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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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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 U50,488H.
- 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.

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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 rechallenged 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.

1. 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

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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(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.

2. 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 to 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 h following adult intravesical treatments, each animal was anesthetized with isoflurane and oxygen (5% for induction, 4% for maintenance, and 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 respirated 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) × 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. (±)-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.

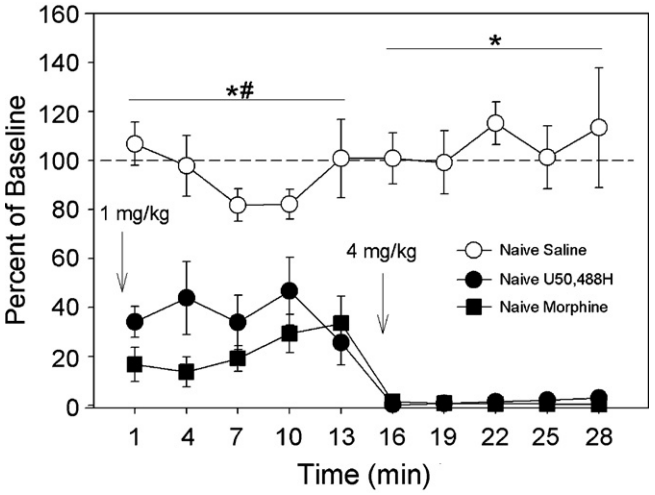

Fig. 1. VMRs to UBD expressed as % of pre-drug baseline normalized EMG activity for animals naïve to any intravesical treatment receiving i.v. administration of U50,488H, morphine, or saline ($n = 7$/group). A 60 mmHg UBD test stimulus (20 s duration) was presented at 3 min intervals and VMRs were recorded. 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 ($^{\#}p = 0.023$).

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 1.5× the interquartile range from the first or third quartile) were excluded.

3. Results

3.1. Naïve animals

Fig. 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 × time interaction. Post hoc comparisons were conducted for data collapsed across time. VMRs of animals treated with 1 mg/kg of U50,488H or