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EARLY-IN-LIFE BLADDER INFLAMMATION ALTERS U50,488H-BUT NOT MORPHINE-INDUCED INHIBITION OF VISCEROMOTOR RESPONSES TO URINARY BLADDER DISTENSION

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Abstract

Previous research has suggested that early-in-life (EIL) exposure to bladder inflammation impairs the function of endogenous opioid inhibitory system(s) and may contribute to the development of chronic bladder pain. This study examined how acute adult and/or prior EIL exposure to bladder inflammation altered the inhibitory effects of systemic κ - and μ -opioid agonists on the visceromotor reflex (VMR) to urinary bladder distension (UBD). Female rats were exposed intravesically EIL (P14–P16) to either the inflammatory agent zymosan or anesthesia-alone, and then rechallenge as adults (12–17 weeks) with either anesthesia-alone or zymosan. The VMR to 60 mmHg UBD was measured after cumulative intravenous (i.v.) administration of 1 mg/kg and 4 mg/kg of either the κ -opioid agonist U50,488H or the μ -opioid agonist morphine. Morphine produced dose-dependent inhibition of the VMR to UBD in all groups, and U50,488H produced dose-dependent inhibition of the VMR to UBD in all but one group. Animals that received bladder inflammation both EIL and as adults showed significantly augmented VMRs to UBD (>100% baseline values) following 1 mg/kg of U50,488H and diminished inhibition of VMRs following 4 mg/kg of U50,488H when compared with other groups. In contrast, neither EIL nor adult bladder inflammation markedly altered the inhibition of the VMR to UBD produced by either 1 or 4 mg/kg of i.v. morphine. These data suggest EIL and adult exposure to bladder inflammation selectively decreases the inhibitory effects of κ -opioids and thereby may enhance bladder hypersensitivity in patients with painful bladder syndromes.

Keywords

Opioid; Visceral Pain; Inflammation; Painful Bladder Syndrome; Animals; Newborn

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Introduction

There is a mounting interest in the long-term effects of exposure to noxious events during early-in-life (EIL) development. Evidence suggests these events alter the development of pain processing systems and can result in permanent changes in pain sensitivity [5]. It has been hypothesized that EIL exposure to noxious stimulation of the bladder also may contribute to the development of some adult painful bladder disorders [13]. Urinary tract infections (UTIs) are relatively common occurrences during the neonatal and childhood periods [2], and bladder pain syndrome (BPS)/interstitial cystitis (IC) has been correlated with both childhood bladder infections and antibiotic use [12]. Animal models designed to study the effects of EIL inflammation of the bladder on adult processing of noxious bladder stimuli are consistent with this hypothesis. EIL exposure to bladder inflammation followed by adult re-inflammation of the bladder produces bladder hypersensitivity as manifested by increased visceromotor reflexes (VMRs), arterial blood pressure, and heart rate responses to graded urinary bladder distension (UBD) [13].

Alterations in the functioning of endogenous opioid inhibitory systems may be responsible for the changes in bladder nociceptive processing produced by EIL inflammation. EIL bladder inflammation impairs an endogenous opioid inhibitory system(s) that can be normally engaged by bladder inflammation and which can be revealed by naloxone treatment [4]. The nature of the opioid inhibitory system affected by EIL bladder inflammation has not been established, but dynorphin and κ -opioid systems may play a critical role since a recent study [16] revealed that EIL bladder inflammation produces a chronic increase in dynorphin A (1–17) in the lumbosacral spinal cord. To functionally evaluate potential changes in opioid responsiveness, VMRs to UBD following EIL and/or adult bladder inflammation were measured in response to intravenous (i.v.) administration of the κ - and μ -opioid receptor agonists U50,488H and morphine, respectively. U50,488H is a highly selective κ -opioid agonist and morphine was used as a μ -opioid agonist because of its extensive clinical use and high standard as a comparison opiate. Altered responses to these ligands could reveal a mechanism that contributes to enhanced bladder sensitivity in painful bladder disorders.

Materials and Methods

This study was approved by the University of Alabama at Birmingham Animal Care and Use Committee and conformed to NIH guidelines for the care and use of laboratory animals. Female pups from timed-pregnant Sprague-Dawley rats were treated with either anesthesia-only or intravesical zymosan (0.1 ml, 1% in saline) for three successive 30 min daily treatments from P14–P16 as described previously [13]. Animals were allowed to mature and at 12–17 weeks received a single 30 min treatment with anesthesia-only or intravesical zymosan (0.5 ml, 1% in saline) as described previously [13]. EIL and adult intravesical treatments resulted in 5 groups: those naïve to any treatment (naïve), those receiving anesthesia-only both EIL and as adults (AA), those receiving anesthesia-only EIL and zymosan as adults (AZ), those receiving zymosan EIL and anesthesia-only as adults (ZA), and those receiving zymosan both EIL and as adults (ZZ). All animals underwent adult intravesical treatments during proestrus (based on daily vaginal lavage for one or more complete cycles) such that most animals were in estrus on the day of testing.

24 hours following adult intravesical treatments, each animal was anesthetized with isoflurane and oxygen (5% for induction, 4% for maintenance, 1% during testing). The external jugular vein was cannulated and the urinary bladder was catheterized with a 22-gauge angiocatheter via the urethra. The rat was artificially respired via tracheostomy and body temperature was maintained at 37°C with a heating pad. Platinum wire electrodes were

inserted into the left external oblique muscle through an incision in the left abdominal skin. Electromyographic (EMG) activity was differentially amplified, rectified, and saved using Spike 2 software (Cambridge Electronic Design). The VMR was quantified as: (EMG activity during UBD - EMG prior to UBD)/(background EMG activity). Data are presented as a % baseline calculated as (test trial VMR/mean VMR of last 2 pre-drug trials) x 100%. Six or more distension trials at 60 mmHg of air pressure (3 min ITI) were presented until the VMRs were stable and varied by less than 10% on 2 consecutive trials. These latter two test trials were averaged and served as the baseline value for a particular animal. In separate groups, 1 mg/kg of either i.v. ($\pm$)-U50,488H or morphine was administered. Test trials were conducted at 1, 4, 7, 10, and 13 min after drug administration. At 15 min after drug administration, 4 mg/kg of either i.v. U50,488H or morphine was administered, and trials were conducted at 16, 19, 22, 25, and 28 min. To determine if i.v. vehicle alone may alter the VMR, a separate group of naïve animals received 1 ml/kg followed by 4 ml/kg saline vehicle in a manner identical to that described for 1 mg/kg and 4 mg/kg U50,488H and morphine.

Three sets of comparisons were conducted using SYSTAT 12 (Systat Software, Inc.). First, responses of naïve animals administered saline were compared to those of naïve animals given either U50,488H or morphine. Data were analyzed separately at each dose (1 mg/kg, 4 mg/kg, 1 ml/kg, or 4 ml/kg) using ANOVAs and Fisher's LSD. Second, VMRs of animals after i.v. administration of U50,488H or morphine were compared between different intravesical treatment groups (AA, AZ, ZA, and ZZ). Data were again analyzed separately at each dose using ANOVAs followed by Fisher's LSD with Holm's correction [6]. Third, a set of comparisons was conducted to determine if U50,488H, morphine, or saline significantly inhibited or facilitated VMRs relative to 100% baseline values using one-sample t-tests. For all comparisons, any outliers (values more extreme than 1.5x the interquartile range from the first or third quartile) were excluded.

Results

Naïve Animals

Figure 1 displays VMRs to 60 mmHg UBD in naïve animals receiving i.v. administration of 1 mg/kg and 4 mg/kg of U50,488H or morphine, or 1 ml/kg and 4 ml/kg saline vehicle. While saline did not significantly alter group mean VMRs, both U50,488H and morphine produced robust inhibition of VMRs. One-sample t-tests revealed that VMRs were significantly less than 100% baseline ($p < 0.001$) after administration of 1 mg/kg and 4 mg/kg of U50,488H or morphine but not saline. A mixed between-within ANOVA was conducted comparing VMRs to UBD after 1 mg/kg of U50,488H, 1 mg/kg of morphine, and 1 ml/kg of saline. This revealed a significant effect of drug (U50,488H, morphine, or saline) [$F(2,13)=42.79$, $p < 0.001$], but no significant effect of time or group x time interaction. Post-hoc comparisons were conducted for data collapsed across time. VMRs of animals treated with 1 mg/kg of U50,488H or morphine were significantly less than those of animals treated with saline ($p < 0.001$ for both comparisons). The inhibition produced by 1 mg/kg of U50,488H was significantly less than that produced by 1 mg/kg of morphine ($p = 0.023$). Similarly, a mixed between-within ANOVA comparing VMRs to UBD after 4 mg/kg of U50,488H, 4 mg/kg of morphine, and 4 ml/kg of saline revealed a significant effect of drug [$F(2,12)=92.93$, $p < 0.001$] but no significant effect of time or group x time interaction. Again, post-hoc comparisons were conducted on data collapsed across time. VMRs of animals treated with 4 mg/kg of U50,488H ($p < 0.001$) or morphine ($p < 0.001$) were significantly less than those of animals treated with saline. VMRs of the former two groups were not significantly different from each other. Therefore, suppression produced by U50,488H and morphine is not attributable to either the vehicle or i.v. volume.

Two-tailed t-tests revealed that 1 mg/kg of U50,488H produced more robust suppression of VMRs in naïve animals compared with those exposed to anesthesia only (group AA) (Figures 1 and 2, $p=0.004$). In addition, VMRs of group AA were significantly greater than those of naïve controls 10 ($p=0.002$) and 13 ($p=0.001$) min after administration of 1 mg/kg of morphine (Figures 1 and 3). To account for the effects of anesthesia exposure, group AA was used as the appropriate control group in the data described below.

1 mg/kg of U50,488H

Figure 2 displays VMRs to 60 mmHg UBD after 1 mg/kg and 4 mg/kg of U50,488H. 1 mg/kg of U50,488H produced modest suppression of VMRs to UBD in most groups. In contrast, the group receiving EIL bladder inflammation followed by adult re-inflammation (group ZZ) showed significant increases in VMRs above 100% baseline. A mixed between-within ANOVA revealed a significant effect of intravesical treatment group [$F(3,23)=9.44$, $p<0.001$], but no significant effect of time or group x time interaction. Post-hoc comparisons were conducted for data collapsed across time. VMRs of group ZZ were significantly greater than those of all other groups ($p<0.001$). VMRs of groups AA, AZ, and ZA were significantly less than 100% baseline ($p<0.001$). In contrast, VMRs of group ZZ were significantly greater than 100% baseline ($p<0.001$) indicating that 1 mg/kg of U50,488H truly facilitated responses in group ZZ. A one-way ANOVA revealed that pre-drug baseline VMRs to 60 mmHg UBD did not differ between intravesical treatment groups, indicating differences in the effects of U50,488H were not due to floor or ceiling effects that could result from very high or very low pre-drug baselines.

4 mg/kg of U50,488H

All groups showed suppression of VMRs to UBD following i.v. administration of 4 mg/kg of U50,488H. A mixed between-within ANOVA revealed significant effects of intravesical treatment group [$F(3,22)=7.11$, $p=0.002$] and time [$F(4,88)=48.04$, $p<0.001$] and a significant group x time interaction [$F(12,88)=2.89$, $p=0.002$]. Post-hoc comparisons were conducted separately at each time point. VMRs of group ZZ were significantly greater than those of groups AA and AZ from 19 to 28 min after the first dose ($p=0.008$). In addition, responses of group ZA were significantly greater than those of AA controls at 22 min ($p=0.012$) and 28 min ($p=0.015$). These data indicate VMRs of group ZZ were less suppressed by 4 mg/kg of U50,488H than those of groups AA and AZ. In addition, VMRs of group ZA were less suppressed by 4 mg/kg U50,488H than group AA. VMRs of groups AA, AZ, and ZA were significantly less than 100% baseline at all time points ($p=0.029$), indicating they were inhibited by 4 mg/kg of U50,488H. VMRs of group ZZ were significantly less than 100% baseline at most time points ($p=0.002$) excluding 28 min after the first dose.

1 mg/kg of Morphine

Figure 3 displays VMRs to 60 mmHg UBD after 1 mg/kg and 4 mg/kg of morphine. 1 mg/kg of morphine suppressed VMRs to UBD in all groups immediately following administration, followed by a gradual return towards baseline. A mixed between-within ANOVA revealed a significant effect of time [$F(4,104)=49.568$, $p<0.001$], but no significant effect of intravesical treatment group or group x time interaction. Therefore, post-hoc tests were not conducted. VMRs of groups AA and ZZ were significantly less than 100% baseline at all time points ($p=0.015$). VMRs of group AZ were significantly less than 100% baseline at all time points ($p=0.004$) except for 13 min after administration of 1 mg/kg of morphine, and VMRs of group ZA were significantly less than 100% baseline only at 1 and 4 min ($p=0.003$). A one-way ANOVA revealed that pre-drug baseline VMRs to UBD did not differ between intravesical treatment groups.

4 mg/kg of Morphine

All groups showed robust suppression of VMRs to UBD after administration of 4 mg/kg morphine. The effect of intravesical treatment group was not significant. Therefore, post-hoc tests were not conducted. VMRs of all groups were significantly less than 100% baseline at all time points ($p < 0.001$).

Discussion

The general suppressive actions of the studied drugs are consistent with results of others showing i.v. administration of either U50,488H or morphine produces inhibition of the VMR to UBD in untreated rats with ED50s of 1.34 mg/kg and 0.69 mg/kg, respectively [18]. However, EIL bladder inflammation followed by adult re-inflammation (group ZZ) produced a significant reduction in the inhibitory effectiveness of the κ -opioid receptor agonist U50,488H on VMRs to UBD, and in fact produced facilitation, without affecting μ -opioid receptor agonist effects. In this treatment group, 1 mg/kg of U50,488H facilitated VMRs above the 100% baseline level, and VMRs of group ZZ were less suppressed than those of the other groups following subsequent administration of 4 mg/kg of U50,488H. In contrast, groups receiving a single exposure to zymosan either EIL (group ZA) or as an adult (group AZ) showed suppression of VMRs similar to those of non-inflamed controls. Surprisingly, EIL and/or adult bladder inflammation had very little impact on the magnitude of VMR to UBD after i.v. administration of morphine. Also surprisingly, EIL and/or adult bladder inflammation did not significantly alter pre-drug baseline VMRs compared to group AA. This is in contrast to the enhanced VMRs during 60 mmHg UBD previously demonstrated in group ZZ [13]. This discrepancy is presumably due to differences in the testing protocol (e.g. number of initial 60 mmHg distensions) between these two studies.

The results of administration of U50,488H suggest κ -opioid agonists normally inhibit bladder nociception but EIL combined with adult bladder inflammation reveals a facilitatory, pro-nociceptive effect. U50,488H has been demonstrated to have both facilitatory and inhibitory effects on the spinal dorsal horn. For example, low doses of U50,488H produce either facilitation or inhibition of spinal dorsal horn neuronal responses to electrical C-fiber stimulation, noxious pinch, or formalin, while greater doses consistently produce inhibition of spinal dorsal horn neuron responses to these stimuli [9, 20]. If U50,488H acted centrally in the present study, then i.v. administration of the 1 mg/kg dose may have been sufficient to produce a net inhibition of spinal dorsal horn neurons to bladder input in groups AA, AZ, and ZA resulting in the decrease in VMRs to UBD. Both EIL and adult bladder inflammation may have impaired this inhibitory effect and thus shifted the dose-response curve for U50,488H to the left in group ZZ, producing more facilitation than inhibition of spinal dorsal horn neurons. At 4 mg/kg of U50,488H, VMRs of group ZZ were initially suppressed but returned toward baseline more quickly than those of groups AA and AZ. The reason behind this different time course is not clear. The pharmacokinetics of U50,488H could be altered in group ZZ such that U50,488H is eliminated from the plasma more rapidly. Alternatively, the pharmacodynamics of U50,488H may differ between intravesical treatment groups. For example, U50,488H could more quickly dissociate from an inhibitory receptor or the receptor could be inactivated more quickly. As inhibition by U50,488H decreases, underlying facilitatory effects at another receptor may be unmasked, enhancing the return toward baseline VMR responses.

The target of U50,488H has proven controversial since attempts to reverse U50,488H-mediated inhibition with selective κ -opioid receptor antagonists [3, 14, 19] or a knockdown of the κ -opioid receptor [8] have frequently failed. Sodium channel blockade has been proposed as a non-opioid mechanism for inhibition of responses to colorectal distension (CRD) and UBD by U50,488H. (+)-U50,488H, the inactive enantiomer, decreased voltage-

activated sodium currents in colon sensory neurons in the dorsal root ganglia (DRG) [7, 17] and inhibited pelvic afferent responses to CRD [17]. However, only ($\pm$)-U50,488H and ($-$)-U50,488H inhibited VMRs to CRD, and the ED50s required for this inhibition (4.7 mg/kg and 0.9 mg/kg, respectively) were less than those required for inhibition of primary afferent activity (8.0 mg/kg and 6.6 mg/kg, respectively) [17]. Further, while pretreatment with nor-BNI did not significantly reduce the inhibition produced by 10 mg/kg ($\pm$)-U50,488H, it was able to reduce the inhibition produced by 5 mg/kg ($-$)-U50,488H [17]. Therefore, at lesser, potentially more selective doses of ($\pm$)-U50,488H such as those used in the current study (1 and 4 mg/kg), inhibition of visceral nociception may be at least partially mediated by κ -opioid receptors.

The facilitatory and/or inhibitory effects of U50,488H may be mediated by actions at both a facilitatory κ -opioid receptor monomer and an inhibitory μ/κ -heterodimer. The μ/κ -heterodimer requires concurrent activation of ER α , ER β , GPR30, and progesterone receptors (PR) [10] and has been shown to mediate morphine anti-nociception in female rats during proestrus [1, 11]. It is proposed to mediate the anti-nociceptive effects of κ -opioids, whereas the κ -opioid receptor monomer has been proposed to facilitate nociception [10]. The interplay between these receptors could provide an explanation for the present data. Normally U50,488H might primarily inhibit VMRs to UBD by acting at the κ -protomer of the inhibitory μ/κ -heterodimer while at the same time producing weak opposing facilitatory effects through activation of the κ -opioid receptor monomer. Bladder inflammation could shift the balance between heterodimer-mediated inhibition and monomer-mediated facilitation. For example, we have shown that EIL intravesical zymosan treatment increases spinal dynorphin [16] and that adult inflammation decreases circulating estrogen and progesterone [15], both which could influence μ/κ -heterodimer expression. Since VMRs were inhibited after adult (group AZ) and EIL (group ZA) bladder inflammation similar to AA controls, the combination of adult inflammation-induced decreases in gonadal hormones and EIL-induced increases in spinal dynorphin may be the necessary condition to alter the effect of U50,488H from inhibition to excitation. ZZ treatment may have little effect on responses to i.v. morphine because the inhibitory influences of morphine are presumably maintained through the μ -opioid receptor monomer when the μ/κ -heterodimer is downregulated because of hormone depletion [10].

Relation to previous studies and BPS/IC

Previous studies have shown that group ZZ displays the greatest amount of bladder sensitivity [13]. Since EIL inflammation increases dynorphin [16], and the current study demonstrates κ -opioid ligands can enhance responses to bladder distension in group ZZ, an increase in facilitation by endogenous κ -opioid ligands could contribute to this hypersensitivity. The demonstration of the presence of κ -opioid facilitation that may counteract μ -opioid receptor mediated inhibition would explain the previously reported lack of effectiveness of the non-specific opioid receptor antagonist naloxone on VMRs to UBD in group ZZ [4]. From a clinical perspective, EIL bladder inflammation may increase susceptibility for the development of BPS/IC by effecting long-term changes in the κ -opioid system. Indeed, women with BPS/IC are more likely to recall recurrent urinary tract infections (UTIs) and antibiotic use as children [12]. Experiencing a second adult UTI could trigger symptom onset in these individuals by shifting a normally analgesic κ -opioid system to an algesic one.

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Abbreviations

EIL	Early-in-life
VMR	Visceromotor reflex
UBD	Urinary bladder distension
AA	Anesthesia EIL and anesthesia adult
AZ	Anesthesia EIL and zymosan adult
ZA	Zymosan EIL and anesthesia adult
ZZ	Zymosan EIL and zymosan adult

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Highlights

- Morphine (i.v.) produced dose-dependent inhibition of the VMR to UBD in all groups.
- U50,488H (i.v.) produced dose-dependent inhibition of the VMR in most groups.
- Bladder inflammation both EIL and as adults augmented VMRs after 1 mg/kg 50,488H.
- The same group demonstrated diminished inhibition of VMRs after 4 mg/kg U50,488H.
- A decrease in inhibition by κ -opioids could contribute to bladder pain states.

Figure 1.

VMRs to UBD expressed as % of pre-drug baseline normalized EMG activity for animals naïve to any intravesical treatment receiving i.v. administration of U50,488H, morphine, or saline ($n=7/\text{group}$). A 60 mmHg UBD test stimulus (20 s duration) was presented at 3 min intervals and VMRs were recorded. 1 mg/kg and 4 mg/kg of U50,488H, 1 mg/kg and 4 mg/kg of morphine, or 1 ml/kg and 4 ml/kg of saline were administered in a cumulative dosing regimen. VMRs of animals treated with 1 mg/kg or 4 mg/kg of U50,488H or morphine were significantly less than those of animals treated with saline ($*p<0.001$). VMRs after administration of 1 mg/kg of U50,488H were significantly less than those after administration of 1 mg/kg of morphine ($\#p=0.023$).

Figure 2.

VMRs to UBD expressed as % of pre-drug baseline normalized EMG activity after i.v. administration of U50,488H (n=7/group). 1 mg/kg and 4 mg/kg of U50,488H were administered in a cumulative dosing regimen. Data after 1 mg/kg of U50,488H were collapsed across time after administration of 1 mg/kg U50,488H. VMRs of group ZZ were significantly greater than those of all other groups (* $p < 0.001$). Because of a significant intravesical treatment x time interaction, data after 4 mg/kg of U50,488H were analyzed individually at each time point. VMRs of group ZZ were significantly greater than those of groups AA and AZ (* $p = 0.008$). VMRs of group ZA were significantly greater than those of AA controls (+ $p = 0.015$).

Figure 3. VMRs to UBD expressed as % of pre-drug baseline normalized EMG activity after i.v. administration of morphine (n=8/group). VMRs to UBD after either administration of 1 mg/kg or 4 mg/kg morphine did not significantly differ between intravesical treatment groups.