA and ISO treated male and female rats. * and + indicate significant group and time effect, respectively. Each group consisted of n=6.

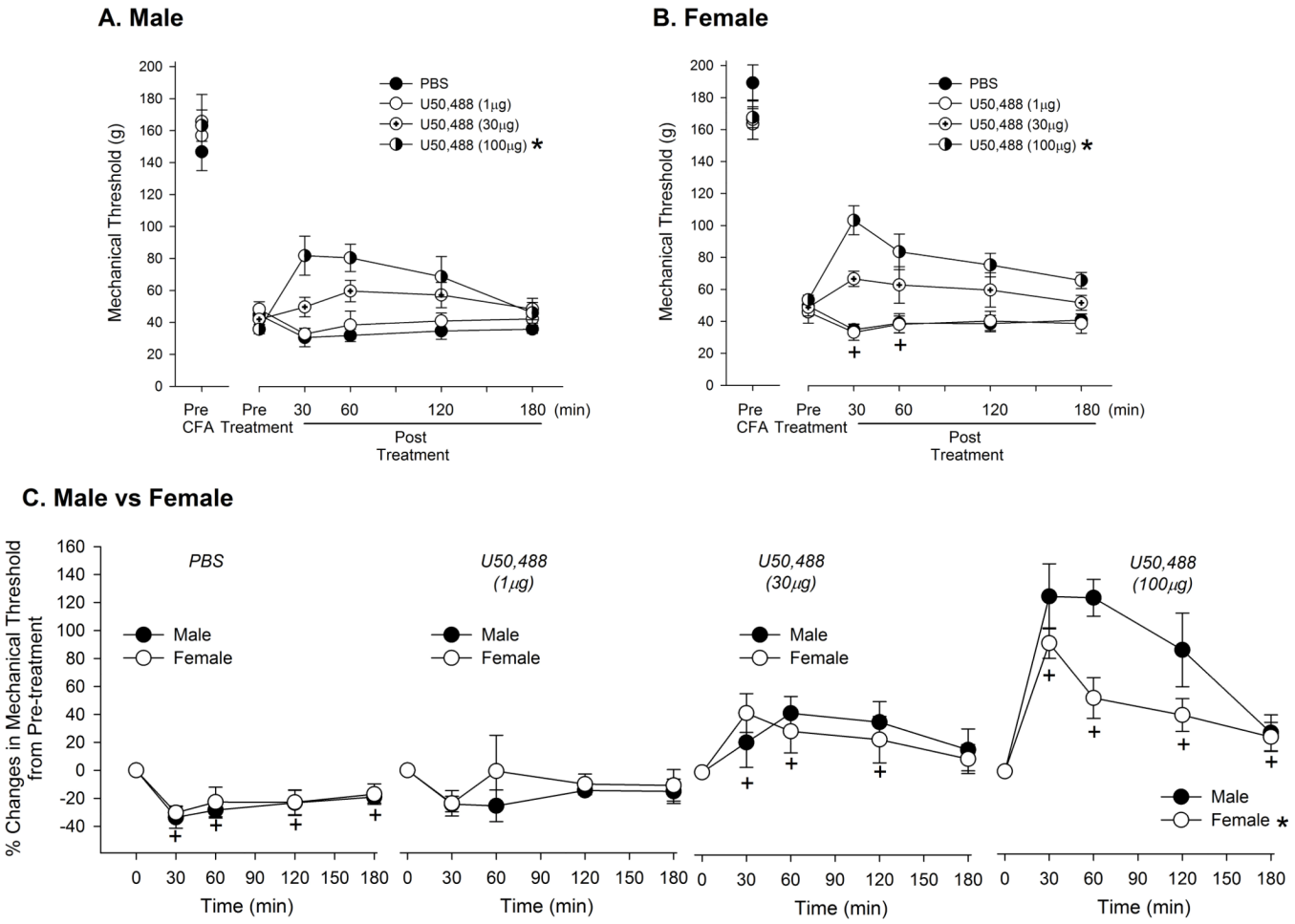

Figure 2.

Effects of local treatment of U50, 488 on CFA-induced mechanical hyperalgesia. On day 3 of CFA injection, changes in mechanical thresholds after local U50, 488 treatment were measured for 180 minutes in male (A) and female (B) rats. The drug was administered 5 minutes after pre-treatment measurement of mechanical threshold. * indicates significant groups effect compared to PBS at $p < 0.05$. (C) Direct comparisons of percent changes in mechanical thresholds between male and female rats following PBS or drug treatment. * and + indicate significant group and time effect, respectively. Each group consisted of n=6.

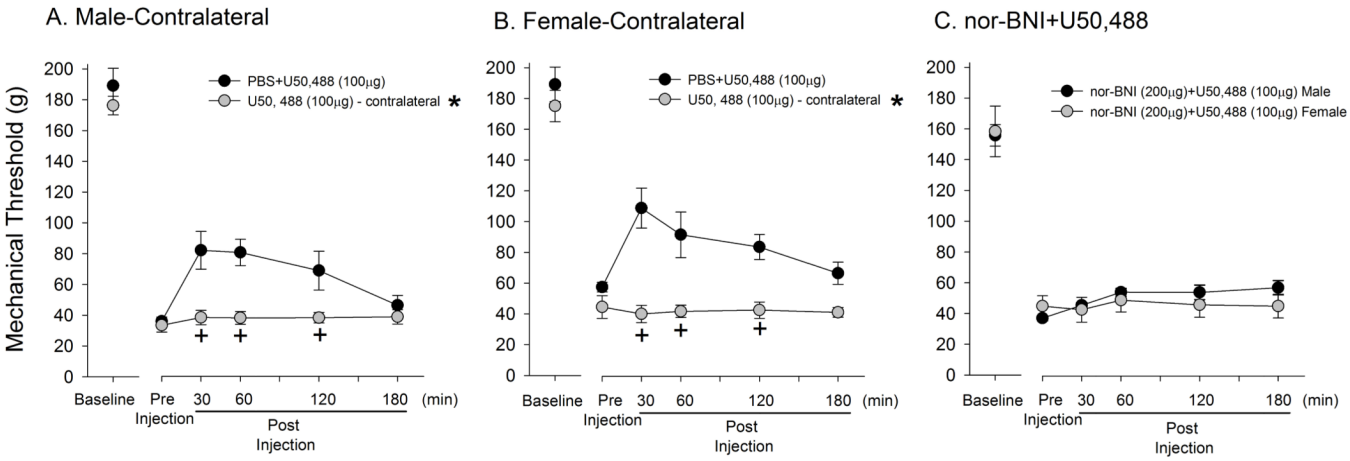

Figure 3.

Local and receptor mediated effects of U50, 488. On day 3 of CFA injection, U50, 488 injected into the hindpaw contralateral to the CFA-inflamed paw had no effect on mechanical thresholds in either males or females (A, B). On day 3 of CFA injection, changes in mechanical thresholds after local U50, 488 treatment with nor-BNI in the same paw were measured in male and female rats (C). The nor-BNI was administered 5 minutes after the pre-treatment measurement of mechanical threshold, followed by U50, 488. *, + indicates significant group and time effect, respectively. Each group consisted of n=6.